ISSN 1330-9862 *original scientific paper*
doi: 10.17113/ftb.54.04.16.4651

Oxygen Consumption by Postfermentation Wine Yeast Lees: Factors Affecting Its Rate and Extent under Oenological Conditions

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Received: February 14, 2016
Accepted: June 30, 2016

Summary

Postfermentation wine yeast lees show antioxidant properties based on their ability to consume dissolved oxygen. The oxygen consumption capacity of suspended yeast lees obtained after fermentations with six commercial active dry yeast strains was investigated in model, white and red wines using fluorescence-based oxygen sensors operating in a non-destructive way. In model solution, the oxygen consumption rate of yeast lees was shown to depend on their amount, yeast strain, sulfur dioxide and temperature. It is slightly lower in red than in white wines. It is strongly decreased by current levels of free sulfur dioxide, thus excluding the complementary use of both as antioxidants in wine. However, in 25 randomly sampled white wines produced under commercial conditions, the rate and extent of oxygen consumption during the first six months of postfermentation had no significant correlation with any of these interacting factors, making it difficult to predict the actual antioxidant effect of yeast lees. In these wines, yeast lees consumed 0 to 47 % of the dissolved oxygen. Although total oxygen consumption capacity of yeast lees is not a limiting factor under commercial winemaking conditions, their oxygen consumption proceeds at a limited rate that reduces but cannot totally prevent concomitant chemical oxidation of the wine.

Key words: wine, yeast lees, dissolved oxygen, oxidation, sulfur dioxide, oxygen consumption

Introduction

At the end of alcoholic fermentation, yeast cells start to autolyze. Yeast autolysis is a slow process characterized by the hydrolysis of intracellular biopolymers by yeast enzymes activated upon cell death. As a result, cytoplasmic and cell wall compounds are released into the wine with various oenological and sensory consequences. With the aim of making deliberate use of this process, ageing on the lees has become an oenological concept for pursuing specific stylistic goals. This concept is based on the assumption that after primary fermentation lees consist predominantly of yeast cells. A distinction is made between light lees that remain in suspension and heavy lees that settle at the bottom of the vessel.

Mannoproteins extracted from yeast lees contribute to volume on the palate and decrease astringency of tannins, thus improving mouthfeel and structure. They can

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also contribute to protein and bitartrate stability. Esters, nucleotides, peptides and amino acids released from postfermentation yeast lees provide flavour precursors and enhance flavour complexity (*1,2*). Some of these amino compounds such as glutathione and cysteine can assist in protection from oxidation (*3*). The yeast cells themselves absorb dissolved oxygen (DO), withdrawing it from oxidation reactions (*4*). However, when yeast lees are left too long and compacted at the bottom of the ageing vessel under anaerobic conditions, they can start to produce volatile sulfur compounds that negatively affect flavour (*5*). The accumulation of yeast compounds in the wine is interrupted by removal of yeast by filtration, while maintaining yeast in suspension by stirring lees accelerates their release.

In its traditional format, ageing on the lees is carried out on white wines stored in barrels over several months to more than a year. During this period, lees left after fermentation are prevented from settling by periodic stirring referred to as bâtonnage, or by rolling the barrels. Bâtonnage as traditionally practiced stimulates the decrease of free sulfur dioxide and the uptake of atmospheric oxygen due to the removal of the bung and the process of stirring. Yet bâtonnage is not inherently oxidative since oxidation is buffered by the absorption of DO by yeast (*4*) and the release of reducing amino acids (*3*). Stirring regime, the amount of yeast, and SO_2 management determine whether ageing on the lees is more reductive or oxidative process (*6*).

The DO uptake rate of yeast during ageing on yeast lees was quantified at 0.003 to 0.011 mg/(L·h) per 10^9 of yeast cells from the second to the sixth month of ageing at 14 °C (*4*). The initial DO consumption rates and their gradual and irregular decay during wine ageing were strongly dependent on yeast strains, with 11 to 100 % of the initial DO consumption rate left after four months of ageing (*4*). In later works, oxygen consumption by non-viable yeasts was ascribed to a largely non-respiratory pathway using the oxygen for oxidation of membrane lipids (*7,8*) and ergosterol (*9*) leading to the production of lipid peroxides and unknown end products, confirming earlier findings on brewer's yeast (*10*). These reactions are NADPH-dependent (*11*) and discontinued after pasteurization (*4*). When oxygen is added in excess during alcoholic fermentation, the reactivity of yeast lees towards oxygen decreases due to oxygen-dependent sterol degradation (*12*). Respiration was shown to be only involved in oxygen uptake by yeast lees during the first three weeks post fermentation (*4*) since 99 % of yeast cells die within two weeks after fermentation (*13*). Contrary to these studies, earlier works have shown that during oxygenation of submerged cultures of postfermentation *S. cerevisiae* cells, oxygen is also used for the formation of acetaldehyde by yeast-bound ethanol dehydrogenase activity (*14–17*). Furthermore, an increase of acetaldehyde concentration was observed when unfiltered white table wines were submitted to inadvertent oxygen uptake during racking and storage, requiring ultimately higher SO_2 additions to bind it (*18*).

