Biotechnological strategies to reduce the doses of sulfur dioxide in white winemaking and avoid problems of browning

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Abstract. This communication studies some possible strategies to reduce or even to replace sulfur dioxide in winemaking. Specifically, the aim of this work was to study the protective effect against oxidation of a commercial inactivated dry yeast (IDY) with very high level of glutathione, and of a selected non-*Saccharomyces* yeast, *Metschnikowia pulcherrima* strain in comparison with sulfur dioxide.

1 Introduction

Sulfur dioxide is without any doubt the most widely used additive (E-220) in winemaking and probably the most difficult to be replaced. The well-known antioxidant, antioxidasic and antimicrobial effects of sulfur dioxide [1] make this molecule a practically essential tool, not only in winemaking, but also in the production of other food products [2]. However, the current trend in winemaking is the reduction and even elimination of this unfriendly additive due to its negative effects on health and environmental, the increasing tendency of minimal intervention and because it can cause headaches in sensitive people. For all these reasons, wine sector is very concerned in searching strategies to reduce and/or even eliminate sulfur dioxide while still preventing oxidation and microbiological spoilage.

Several possible strategies to reduce or even to replace sulfur dioxide have been proposed for protecting wine against oxidation such as the use of ascorbic acid [3], inert gas [4], oenological tannins [5], glutathione [6], inactivated dry yeasts rich in glutathione [7] or that directly consume oxygen [8] and for protecting wine against microbiological spoilage such as the use of lysozyme [9], chitosan [10], fumaric acid [11], bioprotection [12], ultra-high pressure homogenization [13], ozone [14]. The aim of this work was to study the protective effect against oxidation of a commercial inactivated dry yeast (IDY) with a very high level of glutathione and of a selected non-*Saccharomyces* yeast, *Metschnikowia pulcherrima* strain used as bioprotective agent.

2 Materials and methods

2.1 Oxygen consumption measurements

The experimental design for determining the oxygen consumption rate of a commercial IDY with a very high level of glutathione and of a selected non-*Saccharomyces* yeast, *Metschnikowia pulcherrima* was an adaptation of the previously work described by Pascual et al. (2017) [15] for the oxygen consumption rate of oenological tannins. A grape juice of Muscat of Alexandria was immediately after pressing diluted 1:5 with a buffer containing L-(+)-tartaric acid (4 g/L), 3 mg of iron/L, in form of iron (III) chloride hexahydrate, and 0.3 mg of copper/L in the form of copper (II) sulfate pentahydrate, adjusted at pH = 3.5 with sodium hydroxide. This mixture was supplemented with 5 g of SO_2/hL (50 mg/L) or 2 g of SO_2/hL (20 mg/L). Mixtures with 2 g of SO_2/hL were also supplemented or not with 40 g/hL (400 mg/L) of an inactivated dry yeast (IDY-1) with a very high level of glutathione (Glutastar™, Lallemand Inc, Montreal, Canada) or with 40 g/hL (400 mg/L) of a selected non-*Saccharomyces* yeast, *Metschnikowia pulcherrima* MP-1 strain (Level2 Initia™, Lallemand Inc, Montreal, Canada).

This samples were immediately placed in clear glass bottles (750 mL) into which a pill had previously been inserted (PreSens Precision Sensing GmbH, order code: SP-PSt3-NAU-D5-CAF; batch number: 1203-01_PSt3-0828-01, Regensburg, Germany) for the noninvasive measurement of dissolved oxygen by luminescence (Nomasense TM O2 Trace Oxygen Analyzer by Nomacorc S.A., Thimister Clermont, Belgium). The bottles were maintained at 25 ± 2 °C during all this time. Oxygen was measured [16] periodically to determine the oxygen consumption rate. The total oxygen consumption was calculated using the mathematic model previously reported by Pons-Mercadé et al., 2021 [17].

