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Antioxidant activity of yeast derivatives: Evaluation of their application to enhance the oxidative stability of white wine

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ABSTRACT

Maintaining wine oxidative stability and reducing SO_2 addition remains a challenge in white winemaking. This study aimed to evaluate the antioxidant capacity of two yeast derivatives (YDs) with a specific composition: one was rich in reducing compounds, including glutathione (YD_R), and the other was rich in lipid compounds (YD_L). Both YDs were evaluated for their antiradical activity in model wine solution (by the DPPH method) and for their capacity to consume oxygen in model wine solution and in white wine. Antiradical activity in both matrixes and oxygen consumption rate in wine were higher for YD_L than for YD_R. However, the addition of YD_R to wine limited the production of acetaldehyde and preserved glutathione content to a greater extent after wine oxygenation. The sensory analysis confirmed that both YDs, in particular YD_L, limited the occurrence of oxidation off-odours when no SO_2 was added. These data suggest that the use of YDs could be effectively implemented in low-sulfite winemaking in order to improve the antioxidant protection of white wine.

1. Introduction

Oxidation processes constitute the main problem in winemaking, especially in the case of white wines. Enzymatic and chemical oxidation affect the key attributes of white wine organoleptic quality, resulting in colour browning and a decrease in aroma quality (Nikolantonaki & Waterhouse, 2012). Despite the mechanisms involved in wine oxidation having been extensively reviewed (Waterhouse & Laurie, 2006), the protection of wine against oxidative spoilage is still a main goal in winemaking. The protection of young white wines from oxidation becomes particularly challenging when low levels of sulphur dioxide (SO_2) are used. SO_2 is one of the most efficient additives in winemaking, being used to prevent oxidation and microbial spoilage (Fazio & Warner, 1990). However, in the last decade, adverse reactions to SO_2 in sensitive subjects have led to the tendency to reduce its use in winemaking (Vally, Misso, & Madan, 2009). In the context of competitive global winemaking marketing strategies, it has become crucial to reduce or even eliminate the use of SO_2 and to find new healthier strategies.

Different compounds (such as ascorbic acid, tannins and fresh lees, among others) have been proposed as alternatives to SO_2 in winemaking, but none of them have been found to be effective in protecting wines against oxidation (Ugliano, Slaghenaufi, Picariello, & Olivieri, 2020; Fornairon-Bonnefond, Camarasa, Moutounet, & Salmon, 2002). In the last decade, Yeast Derivatives (YDs) have been proposed as new additives for preventing wine chemical oxidation (Bahut et al., 2019; Comuzzo et al., 2015; Pons-Mercadé et al., 2021). YDs are obtained from *Saccharomyces cerevisiae* yeasts after thermal inactivation. These are classified according to production process, composition and degree of purification: inactivated dry yeasts are obtained after thermal inactivation followed by drying; yeast autolysates are obtained after thermal inactivation and enzyme processing; yeast cell walls are obtained by centrifugation of yeast autolysate; yeast extracts are a soluble extract of inactivated yeast or yeast autolysate; and purified mannoproteins are obtained from yeast cell walls, with different degrees of purification (Shurson, 2018). Even though the composition of these products is rarely stated by suppliers, their composition and properties can vary

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depending on yeast strain, yeast culture conditions and manufacture process. YDs are often used during the winemaking process to improve technological and sensorial properties or to remove undesirable wine compounds (Pozo-Bayon, Andujar-Ortiz, Alcaide-Hidalgo, Martin-Alvarez, & Moreno-Arribas, 2009). YDs can improve wine organoleptic properties, because they affect the colour, mouthfeel and chemical-physical stability of wine (Comuzzo, Tat, Tonizzo, & Battistutta, 2006; Escot, Feuillat, Dulau, & Charpentier, 2001). Winemakers have become very interested in the use of yeast preparations to improve wine sensory characteristics and wine stability. Yeast mannoproteins have been shown to act as protective colloids that improve the stability of phenolic compounds (Escot et al., 2001; Riou, Vernhet, Doco, & Moutounet, 2002). This is the reason why the use of YDs enriched in yeast mannoproteins has been reported as stabilising the colour of red wines (François, Alexandre, Granes, & Feuillat, 2007). Moreover, it has been shown that these products can adsorb phenolic compounds and browning products in white wines (Razmkhab et al., 2002). Using yeast preparations to reduce wine astringency and improve wine aromatic profiles has also been explored in some wines (Bautista, Fernández, & Falqué, 2007; Chalier, Angot, Delteil, Doco, & Gunata, 2007; Guadalupe, Martínez, & Ayestarán, 2010; Rinaldi, Gambuti, Moine-Ledoux, & Moio, 2019). YDs may affect wine aroma perception with both direct and indirect effects on the volatile fraction (Comuzzo et al., 2006). In particular, the effect of YDs on wine volatile compounds is the result of several phenomena: i) the adsorption of wine volatiles by yeast walls (Lubbers, Charpentier, Feuillat, & Voilley, 1994), ii) the release of YD volatile compounds (Comuzzo et al., 2006), and iii) the release of soluble colloids able to affect the volatility of wine aroma compounds (Comuzzo et al., 2006; Pozo-Bayon et al., 2009). The entity of these concurrent phenomena depends on several factors, such as wine chemical composition, YD characteristics and YD dosage (Comuzzo et al., 2006). In a recent study, yeasts of specific compositions were prepared to be used as antioxidant in wine (Bahut et al., 2019). Their protective action was mainly due to the glutathione (γ-L-Glutamyl-L-cysteinyl--glycine) present in the yeasts. Glutathione (GSH) is already naturally present in grapes and musts and its concentration in wines can vary between 0.1 and 70 $mg.L^{-1}$, depending on the grape variety, maturity level, vineyard practices and fermentation conditions (Dubourdieu & Lavigne, 2004; Kritzinger, Bauer, & Du Toit, 2013). The addition of GSH to must can prevent enzymatic browning in white wine making (Vaimakis & Roussis, 1996), and the addition of GSH to wine before bottling has been found to reduce oxidation phenomena, thus preserving colour and some varietal aroma compounds, as well as reducing the occurrence of oxidation off-flavour compounds (Dubourdieu & Lavigne, 2003). Nowadays, the addition of pure GSH in winemaking is prohibited (UE 2022/68), its use only being possible during alcoholic fermentation using inactivated dry yeast naturally rich in GSH. Despite the growing interest in yeast derivatives, sound knowledge about their composition and antioxidant action is lacking, and it can thus be difficult for winemakers to choose from different products. For these reasons, the aim of this work was to carry out a preliminary investigation into the antioxidant activity of YDs with different compositions (rich in reducing compounds, including GSH or rich in lipids) in a model wine solution. In order to better understand their antioxidant potential, different methods to evaluate antioxidant activity were applied. In a second step, YDs were added to white wine to simulate their oenological use, and their ability to prevent wine oxidation in oxidative conditions was compared to conventional SO_2 addition.