Although most of these investigations were carried out against the background of barrel ageing, the ability of postfermentation yeast lees to consume DO suggests that they can act as a reducing agent in a more general way. Their consumption of DO picked up during storage and winery operations would protect wine constituents against chemical oxidation. Historical evidence confirms that yeast lees delay the browning of white wines exposed to atmospheric oxygen in the absence of free sulfur dioxide. Thus, the aim of this study is to provide extensive data on the rate and extent to which DO present in wine is consumed by variable amounts of suspended yeast lees as compared to filtered wines, the effect of some technical parameters on the rate of this reaction, its significance under practical winemaking conditions, the possible formation of acetaldehyde as a secondary product, and the feasibility of nephelometric turbidity measurements for evaluating levels of suspended yeast cells in wines.

Materials and Methods

Assessment of yeast biomass and cell number

Suspended yeast lees were quantified by nephelometric turbidity measurements using a Hach 2100Q turbidimeter (Loveland, CO, USA) calibrated with primary formazin standards and expressed in nephelometric turbidity units (NTU). Nephelometric turbidity measurements of serial dilutions in model solution of yeast suspensions obtained after fermentation of a filtered grape juice yielded a correlation curve that showed linearity below 40 NTU with R=0.9995. Consequently, all sample measurements were carried out after dilution with model solution to less than 40 NTU in order to measure in the linear range. The turbidity of the original sample was then computed from the dilution factor. In a second step, the yeast suspensions were used for cell counting in a Fuchs-Rosenthal haemocytometer (Carl Roth, Karlsruhe, Germany) after appropriate dilution from 10 to 40 NTU. The cell count of 40 unit squares on each of eight drops was made and the mean number of cells per mL was calculated. Yeast cell numbers of serial dilutions were correlated with NTU readings. The correlation yielded $6.3 \cdot 10^4$ cells per mL per 1 NTU. It provided the basis for converting yeast concentrations reported in NTU in this work.

Wines and yeasts

Oxygen consumption by yeast cells was studied in model solution and in the wines they originated from. Wines and yeast lees were obtained from local wineries. Vinification was carried out using standard procedures with the exception that juice was clarified to less than 20 NTU of residual turbidity using pectolytic enzymes, followed by the addition of 2 g/L of bentonite three to five hours later, sedimentation, flotation or filtration. Under these conditions of highly clarified grape juices, solids of the resulting wines consisted predominantly (>98 %) of yeast cells. After clarification, the different juices containing 200 to 220 g/L of initial sugar were inoculated with 20 g/hL of commercially available active dry yeast strains. Fermentations and further storage between 8 and 15 °C took place in stainless steel tanks. No oxygen was added during fermentation. Yeast strains used for inoculation and further investigation were Lalvin CY 3079 (Lalle-

mand, Montreal, Canada), Lalvin EC 1118 (Lallemand), Lalvin RC 212 (Lallemand), Fermicru VB 1 (Oenobrands, Montpellier, France), Collection Cépage Sauvignon (Oenobrands), and SIHA Riesling Yeast (Eaton, Langenlonsheim, Germany).

Dissolved oxygen consumption trials

The model solution contained 13 % (by volume) alcohol in distilled water. After addition of 8 g/L of tartaric acid, pH was adjusted to 3.5 using 10 % NaOH.

For monitoring dissolved oxygen (DO) consumption of yeast in model solution, yeast was harvested from young white wines after fermentation to dryness (<2 g/L of sugar) and before the onset of malolactic fermentation (<0.2 g/L of lactic acid). Wine aliquots of 750 mL were sampled into nitrogen-purged bottles and centrifuged (4 min, 3000×*g*). The obtained yeast slurry was washed and diluted in the model solution to desired concentrations as determined by measuring the nephelometric turbidity of the suspensions. Oxygen consumption measurements started immediately after dilution since yeast storage in aqueous solutions has been shown to result in increased values due to physiological changes of the cells (*10*).

For monitoring DO consumption of yeast in unfiltered wines, samples of 750 mL were taken from storage tanks at different time points and split into two aliquots. One aliquot was used for DO consumption measurement without further treatment. The other aliquot was clarified by centrifugation and filtration through a 0.6-μm membrane (model 11305; Sartorius, Göttingen, Germany) to serve as a yeast-deprived reference in DO consumption measurements. For evaluation of the effect of the wine matrix on DO consumption by yeast, sterile-filtered wines were supplemented with yeast slurry to desired concentrations.

