Measuring the oxygen consumption rate of some inactivated dry yeasts: comparison with other common wine antioxidants

Pere Pons-Mercadé[1], Sergi Anguela[1], Pol Giménez[1], José M. Heras[2], Nathalie Sieczkowski[2], Nicolas Rozès[1], Joan Miquel Canals[1] and Fernando Zamora[1*]

[1]Departament de Bioquímica i Biotecnologia, Facultat d'Enologia de Tarragona, Universitat Rovira i Virgili, C/ Marcel.li Domingo s/n, 43007 Tarragona, Spain.
[2] Lallemand Bio S.L. C/ Galileu 303. 1ª planta. 08028-Barcelona, Spain.

*corresponding author: fernando.zamora@urv.cat

Associate editor: Stamatina Kallithraka

ABSTRACT

The aim of the study was to evaluate the oxygen consumption kinetics of some inactivated dry yeasts in comparison with those of sulfur dioxide, ascorbic acid and glutathione.
The oxygen consumption rates of three inactivated dry yeasts, sulfur dioxide, ascorbic acid and glutathione at the usual doses in a model wine solution were determined by carrying out noninvasive fluorescence measurements.
The results indicate that two of the studied inactivated dry yeasts consume oxygen more effectively than sulfur dioxide.
These data suggest that some inactivated dry yeasts may be useful for protecting wine against oxidation.
This study shows for the first time that inactivated dry yeasts can actually consume oxygen, therefore opening up an interesting area for future research.

KEYWORDS

oxygen consumption rate, inactivated dry yeasts, sulfur dioxide, ascorbic acid, glutathione

INTRODUCTION

Wine aging on lees is currently a widespread practice in oenology, especially for white wines (Dubourdieu, 1992; Feuillat, 1994). Due to the phenomenon of autolysis, the presence of lees enriches the wine with many compounds that improve its quality (Fornairon-Bonnefond *et al.*, 2002; Alexandre and Guilloux-Benatier, 2006). It has been reported that lees release many components of yeast membranes and cytoplasm into wine, such as polysaccharides (Martínez-Lapuente *et al.*, 2013), mannoproteins (Martínez-Lapuente *et al.*, 2015) peptides and proteins (Moreno-Arribas *et al.*, 1996; Luguera *et al.*, 1998), amino acids (Martinez-Rodriguez *et al.*, 2002), lipids (Pueyo *et al.*, 2000) and nucleotides (Charpentier *et al.*, 2005). It therefore seems clear that the process of yeast autolysis completely transforms the composition of wine, improving some of its sensory attributes (Kemp *et al.*, 2015).

Yeast autolysis has been reported to have different sensory effects on wine. Polysaccharides and mannoproteins have been found to improve wine mouthfeel (Gawel *et al.*, 2018) and, in combination with some peptides and proteins, to contribute to wine sweetness, smoothing the perception of acidity and astringency (Marchal *et al.*, 2011). Furthermore, some amino acids, peptides and nucleotides can contribute to creating an umami taste (Vilela *et al.*, 2016) and have been described as flavour enhancers, while lipids and amino acids have been described as aroma precursors (Styger *et al.*, 2011). Additionally, mannoproteins seem to exert a protective colloid effect, increasing the stability of proteins (Waters *et al.*, 1994) and tartaric acid salts (Gerbaud *et al.*, 1997).

Lees has also been reported to consume oxygen, slowing down the oxidation of the wine (Salmon *et al.*, 2000; Pons-Mercadé *et al.*, 2020). The mechanism by which this occurs is not known. Some authors associate it with the oxidation of membrane lipids (Fornairon-Bonnefond and Salmon, 2003), but it may also be related to the release of glutathione by yeast (Kritzinger *et al.*, 2013). Whatever the mechanism, oxygen consumption by lees appears to be the main factor involved in white wines aged on lees being able to age for longer than other white wines.

In short, wines aged on lees are generally richer in mouthfeel and have greater depth and flavour complexity, and white wines on lees tend to age better. The practice, however, is laborious and entails certain risks, such as the appearance of

reductive characters (Dubourdieu, 1995) and *Brettanomyces* taints (Renouf *et al.*, 2007). For this reason, inactivated dry yeasts (IDY) are now used, because in theory they have the same effect as lees, but without any of the drawbacks (Pozo-Bayón *et al.*, 2009a; Del Barrio-Galán *et al.*, 2011).

Several studies have examined how IDYs can contribute to wine composition and quality. Many positive effects have been described, such as improving mouthfeel and reducing the perception of acidity (Charpentier, 2010; Del Barrio-Galán *et al.*, 2012), smoothing of astringency (Mekoue-Nguela *et al.*, 2015; González-Royo *et al.*, 2016; Del Barrio-Galán *et al.*, 2019) and bitterness (Pozo-Bayón *et al.*, 2009b; González-Royo *et al.*, 2013; Del Barrio-Galán *et al.*, 2019), improving the foaming properties of sparkling wines (Vanrell *et al.*, 2005; Marti-Raga *et al.*, 2016; Medina-Trujillo *et al.*, 2017), eliminating the presence of ochratoxin (Piotrowska *et al.*, 2013; Petruzzi *et al.*, 2015), preventing browning (Comuzzo and Zironi, 2013; Comuzzo *et al.*, 2015) and ensuring oxidation does not affect aroma (Pozo-Bayón *et al.*, 2009c; Rodríguez-Bencomo *et al.*, 2014) due to the ability of IDYs to release glutathione and other antioxidant compounds (Gabrielli *et al.*, 2017; Bahut *et al.*, 2020).

It has also been recently reported that some IDYs consume oxygen in a similar way to other common antioxidants used in wine, such as sulfur dioxide (Sieczkowski *et al.*, 2016a, Sieczkowski *et al.*, 2016b; Pons *et al.*, 2019). However, little is known about using IDY to protect wine against oxidation. Therefore, the aim of this study was to measure the kinetics of oxygen consumption using different IDYs and to compare them with those of other common wine antioxidants (sulfur dioxide, ascorbic acid and glutathione) in order to determine their real antioxidant capacity for protecting wine from the detrimental effects of oxygen.

MATERIALS AND METHODS

1. Chemicals

Absolute ethanol, L-(+)-tartaric acid, sodium hydroxide, formic acid, Hydrogen chloride and potassium metabisulfite were purchased from Panreac (Barcelona, Spain). Copper (II) sulfate pentahydrate, iron (III) chloride hexahydrate, ascorbic acid, disodium ethylenediaminetetraac-etate dihydrate (EDTA), 5,5′-dithiobis-2-nitro-benzoicacid (DTNB), tris(hydroxymethyl)amino-

methane (TE8), cysteine, γ-L-glutamyl-L-cysteine and glutathione were purchased from Sigma Aldrich (St Louis, USA). Two different commercial inactivated yeasts (IDY-1: Pure-Lees™ Longevity and IDY-2: Noblesse®) and an experimental (IDY-3: Antiox-1), all provided by Lallemand Inc (Montreal, Canada), were used. IDY-1 and IDY-3 were specifically selected for their ability to consume oxygen, whereas IDY-2 was developed as a substitute for lees for its capacity to release polysaccharides and mannoproteins. Pure water was obtained from a Milli-Q purification system (Millipore, USA).