2. Materials and methods

2.1. Chemicals and products

For the preparation of the model wine solution, the following reagents were used: ethanol 99% (VWR Chemicals, Fontenay-sous-Bois Cedex), L (+)-tartaric acid (Acros Organics, Illkirch Cedex), copper (II) sulfate pentahydrate, iron (III) chloride hexahydrate and potassium metabisulfite (Sigma, St. Louis, MO, USA). For the determination of the antiradical activity by the DPPH test the following reagents were used: DPPH (2,2-diphenyl-1-picrylhydrazyl) from Extrasynthèse (Genay, France) and Trolox (±)-6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid 97% from Acros Organics (Illkirch Cedex). For the glutathione and acetaldehyde analysis: organic solvents (acetonitrile, methanol, glacial acetic acid) were purchased from Acros Organics (Illkirch Cedex). Acetaldehyde and glutathione were purchased from Sigma Aldrich (St Louis, MO USA).

Two different commercial yeast derivatives (YDs) (95% dry matter) were tested in the present study (Laffort, France). The two yeast derivatives were selected for their different compositions: one was naturally rich in lipids (YD_L) and the other was naturally rich in reducing compounds, including glutathione (YD_R). YD_L comprised 1.5 mg g^{-1} of total reducing compounds (determined by Ellman's method) and 60 mg g^{-1} of total lipids; YD_R comprised 30 mg g^{-1} of total reducing compounds and 24 mg g^{-1} of total lipids. Both the YDs were obtained from fermentation broth of *S. cerevisiae* under controlled operational conditions in order to obtain the specific composition of the YDs.

2.2. DPPH radical scavenging activity of YDs in model wine solution

The DPPH assay was performed using the method described by Brand-Williams, Cuvelier, and Berset (1995) with some modifications. For the measurements, 190 μL of DPPH reagent (9.10^{-5} M in 50% of ethanol solution) and 10 μL of sample (yeast derivatives at 2 $g.L^{-1}$ in 12% of ethanol solution) or Trolox (0.1–1 mM, used as a standard in 12% of ethanol solution), were put into each cell of a 96-well microplate. After 30 min of reaction at 25 °C in the dark, the absorbance at 515 nm was measured by a spectrophotometer UV–Vis Helios Alpha (Thermo Fisher Scientific Inc., Waltman, MA, USA). All the results were expressed as mg of Trolox equivalent/g of YD. The measurements were repeated six times for each sample.

2.3. Wine treatments

The study was carried out using a Chardonnay wine (PGI Pays D'Oc) from the 2019 vintage. The grapes were harvested at 21 °Brix and the vinification process was carried out following a conventional white winemaking protocol, but with no addition of SO_2. The classical oenological parameters of wine were the following: alcoholic degree = 12.7 vol %, pH = 3.4, total acidity = 6.11 $g.L^{-1}$ of tartaric acid, volatile acidity = 0.7 $g.L^{-1}$ of acetic acid (OenoFoss™, Foss analytical, Denmark). Total and free SO_2 were 3.2 ± 0.7 and 1.1 ± 0.2 $mg.L^{-1}$, respectively (Y15 analyzer, Biosystems S.A., Barcelona). The experimental samples were: wine before oxygenation at saturation (W-NoOx); wine saturated with oxygen (W-Ox); wine supplemented with 0.3 $g.L^{-1}$ of yeast derivatives rich in reducing compounds, including glutathione (YD_R) and saturated with oxygen (WYD_R-Ox); wine with the addition of 0.3 $g.L^{-1}$ of yeast derivatives rich in lipid compounds (YD_L) and saturated with oxygen (WYD_L-Ox); wine with added SO_2 (35 ± 5 and 15 ± 3 $mg.L^{-1}$ of total and free SO_2 respectively) and saturated with oxygen (WSO_2-Ox). For each of these, 1 L of wine was used and for each treatment, 3 aliquots of 320 mL were put into 250 mL glass bottles filled to the brim and subjected to oxygen consumption measurements, as described below.

2.4. Oxidation procedure and oxygen consumption measurements

The Oxygen Consumption Rate (OCR) of the YDs was measured both in the experimental wines described in Section 2.3 and in the model wine solution. The model wine solution was composed of 12% ethanol (v/v), 4 $g.L^{-1}$ of tartaric acid, 3 $mg.L^{-1}$ of Fe (II) and 0.3 $mg.L^{-1}$ of Cu (II) to reproduce the typical catalytic conditions of wine. The pH was adjusted to 3.4 using NaOH 1M. The model wine solution was then

supplemented with 0.5, 4.0, 5.0 and 6.0 g.L^{-1} of one of the YDs (YD_L or YD_R) and saturated with oxygen by bubbling with air (8 ± 0.7 mg.L^{-1}). The oxygen dissolved in the model wine solution saturated with O_2 without YD_s addition was also monitored. 250 mL-bottles (Duran borosilicate glass) equipped with oxygen sensor spots OXSP5 (Bioneuf, Montreuil, France) were used. The bottles were filled to the brim with the experimental wines or model solutions, closed (caps GL45 with septum Bromobutyl, D. Dutscher, Merignac, France) and gently shaken during measurements by a multiple-position stirring plate (Thomas Scientific, New Jersey, USA). The temperature was maintained at 19 ± 1 °C. The dissolved oxygen measurements were performed by the luminescence technique (Pyroscience optical O_2 sensor, red-flash technology, Bioneuf, France). The dissolved oxygen measurements started after 10 min of equilibration and were carried out by automatic on-line measurement at 1h intervals until the total O_2 consumption was achieved.

2.5. Quantification of glutathione (GSH) in wine

The GSH concentration of the experimental wines was determined by the method described by Pons, Lavigne, and Darriet (2015). Before HPLC analyses, the wine samples underwent derivatisation with monobromobimane (MBB), and 40 μL of trifluoroacetic acid solution (70%) was added to stabilise the MBB-GSH adducts. After the addition of these reactants, the samples were stored in the dark for 20 min and then 5 μL of sample was injected into a reversed-phase HPLC system with a fluorescence detector (HPLC Ultimate 3000 system, Dionex, Germering, Germany). Separation was performed on a reverse phase Licrospher analytical column (250 mm × 4.6 mm; 5 μm particle size, Supelco, Dijon, France) using solvent A (10% MeOH, 0.25% acetic acid, adjusted to pH 4.3 with 4 M NaOH) to solvent B (90% MeOH, 0.25% glacial acetic acid, adjusted to pH 4.3 with 4 M NaOH) at a flow rate of 1 mL min^{-1}. Data processing was carried out with Chromeleon 6.8 software (Dionex, Sunnyvale, CA). The amount of GSH was determined by using a calibration curve built from the analyses of standard GSH solutions (0–40 mg.L^{-1}). Measurements were performed in duplicate on each replicate of treatment.