Monitoring oxygen consumption kinetics

Dissolved oxygen was determined by a non-invasive method of luminescence quenching using a Fibox 3 Trace single channel fibre optic oxygen meter coupled with PSt3 luminescent oxygen sensors (PreSens Precision Sensing GmbH, Regensburg, Germany). The Fibox 3 probe measures the luminescence decay time of the luminophore sensor spots mounted inside a vessel of clear glass. The luminophore sensor is excited with a sinusoidal intensity-modulated monochromatic light delivered by the optical fibre. Its decay time causes a time delay in the light signal emitted by the luminophore. The decay time decreases in the presence of oxygen. Thus, the excitation of the sensor generates a response proportional to the amount of DO around the sensor.

The oxygen-sensitive mini sensor spots were glued with silicone at centre positions onto the inner wall of flasks of 100 mL, allowing them to be in contact with the liquid. Then, the flasks were filled to the rim with model solution or wine previously supplied with atmospheric oxygen to near a saturation concentration (approx. 7 mg/L) by intense shaking. Subsequently, the flasks were sealed with screw caps fitted with a plastic cone liner ensuring the exclusion of any air bubbles at the interface between the liquid and internal cap. Supplementary sample treatment and storage conditions are specified in the Results section. DO readings of the liquids were obtained without opening the flasks, pointing the light-transferring optical fibre at the sensor spots through the glass. Measurements were performed with a recording rate of one reading point per three seconds. Readings were taken as valid when the standard deviation was <2 %, after which the mean was calculated. DO measurement range was 0 to 22 mg/L with a detection limit of 0.015 mg/L. Manual calibration was applied using the batch calibration data obtained from the manufacturer.

Recurrent shaking of the closed flasks prevented sedimentation of the lees, thus mimicking more real storage conditions. DO readings were performed periodically until it was mostly depleted (≤0.2 mg/L), and DO data were plotted against time. According to the shape and equation of the obtained curves, interpretation of data was performed in two different ways: in model solutions, the curve linear slope provided the oxygen consumption rate (OCR) in mg/(L·h) of yeast directly. In real wines showing a linear curve shape, the OCR of the yeast was obtained by subtraction of the OCR of a filtered blank. In many wines, however, the change of OCR over time was best fitted with an exponential function regardless of the presence or absence of yeast. This allowed for calculating the percentage and the absolute amount of DO consumed by yeast. For that purpose, the measured DO data of both, the unfiltered aliquot and the filtered blank, were transferred *via* regression analyses into two mathematical functions and normalized both functions to the same zero point before integrating them over time. Dividing the integral of the unfiltered aliquot by the integral of the filtered blank provided the effect of DO consumed in percentage that could not be attributed to the yeast effect but to the chemical oxidation of the wine matrix. The remaining part corresponded to the yeast's effect on the overall decrease of DO in percentage.

Total oxygen consumption capacity

Total oxygen consumption capacity of yeast suspensions was measured in model solution under conditions of potential oxygen supply of up to 80 mg/L, using a standard manometric assay originally devised for assessment of biological oxygen demand of water (OxiTop®, WTW, Weilheim, Germany). Defined volumes of thoroughly washed yeast suspensions were transferred into 500-mL amber bottles tightly sealed with manometric pressure sensors. The bottles were then continuously stirred at 200 rpm and 20 °C on a 6-position magnetic stirrer platform to ensure constant oxygen supply from the headspace during the experiment. The decrease in the monitored pressure was related to oxygen consumption that, in turn, was plotted against time. A control set containing no yeast was used to correct for the oxygen uptake of the model solution matrix. Standard deviation of the measurement was ±10 % of the readings.

Chemical analyses

Acetaldehyde, free sulfur dioxide, lactic acid and residual sugar were measured enzymatically using appropriate test kits (R-Biopharm AG, Darmstadt, Germany).

Statistical analyses

All statistical analyses were performed using the R software v. 3.2.1 (*19*). Graphics were obtained using the 'ggplot2' package (*20*); linear and non-linear regression models were produced using the 'nls2' package (*21*).

Results

Effect of yeast concentration on OCR

The model solution was chosen to study the effect of yeast concentration on its ability to consume DO. Thus, any interference from oxidizable wine constituents was circumvented. Yeast concentrations assayed were 5, 25, 50, 100, 200, 300, and 400 NTU. The oxygen consumption in each lot was almost constant during the tests, following a zero-order kinetics that allowed for expressing the OCR in mg/(L·h) of oxygen (Fig. 1).