2. Experimental design

The experimental design for determining the oxygen consumption rate of IDYs and other antioxidants was an adaptation of those previously described by Navarro *et al*. (2016) and Pascual *et al*. (2017) for the oxygen consumption rate of oak chips and oenological tannins respectively. A model wine solution composed of ethanol (12 % v/v) and L-(+)-tartaric acid (4 g/L) adjusted at pH = 3.5 with sodium hydroxide was used. This solution was enriched with 3 mg of iron/L, in the form of iron (III) chloride hexahydrate, and 0.3 mg of copper/L in the form of copper (II) sulfate pentahydrate. We worked with this model wine solution rather than with real wine, because the naturally occurring phenolic compounds would have competed with the IDY or other antioxidants, making it impossible to determine their oxygen consumption kinetics. This model wine solution was saturated in oxygen (around 8.0 mg/L) by bubbling with air for 10 min just before beginning the experiment.

The usual doses of IDY (400 mg/L), potassium metabisulfite (30 mg/L), ascorbic acid (100 mg/L) or glutathione (20 mg/L) were 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 completely filled with the model wine solution and then immediately closed with a crown cap and bedule so as to minimize the headspace volume. The bottles were then gently shaken to resuspend the IDYs or to dissolve the different antioxidants: sulfur dioxide (added as potassium metabisulfite), ascorbic acid or glutathione. The bottles were maintained at 20 ± 2 °C during all this time. Oxygen was measured (Diéval *et al*., 2011) periodically to determine the oxygen consumption rate: oxygen measurements were made every day for the assays containing IDYs, sulfur dioxide and glutathione over two weeks, and every hour for the assay containing ascorbic acid over one day. Figure 1 is a schematic representation of the experimental design.

FIGURE 1. Experimental design for measuring oxygen consumption.

Two types of control were prepared. Control-A bottles were filled with the oxygen-saturated model wine solution without adding any antioxidant, and Control-B bottles were filled with the same model wine solution, but after bubbling for 10 min with nitrogen to eliminate the oxygen. Control A was used to verify that the model wine solution did not consume oxygen, and Control B was used to verify the tightness of the closure system. All assays were performed in triplicate.

3. Modelisation of the oxygen consumption kinetics

In order to quantify the real oxygen consumption ability of the IDY in comparison with that of the other common antioxidants, we applied the kinetic model proposed by Pascual *et al*. (2017) for estimating the oxygen consumption kinetics of oenological tannins. This model consists in displaying the inverse of consumed oxygen versus the inverse of time. According to this mathematical model, the following equation can be established: $1/[O2] = A/t + B$. This equation, which describes the relationship between oxygen consumed and time, can be used to determine the oxygen consumption rate at time zero (OCRto) and also the total oxygen consumption capacity (TOCC). Figure 2A shows how the consumed oxygen can be cleared up. Figure 2B shows how the OCRto can be determined after calculating the first derivative and considering time equal to zero. Finally, Figure 2C shows how the TOCC is obtained by calculating the limit when time tends towards infinity.

According to this model, the $OCRt_o$ corresponds to the inverse of the slope of the initial equation and the TOCC to the inverse of the y-intercept of the initial equation.

4. Determination of the glutathione content of inactivated dry yeasts

Glutathione extraction and analysis were performed according to an adaptation of the method reported by Nisamedtinov *et al*. (2010).

4.1. Reagents

TE8 solution: 6.05 g/L of tris(hydroxymethyl) aminomethane and 1.12 g/L of EDTA disodium salt dehydrate adjusted at pH = 8.00 with HCl 1N. This solution was stored at 4 °C. DTNB stock-solution: 39.6 mg of DTNB + 8 ml of TE8 solution + 2 ml of NaOH 0.1 N. This solution is stable for at least one month when protected from light. DTNB working-solution: 8 ml of DTNB stock-solution + 92 ml of TE8 solution; fresh solution was prepared and used daily.

4.2. Glutathione extraction

Two grams (dry weight) of each inactivated dry yeast was placed into 15 ml centrifuge tubes to

A. Clear up to obtain the equation of $[O_2]$ versus time

Clear up $[O_2]$

$$\frac{1}{[O_2]} = \frac{A}{t} + B \quad \Longrightarrow \quad [O_2] = \frac{t}{A + Bt}$$

B. Calculation of the Oxygen Consumption Rate at time zero ($OCRt_0$)

$$[O_2] = \frac{t}{A + Bt} \;\xrightarrow{\text{First derivative}}\; \frac{d[O_2]}{dt} = \frac{A}{(A + Bt)^2} \;\Longrightarrow\; \frac{d[O_2]}{dt} = \frac{A}{A^2 + 2ABt + B^2t^2} \;\xrightarrow{\text{For } t = 0}\; \frac{d[O_2]}{dt} = \frac{1}{A} \;\xrightarrow{\text{Ergo}}\; OCRt_0 = \frac{1}{A}$$

C. Calculation of the Total Oxygen Consumption Capacity (TOCC)

$$[O_2] = \frac{t}{A + Bt} \;\xrightarrow{\substack{\text{Limit of the function} \\ \text{as x approaches infinity}}}\; TOCC = \lim_{t \to \infty} \frac{t}{A + Bt} \;\Longrightarrow\; TOCC = \frac{\infty}{\infty} \quad \text{INDETERMINATE FORM}$$

To solve this indeterminate form numerator and denominator are divided by t

$$TOCC = \lim_{t \to \infty} \frac{t/t}{(A + Bt)/t} \;\Longrightarrow\; TOCC = \lim_{t \to \infty} \frac{1}{A/t + B} \;\Longrightarrow\; TOCC = \lim_{t \to \infty} \frac{1}{A/\infty + B} \;\Longrightarrow\; TOCC = \frac{1}{0 + B} \;\xrightarrow{\text{Ergo}}\; TOCC = \frac{1}{B}$$

FIGURE 2. Modeling the Oxygen consumption by the different antioxidant products.

which 5 ml of 0.1 N formic acid solution was immediately added. The samples were maintained at room temperature for one hour with frequent vortex shaking. After one hour had elapsed, the samples were centrifuged (Sorval RC5C) at 11.500 rpm for 10 min at 10 °C. One hundred mL of the supernatant were added to 4.9 mL of DNTB working solution. The sample was immediately vortexed and kept for 10 min at room temperature. The samples were used immediately for UPLC analysis.