2.2 Winemaking conditions

Grapes of Muscat of Alexandria were processed using 5 g or 2 g of SO_2/hL. This last experimental group was subdivided in three new groups. One group (IDY-1) was supplemented with 40 g/hL of an inactivated dry yeast with a very high level of glutathione (Glutastar™, Lallemand Inc, Montreal, Canada). This supplementation was carried out in two times, firstly 20 g/hL immediately after pressing and secondly 20 g/hL after settling. Other group (MP-1) was inoculated with 40 g/hL of a selected non-*Saccharomyces* yeast, *Metschnikowia pulcherrima* strain (Level2 Initia™, Lallemand Inc, Montreal, Canada).

This inoculation was also performed in two times, firstly 20 g/hL immediately after pressing and secondly 20 g/hL after settling. Finally, the last group was not supplemented with any other additive. All the grape juices were then inoculated with 20 g/hL of a commercial Saccharomyces cerevisiae strain (Lalvin EC1118™, Lallemand Inc, Montreal, Canada) and a standard white winemaking was carried out. Once alcoholic fermentation was finished all the wines were racked, slightly sulfited (2 g/hL) and supplemented or not with an experimental IDY with a high capacity to consume oxygen (IDY-2) [17] (Lallemand Inc, Montreal, Canada). Subsequently all the wines were analyzed. All the assays were performed by triplicate.

2.3 Color measurements

The measurement of yellow color (A420 nm) and the CieL*a*b* coordinates of the samples were determined according to Ayala et al. (1997) [18] and data processing was performed with MSCV software [19].

2.4 Hydroxycinnamic acid analysis by HPLC

Hydroxycinnamic acids were analyzed by RP-HPLC-DAD-ESI-MS according with the method reported by Lago-Vanzela et al. (2013) [20].

2.5 Sensory analysis

All the wines were tasted by a trained panel (7 males and 3 females) ageing from 22 to 60 years old. For each wine, tasters evaluated three sensorial attributes on a continuous scale from 1 to 10: colour intensity, fruitiness and olfactory oxidation. The values indicate the intensity of the sensation for each attribute. Tasters were also asked to give the preference order of the different wines from 0 (the lowest rated) to 10 (the highest rated).

2.6 Statistics

All data are expressed as the arithmetic average ± standard deviation of three replicates. One-factor analysis of variance (ANOVA) was carried out using the SPSS 15.0 software (SPSS Inc., Chicago, IL).

3 Results and Discussion

3.1 Oxygen consumption kinetics of the diluted grape juice

Figure 1 shows the evolution of the oxygen consumption kinetics of the different samples.

This graph clearly shows the control consumed oxygen very fast and that when SO_2 or IDY-1 were present this oxygen consumption decreased drastically. The reduction in the oxygen consumption kinetics caused by the presence of SO_2 can be attributed to the well-known inhibitory effect of this additive on the polyphenol oxidases [2]. In turn, the inhibitory effect of the IDY-1 can be attributed to its capacity to release glutathione. Glutathione reacts with orthodiquinones produced by the enzymatic oxidation of hydroxycinnamic acids and other orthodiphenols to form the grape reaction product (GRP) [21].

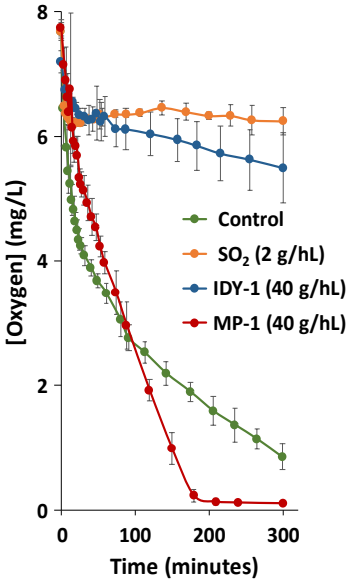

Figure 1. Oxygen consumption kinetics of grape must under different conditions.

This process can reduce the orthodiphenol concentration, causing their depletion and therefore stopping the O_2 consumption by the action of polyphenol oxidases. In contrast the presence of MP-1 accelerated the consumption rate because this non-*Saccharomyces* yeast strain directly consumes oxygen very effectively.