2.6. Base chemical parameters, polyphenols by spectrophotometry and chromatic characteristics of wines

Base chemical parameters (alcoholic degree, pH, total acidity, volatile acidity, and lactic acid) were determined by FT-IR spectroscopy with OenoFoss™ (Foss analytical, Denmark). Total and free SO_2 were determined by enzymatic method with a Y15 analyzer (Biosystems S.A., Barcelona).

The absorbance of wine samples at wavelengths of 280 nm (total polyphenols), 320 nm (phenolic acids) and 420 nm (wine yellow tone) was measured using an UV–vis spectrophotometer (Helios Alpha, Thermo Fisher Scientific Inc., Waltman, MA, USA). The chromatic characteristics of the wine samples were determined using the CIELab universal colour system (method OIV-MA-AS2-11). The results were expressed by L*, which is directly related to wine clarity (L* = 0 black and L* = 100 white), a*, which is related to the green/red colour component (a*>0 red, a*<0 green) and b*, which corresponds to the blue/yellow colour component (b*>0 yellow, b*<0 blue). The L*a*b* values were determined by a Nomasense Color P100 colorimeter. The wine samples were filtered with a 0.8 μm filter before analysis, and the measurements were performed in triplicate for each treatment replicate.

2.7. Acetaldehyde determination

Acetaldehyde in the experimental wines was determined by gas chromatography with flame ionisation detection (GF-FID) according to the OIV method (OIV-MA-AS315-27) with some modifications. For the preparation of the samples, 50 μL of 4-methylpentan-2-ol (14 g.L^{-1}) was added to 5 ml of wine as the internal standard. The mix was directly injected into the GC-FID system. An Agilent 6890 gas chromatograph (Agilent Technologies, Massy, France) equipped with a CP-Wax 57 CB capillary column (50 m × 0.25 mm I.D., 0.2 μm film thickness, Agilent Technologies, Santa Clara, CA) was used. The amount of acetaldehyde was determined by using a calibration curve obtained from the analyses of the standard acetaldehyde solutions. The analyses were performed in duplicate on each treatment replicate.

2.8. Sensory analysis

All the wines used for tasting were treated (in duplicate) in 1 L bottles, which were equipped with oxygen sensor spots OXSP5 (Bioneuf, Montreuil, France), filled to the brim with the different experimental wines, then closed and gently shaken, in order to reproduce the same conditions of the bottles used for the oxygen measurements. The oxygen consumption was evaluated each day and the data were in accordance with the results obtained from the OCR experiments. Once the complete O_2 consumption was achieved, the two replications of 1L were mixed for sensory analysis.

The sensory analysis was carried out by a panel of 19 wine experts (12 females and 8 males, aged 25–45) recruited from the University of Bordeaux (Institut de Science de la Vigne et du Vin, Bordeaux, France). The sensory analysis took place in a standard tasting room equipped with individual booths and air conditioned at 20 ± 1 °C (ISO 8589:2007). The wine samples (30 mL) were presented at room temperature in standard glasses (ISO 3591:1977), covered with glass Petri dishes and coded with random three-digit codes. The order of sample presentation was randomised. The judges were asked to rate the intensity of oxidation off-odour in an orto-nasal evaluation on a 10-cm line scale (0 = absent, 10 = very high). Each sample was evaluated in duplicate.

2.9. Data analysis

The analytical data were submitted to one-way analysis of variance (ANOVA) and the means of the variables were compared by Duncan's post hoc test (for both, $p < 0.05$ was considered significant), according to Hashemi, Jafarpour, & Jouki, 2021. Only the obtained data relative to the DPPH radical scavenging activity and the OCR of YDs in wine model solution were analysed by the two-sample *t*-test (significance level 95%). All the statistical analyses were performed using XLSTAT-Pro 7.5.3 (Addinsoft).

3. Results and discussion

3.1. Radical scavenging activity of yeast derivatives (YD_G and YD_L) in model wine solution

YDs are oenological products that have many applications in winemaking (Comuzzo et al., 2006, 2015; Pozo-Bayon et al., 2009). Although many different yeast derivatives are nowadays available for oenological use, their properties are often not well determined. In particular, in the present study we were interested in the possible application of YDs as antioxidants in winemaking, with a view to reducing SO_2 doses. To achieve this aim, the antioxidant properties of two different yeast derivatives (rich in reducing compounds including glutathione [YD_R] and rich in lipids [YD_L]) were first estimated by measuring the radical scavenging activity in model wine solution. The radical scavenging activity was determined by DPPH assay. The antiradical potential is expressed as mg Trolox equivalent. Fig. 1 shows the values of TEAC (Trolox Equivalent Antioxidant Capacity) of YD_R and YD_L. While both YDs quenched the DPPH radical, YD_L showed an antiradical activity 1.7 times higher than that of YD_R. This difference is probably due to the antioxidant activity of lipids richer in YD_L. Indeed, the antioxidant activity of lipids has already been shown (Gai et al., 2013; Yeo, Jeong, &

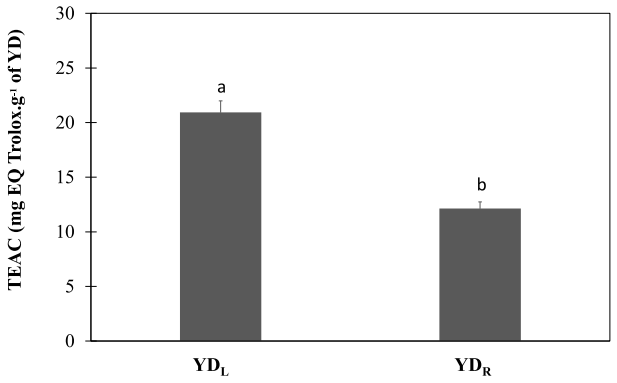

Fig. 1. TEAC (Trolox Equivalent Antioxidant Capacity, mg Trolox.g^{-1} of YDs) of YDs in model wine solution (12% ethanol, 4 g.L^{-1} of tartaric acid, 3 mg.L^{-1} of Fe (II) and 0.3 mg.L-1 of Cu (II)). All data are expressed as the average of 6 replicates ± standard deviation. YD_L = yeast derivative naturally rich in lipids, YD_R: yeast derivative naturally rich in reducing compounds including glutathione. The different letters indicate that the means are significantly different (two-samples *t*-test, 95% significance level).