4.3. Glutathione UPLC Analysis

An ACQUITY UPLC system (Waters, USA), equipped with a C18 column (AccQ Tag Ultra Column, 100 × 2.1 mm, 1.7 mm, Waters, USA) and a photo diode array (PDA) detector ACQUITY PDA 2996 was used for the chromatographic determination of reduced glutathione. A two-gradient mobile phase (A: water + 0.1 % formic acid and B: acetonitrile + 0.1 % formic acid) and a flow rate of 0.3 mL min-1 was used. The standards (cysteine, ³-L-glutamyl-L-cysteine and reduced glutathione) were used for external calibration after derivatisation with DTNB. Results are expressed as mg of reduced glutathione by gram of dry weight.

5. 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).

RESULTS AND DISCUSSION

1. Evolution of oxygen concentration over time

Figure 3 shows the oxygen concentration evolution over time of the initially oxygen-saturated model wine solutions with supplements (the different IDYs and the other common antioxidants) or without supplements.

The oxygen concentration of Control-A (no antioxidant added) remained stable with only slight, erratic variations that are probably linked to the inherent variability of the analytical procedure. The oxygen intake in Control-B (no oxygen) was very low and can be considered negligible (data not shown). By contrast, the oxygen concentration of the model wine solutions supplemented with the different antioxidants decreased over time, with the exception of IDY-2, where the concentration remained stable.

As expected, the model wine solution containing ascorbic acid consumed all the oxygen in a few hours, confirming that it is more effective than the other antioxidants (Barril *et al.*, 2012).

The graph clearly shows that, with the sole exception of IDY-2, all the antioxidants consumed oxygen: the most effective was IDY-3, followed in decreasing order by IDY-1, sulfur dioxide and glutathione.
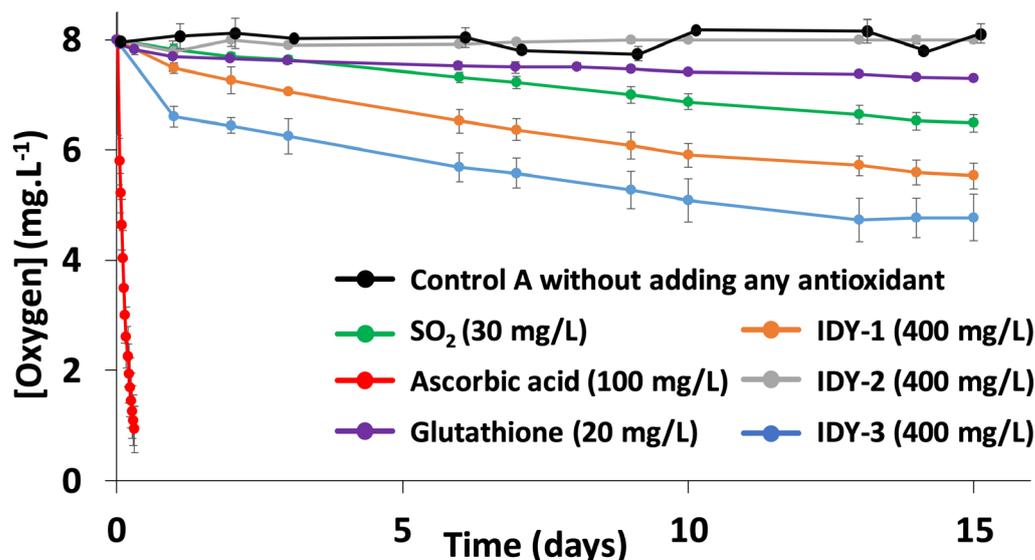
FIGURE 3. Oxygen concentration evolution in the model wine solution supplemented with the different antioxidant products.
All data are expressed as the average of 3 replicates ± standard deviation. IDY = Inactivated Dry Yeasts.

2. Modeling oxygen consumption kinetics

Since Figure 3 does not provide a clear quantitative comparison of the oxygen consumption kinetics of the IDYs and the other antioxidants, we applied the kinetic model proposed by Pascual *et al.* (2017) to better estimate the real capacity of both IDYs to consume oxygen in comparison to that of the other common antioxidants. This model consists in displaying the inverse of consumed oxygen versus the inverse of time, thus obtaining the following equation: $1/[O2] = A/t + B$. Figure 4 shows the results for ascorbic acid, sulfur dioxide, glutathione and both IDYs. In all cases, satisfactory linear regression coefficients were obtained (between 0.9752 and 0.9921), confirming that this mathematical model works reasonably well.

According to the model, the OCRto corresponds to the inverse of the slope and the TOCC corresponds to the inverse of the y-intercept of the above equation (See Figure 2).

After clearing up the oxygen, the equation $[O2] = t/(A + Bt)$ was obtained, which can be used to compare the experimental data (Figure 5A) with the theoretical data (Figure 5B) obtained after applying this model.

As the figures show, the mathematical model reproduces the experimental kinetics of oxygen consumption by all the antioxidants quite well. Once again, these results demonstrate the validity of the model.

3. Oxygen consumption rate at time zero and total oxygen consumption capacity.

As explained above, the kinetic model of Pascual *et al.* (2017) can be used to determine two parameters that define the kinetics of oxygen consumption: OCRto and TOCC. Table 1 shows the resulting values for both IDYs and the other tested antioxidants. It also shows the oxygen consumption rate at time zero (% ROCRto) and the total oxygen consumption capacity (% TOCC) of the different antioxidants compared to those of sulfur dioxide at the usual doses.