3.2 Total oxygen consumption and browning intensity of the diluted grape juice

Figure 2 shows Influence of SO_2, inactivated dry yeast rich on glutathione and *M. pulcherrima* on Total oxygen consumption (TOC) and browning intensity (A420 nm, b* and ΔEab*).

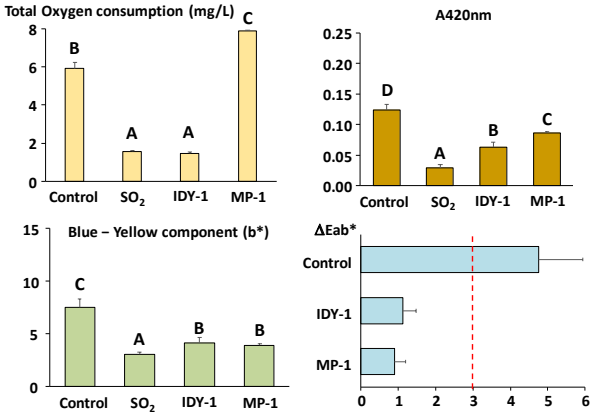

Figure 2. Influence of SO_2, a specific inactivated dry yeast rich on glutathione (IDY-1) and *M. pulcherrima* strain (MP-1) on total oxygen consumption and browning intensity.

The oxygen consumption kinetics data were used to calculate the total oxygen consumption using the mathematic model proposed by Pons-Mercadé et al., 2021

[8]. These data confirm that the presence of SO_2 and the specific IDY rich in glutathione IDY-1 lead to a drastic decrease in total oxygen consumption while *M. Pulcherrima* MP-1 strain increases it. Likewise, the presence of IDY-1 or *M. pulcherrima* MP-1 provided a clear protection against browning, although not as powerful as that of SO_2, as can be seen when comparing the values of A420 and the CIELab blue-yellow coordinate (b*). In any case, it is generally accepted that tasters can only distinguish the color of two wines through the glass when total color differences (ΔEab*) is higher than 3 units [22]. Considering this reference value, it can be asserted that the human eye can perfectly distinguish between the control sample without any additive (more brown) and the sample that was supplemented with SO2 (less brown), whereas cannot distinguish between the sample protected with SO_2 and those protected with the IDY-1 or *M. pulcherrima* MP-1.

3.3 Browning intensity of the real wines

Figure 3 shows the influence of SO_2, the specific inactivated dry yeast rich in glutathione IDY-1 and *M. pulcherrima* MP-1 strain on browning intensity (A420 nm and b*) of real wines.

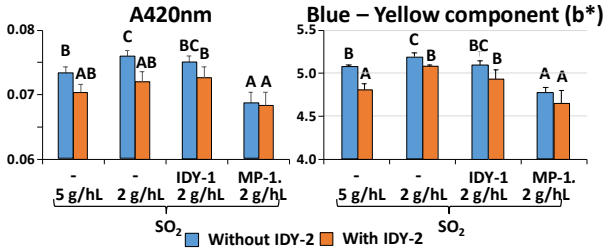

Figure 3. Influence of SO_2, a inactivated dry yeast rich on glutathione (IDY-1) and *M. pulcherrima* strain (MP-1) on browning intensity.

As can be seen from these graphs, both A420 and b* values were significantly higher when SO_2 levels were reduced from 5 to 2 g/hL, confirming that browning intensity was higher when low doses of this antioxidant were used. However, this trend was dampened when IDY-1 and especially *M. pulcherrima* MP-1 were present confirming the protective effect against browning of both treatments. In addition, the supplementation with the experimental IDY with a high capacity to consume oxygen (IDY-2) also seemed to decrease the intensity of browning.

3.4 Hydroxycinnamic acids

Figure 4 shows the influence of SO_2, inactivated dry yeast rich on glutathione and *M. pulcherrima* on the final concentration of hydroxycinnamic acids and GRP.