Lee, 2012). The lipid reaction with oxygen leads to a complex set of free radical reactions followed by their chain oxidation; thus, we can assume that lipid oxidation can lead to the formation of radical scavenging compounds that play a major role in the decrease in DPPH absorbance. The capacity for YD_L to quench radical species could be potentially exploited for the antioxidant protection of wine, as the formation of highly reactive radical oxygen species (ROS), such as hydroperoxyl radical (OH–O•) from molecular oxygen, and the highly reactive hydroxyl radical (HO•) from H_2O_2 through Fenton reaction, are crucial in the oxidative chain of wine (Danilewicz, 2003; Waterhouse & Laurie, 2006).

YD_R also exhibited antiradical activity, although it was lower (- 43%) than YD_L. This antiradical activity may not only be attributable to GSH but also to other reducing compounds co-accumulated with GSH during the enrichment of YDs, as recently shown in different studies (Bahut et al., 2020; Comuzzo et al., 2015). Indeed, the authors observed that GSH was not the only factor to play a role in the radical scavenging capacity of YDs; other components of YDs might be involved, with a non-negligible contribution to the observed effects (Bahut et al., 2020; Comuzzo et al., 2015).

The present results suggest that lipids are mainly responsible for the antiradical properties of the tested YDs, and that it may be possible to use YD_L more effectively as an antioxidant in wine. However, antioxidant activity due to radical quenching is assumed to be of lower importance in real wine, given the non-selectivity of the hydroxyl radical and the relative abundance of ethanol (which is a more likely substrate for radical oxidation) (Kreitman, Laurie, & Elias, 2013); therefore, other antioxidant mechanisms should be considered.

3.2. Oxygen consumption rate (OCR) of YD_R and YD_L in model wine solution

Another antioxidant mechanism exploitable in wine is the consumption of dissolved oxygen (O_2) by chemical reactions other than the "oxidative chain reaction". In order to evaluate the oxygen consumption of the two YDs, the evolution of oxygen concentration over time in model wine solution supplemented with YDs and saturated with O_2 was carried out. The model wine solution contained 3 mg.L^{-1} of Fe (II) and 0.3 mg.L^{-1} of Cu (II) in order to reproduce the typical catalytic conditions of wine (Pascual et al., 2017). From these data, the oxygen consumption kinetics (OCR expressed as mg. of O_2. $days^{-1}.g^{-1}$) of YDs was determined (kinetic model proposed by Pascual et al., 2017).

YD_L consumed O_2 twice as fast as YD_R (OCR = 0.6 and 0.3 mg.O_2. day^{-1}. g^{-1}, for YD_L and YD_R respectively) (Fig. 2). Thus, the oxygen consumption kinetics seem to have been more impacted by the presence of lipids than by the reducing compounds. The higher value obtained for YD_L may be related to the presence of lipids, especially unsaturated fatty acids, which react directly with O_2 (Gunstone, 1984). This reaction involves free radical initiation and peroxyl radical formation (Gunstone, 1984). The capacity of lipids to react rapidly with oxygen could lead to the subtraction of O_2 from the oxidative chain reaction, thus preventing or limiting the oxidation of wine compounds, such as polyphenols and volatile aroma compounds, and preserving the amount of free SO_2 (Danilewicz, 2003).

The OCR of YD_R was lower (−50%) than that of YD_L (Fig. 2). While the global antioxidant capacity of GSH is well reported (Noctor et al., 2012), its capacity to react with oxygen is poorly described in literature. Recently, Pons-Mercadé et al. (2021) evaluated the total oxygen consumption capacity of GSH in model wine solution. They showed that GSH can react with oxygen and consume about 0.85 mg.L^{-1} of O_2.. day^{-1} at 20 mg.L^{-1} of GSH (Pons-Mercadé et al., 2021). In addition, they showed that while inactivated dry yeast rich in GSH (from 1.59 mg.L^{-1} to 5.56 mg.L^{-1}) can consume oxygen (0.6–0.9 mg.L^{-1}.day^{-1} for 400 mg. L^{-1} of YD), their direct oxygen consumption is not correlated with their GSH content (Pons-Mercadé et al., 2021). As for radical quenching activity, it seems that GSH only partially accounts for the oxygen consumption of YDs, the total amount of reducing compounds which are co-accumulated during YD production also playing a role in it (Comuzzo et al., 2015; Pons-Mercadé et al., 2021). In the second part of this study, the application of YD_R and YD_L in white wine was investigated; the results are discussed in the following paragraphs.

3.3. Effect of YD treatments on oxygen consumption rate of wine

In this study, we chose to simulate wine oxidation by supplying oxygen at saturation level (about 8.5 mg.L^{-1} of O_2). A white wine (W) (cv. Chardonnay, vinified without SO_2 addition) was exposed to bubbling air until oxygen saturation was reached, then YD_L (0.3 g.L^{-1}) [WYD_L-Ox], YD_R (0.3 g.L^{-1}) [WYD_R-Ox], or SO_2 (total and free SO_2 at 35 ± 5 and 15 ± 3 mg.L^{-1} respectively) [WSO_2-Ox] were added. Two control wines without antioxidant treatment were implemented: one was submitted to oxygenation at saturation (W-Ox) and the other was not exposed to O_2 (W-NoOx).

First, the Oxygen Consumption Rate (OCR, mg.L^{-1} of O_2.day^{-1}) was evaluated for all the treatments. Fig. 3 shows the OCR of the experimental wines. The O_2 measurements were carried out every 2h until total oxygen consumption (15 days). The method proposed by Pascual

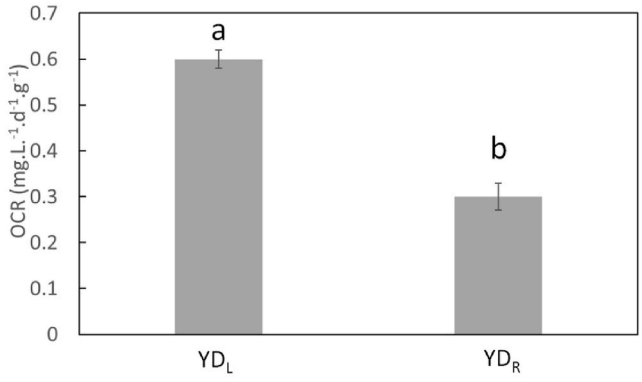

Fig. 2. Oxygen consumption rate (OCR, mg.L^{-1}.d^{-1}.g^{-1}) of Yeast Derivatives naturally rich in lipids (YD_L) and in reducing compounds (YD_R), in model wine solution (12% ethanol (v/v), 4 g.L^{-1} of tartaric acid, 3 mg.L^{-1} of Fe (II) and 0.3 mg.L^{-1} of Cu (II)). Data are the average of 3 replicates ± standard deviation. The different letters indicate that the means are significantly different (two-samples *t*-test, 95% significance level).