Sulfur dioxide, which is by far the most widely used antioxidant in wine production, showed the lowest OCRto of all the studied antioxidants, indicating that its direct reaction with oxygen is slow. However, sulfur dioxide is mainly used as an antioxidant because of its ability to react with hydrogen peroxide formed by oxidation of polyphenols (Danilewicz, 2015), its effectiveness to inhibit polyphenol oxidases (Vignault *et al.*, 2020), and its capacity to react with ethanal and
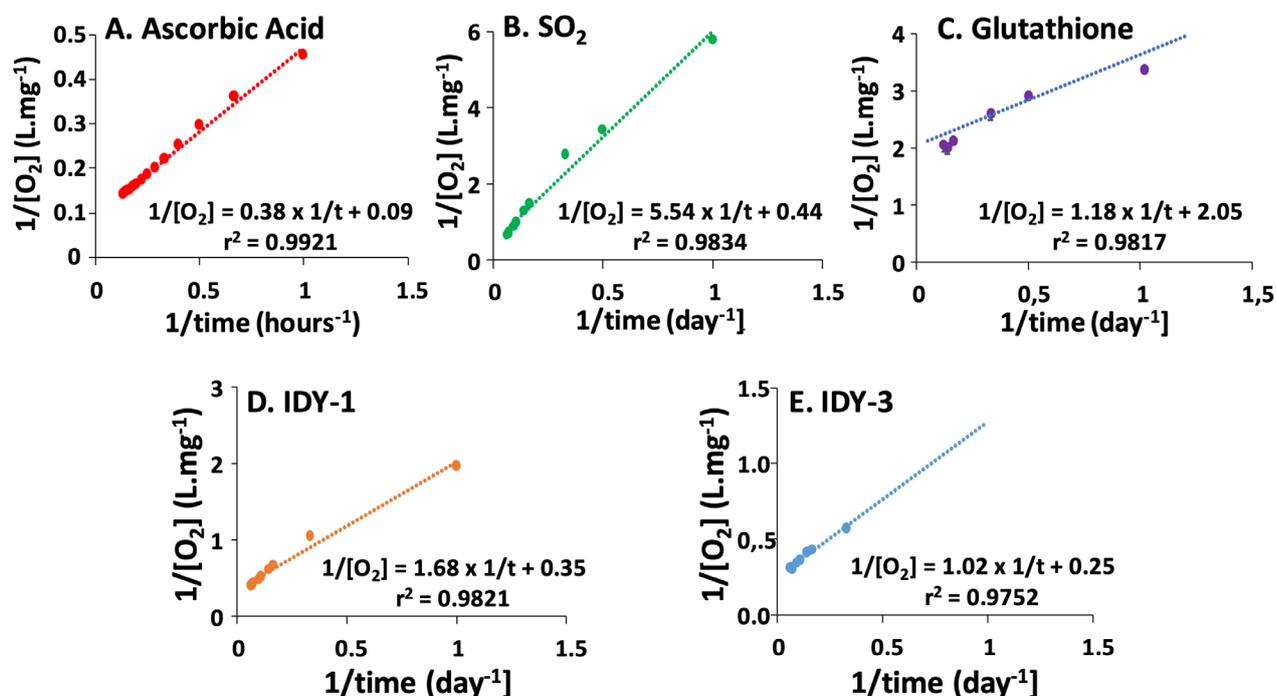
FIGURE 4. Application of the oxygen consumption model to the different antioxidant products.
All data are expressed as the average of 3 replicates ± standard deviation. IDY: Inactivated Dry Yeasts.
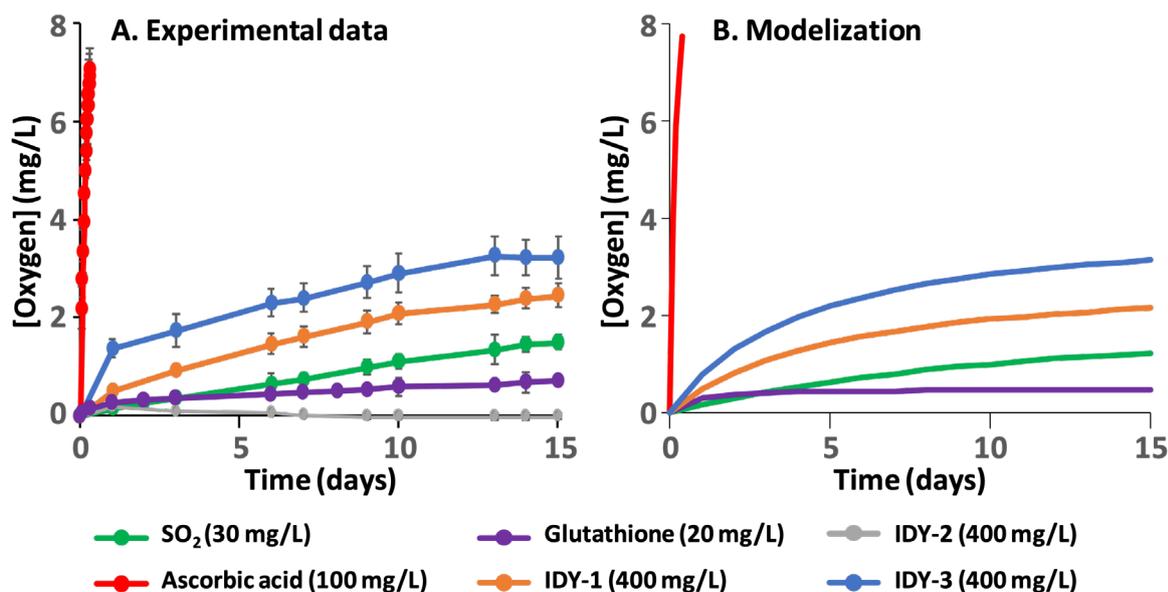
FIGURE 5. Oxygen consumption of the different antioxidant products; Comparison between experimental and model data.
All data are expressed as the average of 3 replicates ± standard deviation. IDY = Inactivated dry yeasts.

TABLE 1. Oxygen consumption rate at time 0 and total oxygen consumption capacity.

Product	Usual dose	OCRto mg/L.day				%ROCRto	TOCC mg/L				%RTOCC
SO₂	30 mg/L	0.18	±	0.06	A	100	2.27	±	0.76	A	100
Ascorbic Acid	100 mg/L	63.16	±	2.34	D	35089	9.52	±	0.41	D	419
Glutathione	20 mg/L	0.85	±	0.09	C	472	0.49	±	0.05	C	22
IDY-1	400 mg/L	0.60	±	0.01	B	333	2.86	±	0.05	B	126
IDY-3	400 mg/L	0.98	±	0.17	C	544	4.02	±	0.40	C	177

All data are expressed as the average of 3 replicates ± standard deviation. IDY: Inactivated Dry Yeasts; OCRto: Oxygen consumption rate at time 0; % ROCRto: Relative OCRto referred to sulfur dioxide; TOCC: Total oxygen consumption capacity; RTOCC: Relative TOCC referred to sulfur dioxide.

therefore eliminate its unpleasant smell (Sheridan and Elias, 2016). The TOCC obtained for sulfur dioxide using this model was lower than the corresponding stoichiometric value of 30 mg of SO₂/L (2.27 mg/L versus 7.50 mg/L respectively). This difference can probably be attributed to the fact that the reaction is very slow, and that after 15 days not all of the sulfur dioxide had been consumed.

The % ROCRto results indicate that ascorbic acid initially consumes oxygen around 350 times faster than sulfur dioxide, and the % TOCC indicates

that it can consume around 4.2 times more oxygen than sulfur dioxide at the usual doses. These data confirm the high effectiveness of ascorbic acid reported in the literature (Barril *et al.*, 2012). However, the fact that ascorbic acid generates hydrogen peroxide after consuming oxygen must be taken into account, as its use in wine may cause subsequent oxidations (Gibson, 2006; Oliveira *et al.*, 2011) which can affect the sensory quality of the wine. For this reason, ascorbic acid must be always used in the presence of sulfur dioxide to prevent wine oxidation (Barril *et al.*, 2016). The TOCC obtained using this model was very

close to the corresponding stoichiometric value of 100 mg of ascorbic acid/L (9.52 mg/L versus 9.08 mg/L respectively).