The results clearly indicate that the total concentration of hydroxycinnamic acids decrease drastically when the SO_2 concentration was reduced from 5 to 2 g/hL. This trend was in general similar for all the hydroxycinnamic acids. However, the presence of IDY-2 and *M. pulcherrima MP-1 strain* kept hydroxycinnamic acids at significantly higher levels than with only 2 g of SO_2/hL were used. These data confirm again the protective effect against enzymatic browning of this treatments since hydroxycinnamic acids are the main substrates of polyphenol oxidases. In that case, no significant differences were found when IDY-2 was added in any of the experimental conditions.

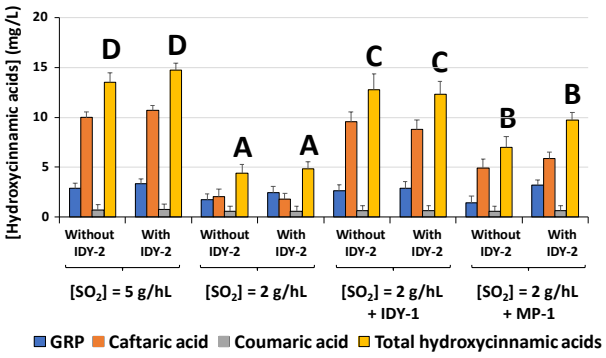

Figure 4. Influence of SO_2, a specific inactivated dry yeast rich in glutathione (IDY-1) and *M. pulcherrima* strain (MP-1) on hydroxycinnamic acids and GRP concentration.

3.5 Sensory analysis

Figure 5 shows the results of the sensory analysis of the different wines. The panelists in general considered that the wine elaborated with 5 g of SO_2/hL has a less intense color, lower levels of olfactory oxidation and higher intensity of fruitiness than all the wines elaborated with 2 g of SO_2/hL. In addition, the wine elaborated with 5 g of SO_2/hL was classed as the first in the preference order.

However, tasters also considered that the wines that were also supplemented with the IDY-1 and *M. pulcherrima* MP-1 presented better characteristics in color, olfactory oxidation and fruitiness than the wine elaborated only with 2 g of SO_2/hL. Moreover, in all the cases the wine elaborated only with 2 g of SO_2/hL was considered as the worse. This trend was in general terms similar when the wines were supplemented with IDY-2.

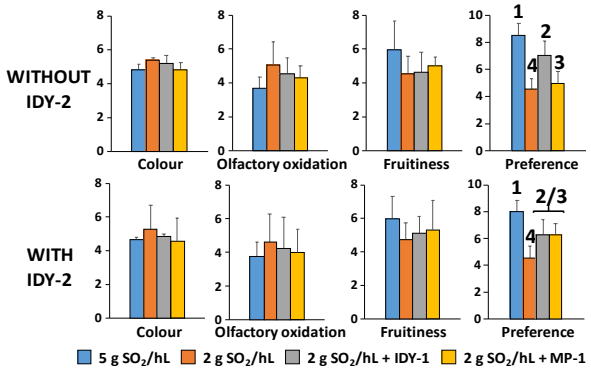

Figure 5. Sensory analysis.

4 Conclusions

It can be concluded that the supplementation with a specific IDY rich in glutathione IDY-1 and the inoculation

of *M. pulcherrima* MP-1 strain used as bioprotective agent are interesting and useful tools to protect wine against browning. Both treatments resulted in wines with better color and that were better valued in the tasting compared to the wine that only contained 2 g of SO2/hL which suggests their usefulness to reduce the doses of SO_2 in winemaking.

The mechanism by which IDY-1 appears to be related to the protective effect exerted by glutathione and other nucleophilic peptides identified in this IDY [23], which is combined with the orthodiquinone produced by the enzymatic oxidation of hydroxycinnamic acids and other orthodiphenols to form the grape reaction product (GRP), stopping thereby the formation of brown melanins. In contrast, the protective effect of the *M. pulcherrima* MP-1 strain seems to be related with the fact that it consumes oxygen very effectively preventing it from being consumed by polyphenol oxidases. Both treatments seem to avoid the consumption of hydroxycinnamic acids which would explain the lower browning.

Acknowledgements

This research was funded by the Spanish Ministry of Science and Innovation, Interministerial Commission for Science and Technology (CICYT - project RTI2018-095658-B-C33).

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