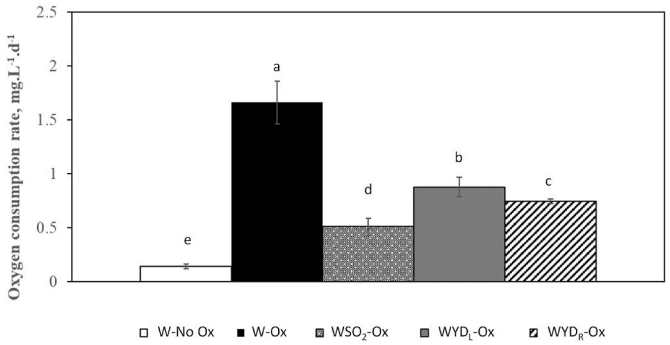

Fig. 3. Oxygen consumption rate of the experimental wines. W-NoOx: control wine without oxygenation at saturation; W-Ox: control wine saturated with O_2; WSO_2-Ox: wine with added SO_2 (total SO_2: 35 ± 5 mg.L^{-1} and free SO_2: 15 ± 3 mg.L^{-1}) and saturated with O_2; WYD_R-Ox: wine with added 0.3 g.L^{-1} of yeast derivatives rich in reducing compounds and saturated with O_2; WYD_L-Ox: wine with added 0.3 g.L^{-1} of yeast derivatives rich in lipid compounds and saturated with O_2. All data are expressed as the average of 3 replicates ± standard deviation. Different letters indicate a significant difference ($p < 0.05$).

et al. (2017) was used to estimate the oxygen consumption rate (OCR) expressed as mg.L^{-1} of O_2.day^{-1}.

The oxygen consumption rate of W-NoOx was very slow (0.1 mg.L^{-1}.day^{-1}), which can be considered negligible. The range of the oxygen consumption rates of the wines after air saturation varied from 1.6 ± 0.2 to 0.5 ± 0.05 mg.L^{-1}.day^{-1}. For the air-saturated wines, the oxygen consumption rate was in the following order (from highest to lowest OCR): W-Ox > WYD_L-Ox > WYD_R-Ox > WSO_2-Ox. The mean OCRs of WYD_L-Ox, WYD_R-Ox and WSO_2-Ox were 46%, 53% and 68% lower than W-Ox respectively (Fig. 3). These results show that the addition of both YDs reduced the oxygen consumption kinetics in wine at levels almost comparable to the addition of a conventional dose of SO_2. The oxygen consumption observed in the W-Ox sample is due to the well-known reaction of O_2 with wine constituents, particularly polyphenols, as they are among the most readily oxidised wine constituents in the presence of the metal ions Cu^{2+} and Fe^{2+} (Danilewicz, 2003; Waterhouse & Laurie, 2006). Quinones, which are derived from the oxidation of *o*-diphenols, are unstable and undergo further reactions (Danilewicz, 2003; Waterhouse & Laurie, 2006). Quinones can combine with nucleophilic compounds, including some phenols, thiols and amines due to their high electrophilic character, thus their oxidation results in an acceleration of reactive oxygen species formation that increases the kinetics of oxygen consumption (Danilewicz, 2003; Waterhouse & Laurie, 2006). The wine treated with SO_2 showed the lowest OCR out of all the treatments (0.2 mg.L^{-1}.d^{-1}). This contrasts with the behaviour reported in the literature; indeed, Danilewicz, Seccombe, and Whelan (2008) showed that the rate of oxidative reactions is accelerated by SO_2 addition (experiments in model wine solution). These authors explained that sulphites can react with quinones, reducing them back to *o*-diphenols (reactive species), thus increasing OCR (Danilewicz et al., 2008). However, in a recent study on white wine, Comuzzo and his collaborators (Comuzzo et al., 2015) showed that while SO_2 did not affect the kinetics of oxygen consumption in young wine, the kinetics were accelerated in aged wines. It is difficult to explain these differences in SO_2 behaviour. In fact, if we consider that sulphites can accelerate catechol autoxidation, we can assume that the addition of SO_2 accelerates the conversion of polyphenols into quinonic species. Thus, the low total polyphenol index of the studied wines and their low amounts of SO_2 may slow down oxygen consumption simply because of the low content of reactive substances like polyphenols (Comuzzo et al., 2015).

We also observed that the treatment WYD_R-Ox and WYD_L-Ox decreased the kinetics of O_2 consumption almost 2-fold compared to W-Ox. The action mechanisms of these YDs have not yet been elucidated. The oxidation of lipids leads to the formation of lipid peroxy radical (LOO•), which are reactive oxygen species (ROS). This is the first time that a YD naturally rich in lipids has been shown to preserve wines from oxidation. This capacity for lipids to react rapidly with oxygen may allow them to compete with less reactive wine compounds (like polyphenols and varietal aroma) and to prevent or limit their oxidation. However, the fate of the radical species derived from lipid oxidation needs to be elucidated.

3.4. Effects of treatments on base chemical parameters and colour of wine

Classical oenological parameters of the experimental wines were determined at the beginning of the experiment (W-NoOx) and at the end of oxygen consumption for all the treated wines. The differences in terms of pH and total acidity were not significant, while the differences found for ethanol, lactic acid and volatile acidity were in the range of instrumental error (Table 1). Due to the absence of any differences between the untreated wine (W-NoOx) and treated wines after O_2 consumption it was possible to exclude potential bacterial spoilage (such as lactic acid bacteria or acetic acid bacteria) caused by the addition of YDs and to confirm that oxygen consumption can be attributed to YDs and wine compounds only. For the wine treated with sulphur dioxide, total and free SO_2 before oxygen consumption was 35 mg.L^{-1} and 15 mg.L^{-1} respectively. As expected, free SO_2 decreased after oxidation, from 15 mg.L^{-1} to 5 mg.L^{-1} (Table 1), due to it combining with oxidation products (quinones and hydrogen peroxide).