The % ROCRto of glutathione is around 4.7 times greater than that of sulfur dioxide but the % TOCC is around 5 times lower. These data indicate that glutathione reacts faster with oxygen than sulfur dioxide; however, the maximal dose of this antioxidant (20 mg/L) authorised by the International Organization of Vine and Wine (OIV, 2015) can only directly consume small amounts of oxygen. To our knowledge, there are no data on the kinetics of oxygen consumption by glutathione in a medium similar to wine. There is extensive literature, however, on its protective effect against browning (El Hosry *et al.*, 2009; Kritzinger *et al.*, 2013) and the loss of certain aromas (Roussis *et al.*, 2007; Rodríguez-Bencomo *et al.*, 2014), especially volatile thiols (Ugliano *et al.*, 2011; Nikolantonaki *et al.*, 2018). The mechanism by which glutathione manifests these protective effects seems to be more closely related to its ability to block the orthoquinones that form grape reaction product (GRP) than to its direct reactivity to oxygen (Nikolantonaki *et al.*, 2014; Webber *et al.*, 2017). The orthoquinones formed by the reaction of oxygen with orthophenols can subsequently react with volatile thiols to form adducts that are no longer volatile, or with other phenols to form melanins that cause browning. Consequently, its blockage by glutathione prevents the loss of volatile thiols and browning. The TOCC obtained using this model was also very close to the corresponding stoichiometric value of 20 mg of ascorbic acid/L (0.49 mg/L versus 0.52 mg/L).

Table 1 also shows the oxygen consumption kinetic parameters of IDY-1 and IDY-3. No data are shown for IDY-2, because this inactivated dry yeast did not show any effects (see Figure 3). The % OCRto indicates that oxygen is consumed 3.3 times faster by IDY-1 and 5.4 times faster by IDY-3 than sulfur dioxide. Furthermore, the TOCC values for both IDYs were also higher than those of sulfur dioxide: comparison of the % TOCC values shows that IDY-1 and IDY-3 can consume around 1.2 and 1.8 more oxygen respectively. These TOCC values were similar to those reported by Sieczkowski *et al.* (2016a, 2016b) for an equivalent dose of IDY.

4. Glutathione content of the inactivated dry yeasts

Table 2 shows the glutathione content of the different IDY. The highest glutathione concentration was found in IDY-3 followed in decreasing order by IDY-1 and IDY-2. Table 2 also shows the corresponding glutathione concentration corresponding to the total glutathione released to the model wine solution by the different IDYs and the corresponding stoichiometric oxygen consumption capacity (SOCC).

Two clear conclusions can be drawn from these results: first, the glutathione concentration released by all the IDYs is much lower than the maximal authorised dose of pure glutathione; second, the IDYs better able to consume oxygen were precisely those that released more glutathione (IDY-1 and especially IDY-3). However, the levels of glutathione released by the different IDY does not justify all the oxygen consumption. Figure 6 displays the total oxygen consumption capacity (TOCC) of pure glutathione, and the different IDYs versus their corresponding values of the

TABLE 2. Glutathione content and oxygen consumption.

Inactivated dry yeast	Glutathione (mg/g dry weight)			Glutathione (mg/L)			Stoichiometric oxygen consumption capacity (mg/L)			TOCC (mg/L)		
IDY-1	8.87	±	0.95 B	3.55	±	0.37 B	0.09	±	0.01 B	2.86	±	0.25 B
IDY-2	3.95	±	0.80 A	1.59	±	0.32 A	0.04	±	0.01 A		n.d	
IDY-3	13.90	±	1.41 C	5.56	±	0.56 C	0.14	±	0.01 C	4.02	±	0.40 C
Glutathione		-			20.00			0.52		0.49	±	0.22 C

All data are expressed as the average of 3 replicates ± standard deviation. IDY: Inactivated Dry Yeasts.
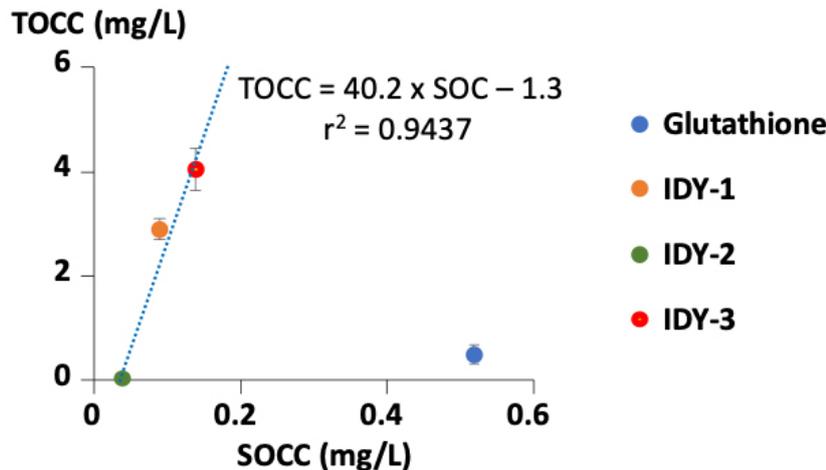
FIGURE 6. Total oxygen consumption capacity versus stoichiometric oxygen consumption capacity of glutathione and inactivated dry yeasts**.**
All data are expressed as the average of 3 replicates ± standard deviation. IDY: Inactivated Dry Yeasts; TOCC: Total oxygen consumption capacity; SOCC: Stoichiometric oxygen consumption capacity.

stoichiometric oxygen consumption capacity (SOCC).

This figure indicates a reasonably good correlation between TOCC and SOCC for the three studied IDYs. It also shows that IDY-2 does not consume oxygen and that IDY-1 and IDY-3 consume much more than the corresponding stoichiometric oxygen consumption capacity of the glutathione they are able to release. Therefore, these data indicate that the oxygen consumption by IDYs must be related to mechanisms other than glutathione release.

CONCLUSION

This work showed that nearly all the studied antioxidants directly consume oxygen in a model wine solution. Ascorbic acid consumed oxygen much faster than the other antioxidants, followed in decreasing order by IDY-3, glutathione, IDY-1 and sulfur dioxide at the usual doses. Ascorbic acid also showed the highest total oxygen consumption capacity, followed in decreasing order by IDY-3, IDY-1, sulfur dioxide and glutathione.

Furthermore, these results confirm that some inactivated dry yeasts are more effective than sulfur dioxide at directly consuming oxygen; they could therefore be a very useful tool for protecting wine against oxidation. However, the direct oxygen consumption by IDYs cannot be justified by their content in glutathione since the corresponding stoichiometric consumption is much lower than their real oxygen consumption capacity. Further research is needed to investigate the mechanisms by which some inactivated dry yeasts consume oxygen, and to determine whether they can be

used to reduce the dose of sulfur dioxide while still preventing spoilage due to oxidation.

REFERENCES

Alexandre, H., & Guilloux-Benatier, M. (2006). Yeast autolysis in sparkling wine: a review. *Australian Journal of Grape and Wine Research*, 12, 119–127. https://doi.org/10.1111/j.1755-0238.2006.tb00051.x

Bahut, F., Romanet, R., Sieczkowski, N., Schmitt-Kopplin, P., Nikolantonaki, M., & Gougeon, R.D. (2020). Antioxidant activity from inactivated yeast: Expanding knowledge beyond the glutathione-related oxidative stability of wine. *Food Chemistry*, 325, 126941. https://doi.org/10.1016/j.foodchem.2020.126941

Barril, C., Clark, A.C., & Scollary, G.R. (2012). Chemistry of ascorbic acid and sulfur dioxide as an antioxidant system relevant to white wine. *Analytica Chimica Acta*, 732, 186–193. https://doi.org/10.1016/j.aca.2011.11.011

Barril, C., Rutledge, D.N., Scollary, G.R., & Clark, A.C. (2016). Ascorbic acid and white wine production: a review of beneficial versus detrimental impacts. *Australian Journal of Grape and Wine Research*, 22, 169–181. https://doi.org/10.1111/ajgw.12207

Charpentier, C. (2010). Ageing on lees (sur lies) and the use of speciality inactive yeasts during wine fermentation. In: Reynolds AG (ed.) Managing wine quality. Oenology and wine quality, vol. 2, pp 165–

187. Canada: Woodhead Publishing Series in Food Science, Technology and Nutrition No. 192. https://doi.org/10.1016/B978-1-84569-798-3.50006-1

Charpentier, C., Aussenac, J., Charpentier, M., Prome, J. C., Duteurtre, B., & Feuillat M. (2005). Release of nucleotides and nucleosides during yeast autolysis: kinetics and potential impact on flavor. *Journal of Agricultural and Food Chemistry*, 53, 3000−3007. https://doi.org/10.1021/jf040334y

Comuzzo, P.G., Battistutta, F., Vendrame, M., Páez, M.S., Luisi, G., & Zironi R. (2015). Antioxidant properties of different products and additives in white wine. Food Chemistry, 168, 107–114. https://doi.org/10.1016/j.foodchem.2014.07.028

Comuzzo, P.G., & Zironi, R. (2013). Biotechnological Strategies for Controlling Wine Oxidation. Food Engineering Reviews, 5, 217–229. https://doi.org/10.1128/AEM.02547-10

Danilewicz, J.C. (2015). Reaction of oxygen and sulfite in wine. *American Journal of Enology and Viticulture*, 67, 13–17. https://doi.org/10.5344/ajev.2015.15069

Del Barrio-Galán, R., Pérez-Magariño, S., Ortega-Heras, M., Williams, P., & Doco T. (2011). Effect of aging on lees and of three different dry yeast derivative products on verdejo white wine composition and sensorial characteristics. *Journal of Agricultural and Food Chemistry*, 59, 12433–12442. https://doi.org/10.1021/jf204055u

Del Barrio-Galán, R., Pérez-Magariño, S., Ortega-Heras, M., Guadalupe, Z., & Ayestarán, B. (2012). Polysaccharide characterization of comercial dry yeast preparations and their effect on white and red wine composition, *LWT Food Science and Technology*, 48, 215–223. https://doi.org/10.1016/j.lwt.2012.03.016

Del Barrio-Galán, R., Úbeda, C., Gil, M., Medel-Maraboll, M., Sieczkowski, N., & Peña-Neira A. (2019). Evaluation of Yeast Derivative Products Developed as an Alternative to Lees: The Effect on the Polysaccharide, Phenolic and Volatile Content, and Colour and Astringency of Red Wines. *Molecules*, 24(13), 2385. https://doi.org/10.3390/molecules24081478

Diéval, J.B., Vidal, S., & Aagaard, O. (2011). Measurement of the oxygen transmission rate of co-extruded wine bottle closures using a luminescence-based technique. *Packaging Technology and Science*, 24, 375–385. https://doi.org/10.1002/pts.945

Dubourdieu, D. (1992). Vinification des vins blancs secs en barriques. In "Le bois et la qualité des vins et des eaux-de-vie". *Journal International des Sciences de la Vigne et du Vin*, (numéro hors série), 137–145.

Dubourdieu, D. (1995). Intérêts œnologiques et risques associés à l'élevage des vins blancs sur lies en barriques. *Revue Française d'Oenologie*, 155, 30–35.

El Hosry, L., Auezova, L., Sakr, A., & Hajj-Moussa, E. (2009). Browning susceptibility of white wine and antioxidant effect of glutathione. *International Journal of Food Science and Biotechnology*, 44, 2459–2463. https://doi.org/10.1111/j.1365-2621.2009.02036.x

Feuillat, M. (1994). Fermentation dans le bois et élevage sur lies des vins blanc; Influence des macromolécules de levures sur les arômes. *Revue des Œnologues*, 71, 19–21.

Fornairon-Bonnefond, C., Camarasa, C., Moutounet, M., & Salmón, J.M. (2002). New trends on yeast autolysis and wine ageing on lees: A bibliographical review. *Journal International des Sciences de la Vigne et du Vin,* 36, 49–69. https://doi.org/10.20870/oeno-one.2001.35.2.990

Fornairon-Bonnefond, C. & Salmon, J.M. (2003). Impact of oxygen consumption by yeast lees on the autolysis phenomenon during simulation of wine aging on lees. *Journal of Agricultural and Food Chemistry*, 51, 2584−2590. https://doi.org/10.1021/jf0259819

Gabrielli, M., Aleixandre-Tudo, J.L., Kilmartin, P.A., Sieczkowski, N., & du Toit W.J. (2017). Additions of Glutathione or Specific Glutathione-rich Dry Inactivated Yeast Preparation (DYP) to Sauvignon blanc Must: effect on wine chemical and sensory composition. *South African Journal of Enology and Viticulture*, 38, 18–28. https://doi.org/10.21548/38-1-794

Gawel, R., Smith, P.A., Cicerale, S., & Keast, R. (2018). The mouthfeel of white wine. *Critical Reviews in Food Science and Nutrition*, 58, 2939–2956. https://doi.org/10.1080/10408398.2017.1346584

Gerbaud, V., Gabas, N., Blounin, J., Pellerin, P., & Moutounet, M. (1997). Influence of wine polysaccharides and polyphenol on the crystallisation of potassium hydrogen tartrate. *Journal International des Sciences de la Vigne et du Vin,* 31, 65–83. https://doi.org/10.20870/oeno-one.1997.31.2.1087

Gibson, R. (2006). Ascorbic acid − Friend or foe? In 35th annual new york wine industry workshop (pp. 60). Australia: Scorpex Services.