Because oxidation phenomena have an impact on wine browning, absorbance at 280 nm, 320 nm and 420 nm and chromatic characteristics (CIELab) were measured in order to determine the impact of treatments on total polyphenols (280 nm), phenolic acid contents (320 nm) and yellow colour evolution (browning) (420 nm) in wines (Voltea, Karabagias, & Roussis, 2022). Table 2 shows the optical densities and the chromatic characteristics of the experimental wines.

The absorbance of oxygen-saturated wines at 280 did not show any significant differences among wines. As concerns absorbance at 320 and 420 nm, even when statistically significant differences were found, they were very little and of negligible relevance for wine quality characteristics.

To explore the chromatic characteristics of wine after the treatments, a CIELab method (absorption of the entire visible spectrum L*a*b) was used. In CIELab space, L* indicates that the brightness varied from 0 (black) to 100 (white), a* and b* indicates the colour direction: positive and negative a* values indicate the red and green ends of the colour range respectively, while positive and negative b* values indicate the yellow and blue ends respectively. W-Ox showed higher L* a* b* chromatic values than W-NoOx (Table 2). The increase in red (+a) and yellow (+b) components is typical of colour oxidation, while the L* value indicates wine clarity; therefore, the increase in L* for W-Ox indicates a decrease in its colour clarity.

When comparing W-Ox and WSO_2-Ox, it can be seen that the values of a*, b*components are not different to those of W-NoOx (Table 2). As expected, the presence of SO_2 in the wine prevented oxidation and thus preserved the colour. The addition of YD_R and YD_L showed a good efficacy, quite similar to those of SO_2 (WSO_2-Ox), in preserving colour, with a*, b* and L* parameters significantly lower than W-Ox and (Table 2). These results are promising in terms of the potential use of both the studied YDs as alternative treatments to SO_2 for preventing white wine from browning.

3.5. Impact of treatments on acetaldehyde content of wine

Of the oxidation markers, acetaldehyde is the principal compound to be derived from the chemical oxidation of wine (Waterhouse & Laurie, 2006). Indeed, acetaldehyde is derived from the oxidation of ethanol, which is the most abundant non-water component of wine, and therefore acetaldehyde is the most abundant product of the Fenton reaction (Waterhouse & Laurie, 2006). In red wine acetaldehyde is often

Table 1
Base chemical parameters of the experimental wines at the end of oxygen consumption. W-NoOx: control wine before oxygenation at saturation; W-Ox: control wine saturated with O_2; WSO_2-Ox: wine with added SO_2 (total SO_2: 35 ± 5 mg.L^{-1} and free SO_2: 15 ± 3 mg.L^{-1}) and saturated with O_2; WYD_R-Ox: wine with added 0.3 g.L^{-1} of yeast derivatives rich in reducing compounds and saturated with O_2; WYD_L-Ox: wine with added 0.3 g.L^{-1} of yeast derivatives rich in lipid compounds and saturated with O_2. Data are expressed as mean of 3 replicates (for each replicate of treatment) ± standard deviation. Different letters in a column indicate a significant difference ($p < 0.05$).

	Ethanol % (v/v)	pH	Lactic acid (g. L^{-1})	Volatile acidity (acetic acid g. L^{-1})	Total acidity (tartaric acid g. L^{-1})	Free (SO_2 mg. L^{-1})	Total SO_2 (mg. L^{-1})
W-noOx	12.70 ± 0.02 ^{b}	3.44 ± 0.004 ^{a}	4.10 ± 0.15 ^{a}	0.76 ± 0.01 ^{ab}	6.11 ± 0.11 ^{a}	1.00 ± 0.62 ^{b}	3.40 ± 0.55 ^{b}
W-Ox	12.92 ± 0.01 ^{a}	3.41 ± 0.002 ^{a}	4.20 ± 0.16 ^{a}	0.73 ± 0.02 ^{a}	6.10 ± 0.10 ^{a}	1.00 ± 0.55 ^{b}	3.50 ± 0.45 ^{b}
WSO_2-Ox	12.91 ± 0.01 ^{a}	3.41 ± 0.004 ^{a}	3.80 ± 0.15 ^{b}	0.76 ± 0.02 ^{ab}	6.12 ± 0.12 ^{a}	5.00 ± 0.68 ^{a}	34.20 ± 2.10 ^{a}
WYD_R-Ox	12.95 ± 0.02 ^{a}	3.42 ± 0.003 ^{a}	4.20 ± 0.16 ^{a}	0.78 ± 0.02 ^{b}	6.13 ± 0.10 ^{a}	1.50 ± 0.50 ^{b}	3.50 ± 0.65 ^{b}
WYD_L-Ox	12.90 ± 0.02 ^{a}	3.41 ± 0.004 ^{a}	4.10 ± 0.17 ^{a}	0.76 ± 0.01 ^{ab}	6.09 ± 0.12 ^{a}	1 .00 ± 0.65 ^{b}	3.50 ± 0.50 ^{b}

Table 2
Analysis of wine colour of experimental wines at the end of oxygen consumption. W-NoOx: control wine before oxygenation at saturation; W-Ox: control wine saturated with O_2; WSO_2-Ox: wine with added SO_2 (total SO_2: 35 ± 5 mg.L^{-1} and free SO_2: 15 ± 3 mg.L^{-1}) and saturated with O_2; WYD_R-Ox: wine with added 0.3 g.L^{-1} of yeast derivatives rich in reducing compounds and saturated with O_2; WYD_L-Ox: wine with added 0.3 g.L^{-1} of yeast derivatives rich in lipid compounds and saturated with O_2. Data are expressed as mean of 3 analytical replicates for each replicate of treatment ± standard deviation. Mean values followed by different letters on the column are significantly different ($p < 0.05$).