González-Royo, E., Esteruelas, M., Kontoudakis, N., Fort, F., Canals, J.M., & Zamora, F. (2016). The effect of supplementation with three commercial inactive dry yeasts on the color, phenolic compounds, polysaccharides and astringency of a model wine solution and red wine. *Journal of the Science of Food and Agriculture*, 97, 172–181. https://doi.org/10.1002/jsfa.7706

González-Royo, E., Urtasun, A., Gil, M., Kontoudakis, N., Esteruelas, M., Fort, F., Canals, J.M., & Zamora, F. (2013). Effect of yeast strain and supplementation with inactive yeast during alcoholic fermentation on wine polysaccharides. *American Journal of Enology and Viticulture*, 64, 268–273. https://doi.org/10.5344/ajev.2012.12071

Kemp, B., Alexandre, H., Robillard, B., & Marchal, R. (2015). Effect of production phase on bottle-fermented sparkling wine quality. *Journal of Agricultural and*

Food Chemistry, 63, 19–38. https://doi.org/10.1021/jf504268u

Kritzinger, E.C., Bauer, F.F., & du Toit, W.J. (2013). Role of glutathione in winemaking: a review. *Journal of Agricultural and Food Chemistry*, 61, 269–277. https://doi.org/10.1021/jf303665z

Luguera, C., Moreno-Arribas, M.V., Puey, E., Bartolome, B., & Polo, M.C. (1998). Fractionation and partial characterisation of protein fractions present at different stages of the production of sparkling wines. *Food Chemistry*, 63, 465–471. https://doi.org/10.1016/S0308-8146(98)00063-6

Marchal, A., Marullo, P., Moine, V., & Dubourdieu, D. (2011). Influence of yeast macromolecules on sweetness in dry wines: role of the Saccharomyces cerevisiae protein Hsp12. *Journal of Agricultural and Food Chemistry*, 59, 2004–2010. https://doi.org/10.1021/jf103710x

Marti-Raga, M., Martin, V., Gil, M., Sancho, M., Zamora, F., Mas, A., & Beltran, G. (2016). Contribution of yeast and base wine supplementation on sparkling wine composition. *Journal of the Sciences of Food and Agriculture*, 96, 4962–4972. https://doi.org/10.1002/jsfa.7905

Martínez-Lapuente, L., Guadalupe, Z., Ayestaran, B., Ortega-Heras, M., & Perez-Magariño, S. (2013). Changes in polysaccharide composition during sparkling wine making and aging. *Journal of Agricultural and Food Chemistry*, 61, 12362–12373. https://doi.org/10.1021/jf403059p

Martínez-Lapuente, L., Guadalupe, Z., Ayestarán, B., & Pérez-Magariño, S. (2015). Role of major wine constituents in the foam properties of white & rosé sparkling wines. *Food Chemistry*, 174, 330–338. https://doi.org/10.1016/j.foodchem.2014.10.080

Martinez-Rodriguez, A.J., Carrascosa, A.V., Martin-Alvarez, P.J., Moreno-Arribas, M.V., & Polo, M.C. (2002). Influence of the yeast strain on the changes of the amino acids, peptides and proteins during sparkling wine production by the traditional method. *Journal of Industrial Microbiology and Biotechnology*, 29, 314–322. https://doi.org/10.1038/sj.jim.7000323

Medina-Trujillo, L., González-Royo, E., Sieczkowski, N., Heras, J., Fort, F., Canals, J.M., & Zamora, F. (2017). Influence of supplementation with inactivated dry yeasts during the tirage of sparkling wines (Cava) on its composition, its foaming properties and its sensorial quality. *BIO Web of Conferences*, 9, 02031. https://doi.org/10.1051/bioconf/20170902031

Mekoue-Nguela, J., Sieczkowski, N., Roi, S. & Vernhet, A. (2015). Sorption of grape proanthocyanidins and wine polyphenols by yeasts, inactivated yeasts, and yeast cell walls. *Journal of Agricultural and Food Chemistry*, 63, 660–670. https://doi.org/10.1021/jf504494m

Moreno-Arribas, M.C., Pueyo, E., & Polo, M.C. (1996). Peptides in musts and wines. changes during the manufacture of cavas (sparkling wines) *Journal of Agricultural and Food Chemistry*, 44, 3783–3788. https://doi.org/10.1021/jf960307a

Navarro, M., Kontoudakis, N., Giordanengo, T., Gómez-Alonso, S., García-Romero, E., Fort F., Canals, J.M., Hermosín-Gutíerrez, I., & Zamora, F. (2016). Oxygen consumption by oak chips in a model wine solution; Influence of the botanical origin, toast level and ellagitannin content. *Food Chemistry*, 199, 822–827. https://doi.org/10.1016/j.foodchem.2015.12.081

Nikolantonaki, M., Julien, P., Coelho, C., Roullier-Gall, C., Ballester J., Schmitt-Kopplin, P., & Gougeon R.D. (2018). Impact of glutathione on wines oxidative stability: a combined sensory and metabolomic study. *Frontiers in Chemistry*, 6, 00182. https://doi.org/10.3389/fchem.2018.00182

Nisamedtinov, I., Kevvai, K., Orumets, K., Rautio, J. J., & Paalme, T. (2010). Glutathione accumulation in ethanol-stat fed-batch culture of Saccharomyces cerevisiae with a switch to cysteine feeding. *Applied Microbiology and Biotechnology*, 87(1), 175–183. https://doi.org/10.1007/s00253-010-2502-x

Nikolantonaki, M., Magiatis, P., & Waterhouse, A.L. (2014). Measuring protection of aromatic wine thiols from oxidation by competitive reactions vs wine preservatives with orthoquinones. *Food Chemistry*, 163, 61–67. https://doi.org/10.1016/j.foodchem.2014.04.079

OIV (2015) Resolutions OIV-OENO446-2015 «Treatment of wine with glutathione» https://www.oiv.int/public/medias/1687/oiv-oeno-446-2015-en.pdf

Oliveira, C.M., Ferreira, A.C.S., De Freitas, V., & Silva, A.M.S. (2011). Oxidation mechanisms occurring in wines. *Food Research International*, 44, 1115–1126. https://doi.org/10.1016/j.foodres.2011.03.050

Pascual, O., Vignault, A., Gombau, J., Navarro, M., Gómez-Alonso, S., García-Romero, E., Canals, J.M., Hermosín-Gutíerrez, I., Teissedre, P.L., & Zamora, F. (2017). Oxygen consumption rates by different oenological tannins in a model wine solution. *Food Chemistry*, 234, 26–32. https://doi.org/10.1016/j.foodchem.2017.04.148

Petruzzi, L., Baiano, A., De Gianni, A., Sinigaglia, M., Corbo, M.R., & Bevilacqua, A. (2015). Differential Adsorption of Ochratoxin A and Anthocyanins by Inactivated Yeasts and Yeast Cell Walls during Simulation of Wine Aging. *Toxins*, 7, 4350–4365. https://doi.org/10.3390/toxins7104350

Piotrowska, M., Nowak, A., & Czyzowska, A. (2013). Removal of ochratoxin A by wine *Saccharomyces cerevisiae* strains. *European Food Research and Technology*, 236, 441–447. https://doi.org/10.1007/s00217-012-1908-3

Pons, P., Heras, J.M., Sieczkowski, N., Canals, J.M., & Zamora, F. (2019). Determinación de la tasa de consumo de oxígeno por parte de una levadura seca

inactivada (LSI); Comparación con el ácido ascórbico y con el dióxido de azufre. *Enólogos*, 117, 62–67.

Pons-Mercadé, P., Giménez, P., Gombau, J., Vilomara, G., Conde, M., Cantos, A., Rozès, N., Canals, J.M., & Zamora, F. (2020) Oxygen consumption rate by the lees during sparkling wine (Cava) aging; Influence of the aging time. Food Chemistry, 2020.128238. *Food Chemistry*, 2020. 126941. https://doi.org/10.1016/j. foodchem.2020.128238

Pozo-Bayón, M.A., Andujar-Ortiz, I., Alcaide-Hidalgo, J.M., Martín-Álvarez, P.J., & Moreno-Arribas, M.V. (2009a). Characterization of commercial inactive dry yeast preparations for enological use based on their ability to release soluble compounds and their behavior toward aroma compounds in model wines *Journal of Agricultural and Food Chemistry*, 57, 10784–10792. https://doi.org/10.1021/jf900904x

Pozo-Bayón, M.A., Andújar-Ortiz, I., & Moreno-Arribas, M.V. (2009b). Scientific evidences beyond the application of inactive dry yeast preparations in winemaking. *Food Research International*, 42, 754–761. https://doi.org/10.1016/j.foodres.2009.03.004

Pozo-Bayón, M.A., Andújar-Ortiz, I., & Moreno-Arribas, M.V., (2009c). Volatile profile and potential of inactive dry yeast-based winemaking additives to modify the volatile composition of wines. *Journal of the Science of Food and Agriculture*, 89, 1665–1673. https://doi.org/10.1002/jsfa.3638

Pueyo, E., Martínez-Rodríguez, A., Polo, M.C., Santa-María, G., & Bartolomé, B. (2000). Release of lipids during yeast autolysis in a model wine System. *Journal of Agricultural and Food Chemistry*, 48, 116−122. https://doi.org/10.1021/jf990036e

Renouf, V., Lonvaud-Funel, A., & Coulon, J. (2007). The origin of Brettanomyces bruxellensis in wines: a review. *Journal International des Sciences de la Vigne et du Vin*, 41, 161–173. https://doi.org/10.20870/oeno-one.2007.41.3.846

Rodríguez-Bencomo, J.J., Andújar-Ortiz, I., Moreno-Arribas, M.V., Simó, C., González, J., Chana, A., Dávalos, J., & Pozo-Bayón, M.A. (2014). Impact of glutathione-enriched inactive dry yeast preparations on the stability of terpenes during model wine aging. *Journal of Agricultural and Food Chemistry*, 62, 1373–1383. https://doi.org/10.1021/jf402866q

Roussis, I.G., Lambropoulos, I., & Tzimas P. (2007). Protection of volatiles in a wine with low sulfur dioxide by caffeic acid or glutathione. *American Journal of Enology and Viticulture*, 58, 274–278. https://doi.org/10.21548/34-2-1103

Salmon, J.M., Fornairon-Bonnefond, C., Mazauric, J.P., & Moutounet, M. (2000). Oxygen consumption by wine lees: impact on lees integrity during wine ageing. *Food Chemistry*, 71, 519–528. https://doi.org/10.1016/S0308-8146(00)00204-1

Sheridan, M.K., & Elias, R.J. (2016). Reaction of acetaldehyde with wine flavonoids in the presence of sulfur dioxide. *Journal of Agricultural and Food Chemistry*, 64, 8615–8624. https://doi.org/10.1021/acs.jafc.6b03565

Sieczkowski, N., Ortiz-Julien, A., Garcia, E., Samson, A., Caillè, S., & Salmon, J.M. (2016a). Une nouvelle levure inactivée spécifique comme solution naturelle pour la maîtrise de l'oxygène et la conservation de la qualité des vins en élevage. Partie 1/2: Mise au point et preuve de concept. *Revue des Œnologues*, 159, 25–28.

Sieczkowski, N., Ortiz-Julien, A., Garcia, E., Samson, A., Caillè, S., & Salmon, J-M. (2016b). Une nouvelle levure inactivée spécifique comme solution naturelle pour la maîtrise de l'oxygène et la conservation de la qualité des vins en élevage. Partie 2/2: Validation en situations pratiques de cave. *Revue des Œnologues*, 160, 38–40.

Styger, G., Prior, B., & Bauer, F.F. (2011). Wine flavor and aroma. *Journal of Industrial Microbiology and Biotechnology*, 38, 145–1159. https://doi.org/10.1007/s10295-011-1018-4

Ugliano, M., Kwiatkowski, M., Vidal, S.P., Capone, D., Siebert, T., Dieval, J.B., Aagaard, O., & Waters E.J. (2011). Evolution of 3-mercaptohexanol, hydrogen sulfide, and methyl mercaptan during bottle storage of Sauvignon blanc wines. Effect of glutathione, copper, oxygen exposure, and closure-derived oxygen. *Journal of Agricultural and Food Chemistry*, 59, 2564–2572. https://doi.org/10.1021/jf1043585

Vanrell, G., Esteruelas, M., Canals, J.M., Zamora, F., Poinsaut, P., Sieczkowski, N., & Lebouef, D. (2005). Influence du type de clarification du vin de base et des adjuvants de tirage sur la qualité de la mousse des vins effervescents. *Revue des Oenologues*, 114, 28–30.

Vignault, A., Gombau, J., Jourdes, M., Moine, V., Canals, J.M., Fermaud, M., Roudet, J., Teissedre, P.L., & Zamora F. (2020), Oenological tannins to prevent Botrytis cinerea damages: kinetics and electrophoresis characterization. *Food Chemistry*, 316 (2020) 126334. https://doi.org/10.1016/j.foodchem.2020.126334

Vilela, A., Ines, A., & Cosme, F. (2016). Is wine savory? Umami taste in wine. *SDRP Journal of Food Science and Technology*, 1, 100−105.

Waters, E.J., Pellerin, P., & Brillouet, J.M. (1994). A wine arabinogalactan-protein that reduces heat-induced wine protein haze. *Bioscience, Biotechnology, and Biochemistry*, 58, 43–48. https://doi.org/10.1271/bbb.58.43

Webber, V., Dutra, S.V., Rodrigues-Spinelli, F., Carnieli, G.J., Cardozo, A., & Vanderlinde, R. (2017). Effect of glutathione during bottle storage of sparkling wine. *Food Chemistry*, 216, 254–259. https://doi.org/10.1016/j.foodchem.2016.08.042.