Treatement	Absorbance UV			CIELab		
	280 nm	320 nm	420 nm	L*	a*	b*
W-NoOx	3.5 ± 0.2^{a}	2.8 ± 0.3^{a}	0.17 ± 0.01^{ab}	64.1 ± 6.0^{c}	3.1 ± 0.1^{b}	10.4 ± 0.1^{c}
W-Ox	3.5 ± 0.1^{a}	2.8 ± 0.2^{a}	0.16 ± 0.02^{bc}	72.8 ± 0.6^{ab}	4.1 ± 0.1^{a}	14.3 ± 1.2^{a}
WSO_2-Ox	3.4 ± 0.2^{a}	2.3 ± 0.1^{b}	0.15 ± 0.02^{c}	75.2 ± 0.1^{a}	2.8 ± 0.2^{b}	10.3 ± 0.3^{c}
WYD_R-Ox	3.5 ± 0.1^{a}	2.8 ± 0.2^{a}	0.18 ± 0.01^{a}	74.4 ± 1.5^{a}	3.1 ± 0.2^{b}	11.5 ± 0.1^{b}
WYD_L-Ox	3.5 ± 0.2^{a}	2.9 ± 0.3^{a}	0.16 ± 0.02^{bc}	68.0 ± 1.4^{bc}	3.0 ± 0.2^{b}	11.5 ± 0.2^{b}

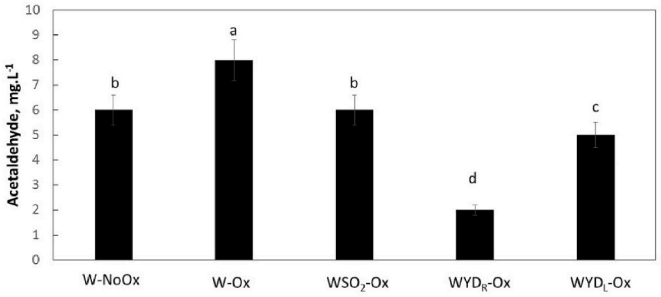

Fig. 4. Acetaldehyde concentration in the experimental wines at the end of oxygen consumption. W-NoOx: control wine before oxygenation at saturation; W-Ox: control wine saturated with O_2; WSO_2-Ox: wine with added SO_2 (total SO_2: 35 ± 5 mg.L^{-1} and free SO_2: 15 ± 3 mg.L^{-1}) and saturated with O_2; WYD_R-Ox: wine with added 0.3 g.L^{-1} of yeast derivatives rich in reducing compounds and saturated with O_2; WYD_L-Ox: wine with added 0.3 g.L^{-1} of yeast derivatives rich in lipid compounds and saturated with O_2. Data are expressed as mean of 6 values (2 analytical replicates for each replicate of treatment) ± standard deviation. Different letters indicate a significant difference ($p < 0.05$).

responsible for some beneficial reactions, mainly involving phenolics, which can improve wine colour stability and astringency over time (Drinkine, Lopes, Kennedy, Teissedre, & Saucier, 2007; Sheridan & Elias, 2015); meanwhile, in white wine the accumulation of acetaldehyde should always be viewed negatively due to its contribution to oxidative off-flavour and browning (Silva-Ferreira, Barbe, & Bertrand, 2002; Li, Guo, & Wang, 2008). Moreover, acetaldehyde rapidly reacts with SO_2, forming stable adducts and thus reducing its activity against microbial spoilage and chemical oxidation (Waterhouse, Sacks, & Jeffery, 2016). Fig. 4 shows the concentration of acetaldehyde determined in the control wines (W-NoOx and W-Ox), the sulfited wine (WSO_2-Ox) and the wines with added yeast derivatives (WYD_R-Ox and WYD_L-Ox). In the absence of oxygenation (W-NoOx, Fig. 4), the acetaldehyde content was around 6 mg.L^{-1}, whereas in W-Ox the acetaldehyde concentration increased to 8 mg.L^{-1}, indicating its formation after wine oxidation. The sulfited wine (WSO_2-Ox) contained the same amount of acetaldehyde present in the wine not exposed to oxygen (W-NoOx) (Fig. 4).

SO_2 is able to remove H_2O_2 by reducing it to water and hence blocking the Fenton reaction and, in turn, any acetaldehyde production (Danilewicz, 2003). The results obtained for YD wines were very interesting: WYD_R-Ox, followed by WYD_L-Ox, had the lowest acetaldehyde concentration, and performed better than WSO_2-Ox (Fig. 4).

These results indicate that YDs may contribute to effectively reducing acetaldehyde accumulation during white wine chemical oxidation, the YD rich in reducing compounds (YD_R) being the most effective. The presence of residual thiol nucleophile species (such as glutathione) in YDs (higher in YD_R than in YD_L) may explain this result. The addition of these thiol nucleophiles to acetaldehyde yields thiohemiacetals; this is a possible pathway that would explain the lower content of acetaldehyde in YD wines (Sonni et al., 2011). While supplementary experiments would be necessary to understand the link between the composition of YDs and their ability to reduce acetaldehyde accumulation in wine, these results promote the potential use of YDs as antioxidants to reduce the accumulation of acetaldehyde during winemaking carried out without or with low doses of SO_2.

3.6. Impact of treatments on amount of glutathione in wine

The effect of the addition of YDs on the glutathione (GSH) content of wine was also studied. GSH is naturally present in grapes, thus transferred to must and wine during winemaking (Dubourdieu & Lavigne, 2004). GSH can form colourless adducts with quinone, which delays the browning of wines and limits the oxidation of volatile esters, terpenes and thiols in wine (Dubourdieu & Lavigne, 2004). The initial amount of GSH in the wine (W-NoOx) was 2.5 mg.L^{-1}, and it decreased to 1.4 mg.L^{-1} (- 44%) in W-Ox, owing to the oxidation of GSH after O_2 consumption (Fig. 5). The wines treated with SO_2, YD_L and YD_R, after total consumption of oxygen showed different GSH concentrations: 1.7 mg.L^{-1}, 1.2 mg.L^{-1} and 2.5 mg.L^{-1} respectively (Fig. 5). In WSO_2-Ox, GSH

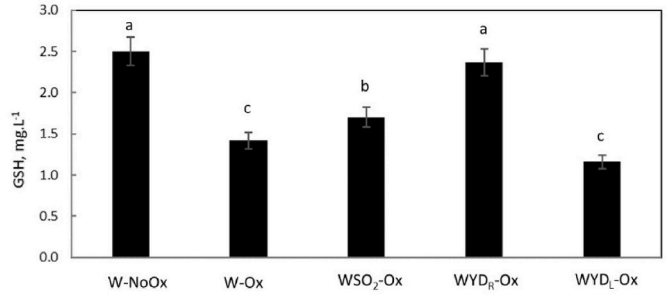

Fig. 5. Glutathione (GSH) concentration (in $mg.L^{-1}$) in the experimental wines at the end of oxygen consumption. W-NoOx: control wine before oxygenation at saturation; W-Ox: control wine saturated with O_2; WSO_2-Ox: wine with added SO_2 (total SO_2: 35 ± 5 $mg.L^{-1}$ and free SO_2: 15 ± 3 $mg.L^{-1}$) and saturated with O_2; WYD_R-Ox: wine with added 0.3 $g.L^{-1}$ of yeast derivatives rich in reducing compounds and saturated with O_2; WYD_L-Ox: wine with added 0.3 $g.L^{-1}$ of yeast derivatives rich in lipid compounds and saturated with O_2. Data are expressed as mean of 6 values (3 analytical replicates for each replicate of treatment) ± standard deviation. Different letters indicate a significant difference ($p < 0.05$).

was slightly higher than W-Ox (+12%) (Fig. 5) indicating that SO_2 helped to preserve GSH in oxidative conditions. WYD_L-Ox contained almost the same amount of GSH (no significant difference) as W-Ox (Fig. 5), thus, in these conditions, YD_L addition did not seem to preserve the GSH content of wine, despite the contribution of YD_L itself (containing 1.5 mg g^{-1} of total reducing compounds). In contrast, with the addition of YD_R, GSH was preserved in oxidative conditions; indeed, WYD_R-Ox had the same GSH concentration as W-NoOx (Fig. 5).

It can be assumed that (i) reducing compounds limited GSH oxidation, and (ii) the GSH released from YD_R into the wine compensated for the depleted GSH. The release of GSH into wine by some YDs has already been observed in other studies (Andujar-Ortiz et al., 2012; Gabrielli, Aleixandre-Tudo, Kilmartin, Sieczkowski, & Du Toit, 2017). In order to verify the latter hypothesis, GSH in model wine solution after oxygenation and in the presence of 0.3 $g.L^{-1}$ of YD_R was measured. GSH concentration was found to increase in the presence of YD_R, demonstrating its capacity to release glutathione into the medium (data not shown). Additional experiments could be implemented to better understand the link between the amount of GSH and other reducing compounds in YDs, as well as the capacity of YDs to act as a reservoir for GSH and thus to maintain GSH levels in wine.

3.7. Sensory analysis

In order to determine the ability of YDs to prevent the occurrence of oxidation off-odour following oxygen exposure, the experimental wines were submitted to sensory analysis (Fig. 6). The judges were asked to rate the perceived intensity of oxidation off-odour. In a preliminary knowledge-sharing session, all the expert judges smelt several white wines naturally oxidised to different degrees, rated the intensity of the oxidation off-odour and discussed the results.

From the results of the sensory analysis, as expected W-Ox wine was found to be the most oxidised from a sensory point of view (Fig. 6), with a mean intensity of oxidation off-odour corresponding to the mean point of the measure scale. The wines containing added antioxidants (SO_2 or YDs) obtained a lower score for oxidation off-odour intensity (Fig. 6). Interestingly, the intensity scores of the oxidation off-odours obtained for WYD_R-Ox did not differ from the score obtained for the wine treated with the conventional SO_2 (WSO_2-Ox) and the score of WYD_L-Ox was lower both than SO_2 treated wine and than W-NoOx wine. The results of the sensory analysis indicate that the yeast derivatives performed as well as SO_2, or even better, in preventing the occurrence of oxidation off-odours following the exposure of wine to oxygen, and the results are consistent with those obtained by chemical analysis (acetaldehyde concentration).

These results indicate that YD_R and YD_L may be able to preserve the organoleptic properties of wine not protected by conventional SO_2 after oxygen exposure.

4. Conclusions

This study shows for the first time that the addition of YDs to white wine vinified without SO_2 prevents it from browning and limits the accumulation of oxidation products like acetaldehyde. The two YDs differed in efficiency as they have different compositions, being either naturally rich in reducing compounds (YD_R) or lipids (YD_L). Compared to than YD_R, YD_L showed higher antiradical activity in the model solution and a higher capacity to consume oxygen in both the model solution and in the white wine. However, YD_R performed better in maintaining the GSH content of the wine as well as in reducing acetaldehyde accumulation. The sensory analysis of the wines confirmed that the levels of oxidation off-odours were lower in wines treated with YDs (in particular with YD_L) than in non-treated wine. YD_L and YD_R showed interesting antioxidant properties that could be exploited in low- or no-added sulphite winemaking. Further research is needed to investigate the mechanisms by which YDs consume oxygen and prevent acetaldehyde accumulation and glutathione loss, as well to test their activity in different wine types, and in combination with reduced doses of SO_2.

CRediT authorship contribution statement

Claudia Nioi: Conceptualization, Methodology, Validation, Formal

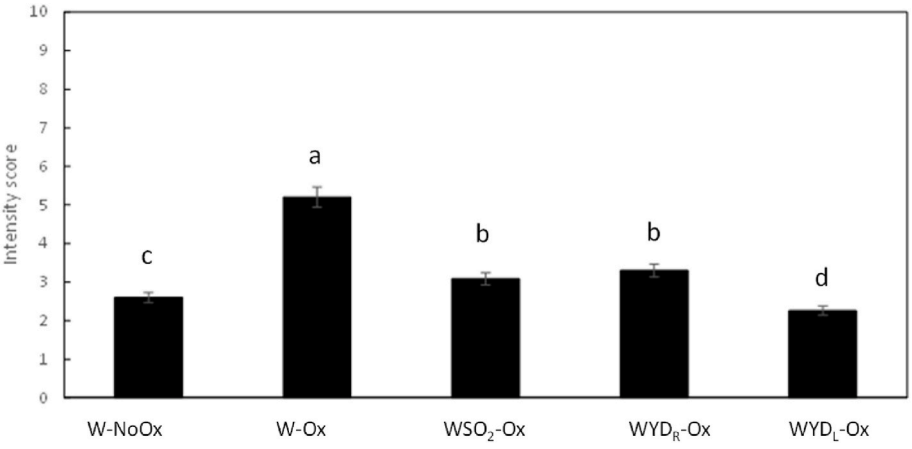

Fig. 6. Sensory analysis (intensity of oxidation off-odour) of the experimental wines analysed at the end of oxygen consumption. W-No-Ox: control wine before oxygenation at saturation; W-Ox: control wine saturated with O_2; WSO_2-Ox: wine added with SO_2 (total SO_2: 35 ± 5 $mg.L^{-1}$ and free SO_2: 15 ± 3 $mg.L^{-1}$) and saturated with O_2; WYD_R-Ox: wine added with 0.3 $g.L^{-1}$ of yeast derivatives rich in reducing compounds and saturated with O_2; WYD_L-Ox: wine added with 0.3 $g.L^{-1}$ of yeast derivatives rich in lipid compounds and saturated with O_2. Different letters indicate a significant difference ($p < 0.05$).

analysis, Investigation, Writing – original draft, Writing – review & editing, Visualization, Supervision, Project administration. **Maria Tiziana Lisanti:** Formal analysis, Writing – original draft, Writing – review & editing, Visualization. **Fabrice Meunier:** Investigation. **Pascaline Redon:** Investigation, Formal analysis. **Arnaud Massot:** Resources, Founding acquisition. **Virginie Moine:** Resources, Supervision, Founding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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