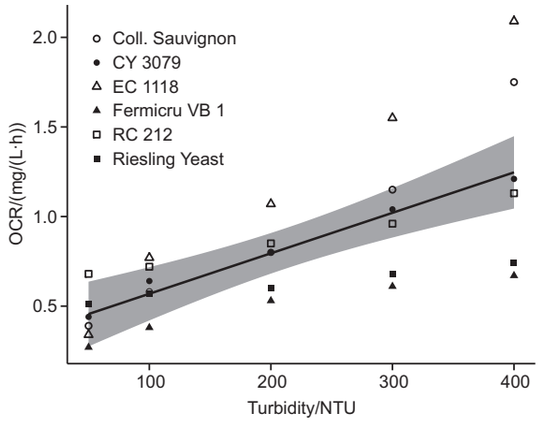

Fig. 1. Oxygen consumption rate (OCR) of yeast strains in model solution at 20 °C as affected by yeast concentration. Yeasts were harvested and trialled one to three weeks after the end of alcoholic fermentation. $\gamma(SO_2)_{free}$=0 mg/L; *N*=2; NTU=nephelometric turbidity units

Although all yeasts were harvested and trialled within one month after the end of alcoholic fermentation, there were remarkable differences in the OCR among yeast strains at given concentrations. However, these differences should be considered with caution since they might be impacted by matrix and technical variables as well as some residual, respiratory DO consumption. Reproducible consumption rates required a minimum of 50 NTU of yeast. Above that minimum level, OCR ranged from 0.27 to 2.09 mg/(L·h).

Interestingly, OCR does not always increase with increasing yeast concentrations in a proportional way, *i.e.* the OCR per cell declines at higher cell densities. In these cases, when OCR is plotted against NTU, the slope of the obtained curve suggests a Michaelis-Menten type behaviour. This pattern has been observed by other authors (*11*) and is believed to be caused by a steric hindrance of oxygen diffusion at the particle level as a result of the physical presence of yeast cells, making DO diffusion within the liquid the rate-limiting step. However, since this behaviour could not be verified over the entire data set, a linear model of regression analysis was used to test the effect of yeast concentration on the OCR. As expected, it could be confirmed that higher yeast concentrations lead to an increase of OCR (99.9 % confidence level). This general trend is valid for all yeast strains under consideration. Data below 50 NTU were neglected as the quantification limit did not allow reproducible measurements.

Effect of temperature on OCR

Since the DO consumption of yeast cells is based on the oxidation of complex yeast membrane compounds, it is expected to depend also on temperature. The temperature effect on the yeast strains with concentrations adjusted to 200 NTU was evaluated in model solution at 20, 15 and 5 °C. The DO consumption followed a zero-order kinetics for each temperature (Table 1).

Table 1. Effect of temperature on the oxygen consumption rate (OCR) by different yeast strains in model solution at 20 °C

Yeast strain	Temperature/°C		
	5	15	20
	OCR/(mg/(L·h))		
Coll. Sauvignon	0.030	0.469	0.507
CY 3079	0.029	0.041	0.048
EC 1118	0.136	0.193	0.244
Riesling-Yeast	0.017	0.046	0.099
RC 212	0.573	0.614	0.706
Fermicru VB 1	0.591	0.781	0.833
$\bar{x}$	0.278	0.357	0.406
($\bar{x}/\bar{x}$ at 20 °C)/%	68.5	87.9	100

Yeasts were harvested two weeks after the end of alcoholic fermentation and adjusted to 200 NTU
NTU=nephelometric turbidity units; *N*=2

Using a linear regression model to examine the effect of temperature and yeast strains on the OCR shows that temperature has a significant influence (99 % confidence level). This behaviour was found of each of the different yeast strains to a varying degree, with confidence levels for differences among yeast strains ranging from 90 to 99 %.

Under the conditions of cool wine storage, the OCR of yeast cells decreases markedly. This temperature effect is in agreement with the results of a classical study showing that DO consumption rates obey the Arrhenius equation when the relation of consumption rate to temperature is calculated as a kinetic constant (*22*). However, the oxygen consumption rate of filtered wines also proceeds at a lower rate when the temperature is decreased (*6*). Therefore, when yeast lees and oxidizable wine matrix compounds compete for DO in real wines, the yeast percentage contribution to total oxygen consumption is not necessarily affected by temperature.

Effect of free sulfur dioxide on OCR

Since most wines are stored with variable concentrations of free sulfur dioxide after fermentation, another pertinent question was whether SO_2 additions would have

any effect on the OCR of postfermentation yeast cells. OCR measurements of the six yeast strains suspended at 200 NTU in model solution spiked with increasing amounts of SO_2 showed a strong suppressive effect of SO_2 (Fig. 2). At an average wine pH=3.5, there is a critical limit around 20 mg/L of free SO_2 above which the OCR of yeast becomes almost insufficient to have any practical effect. Depletion of DO by reactions with SO_2 during the course of the assay cannot explain this behaviour since it would increase the OCR when initial SO_2 concentrations increase. The reason why SO_2 strongly inhibits DO uptake by yeast remains unknown, but inhibition of unidentified enzymes by SO_2 might be a cause.

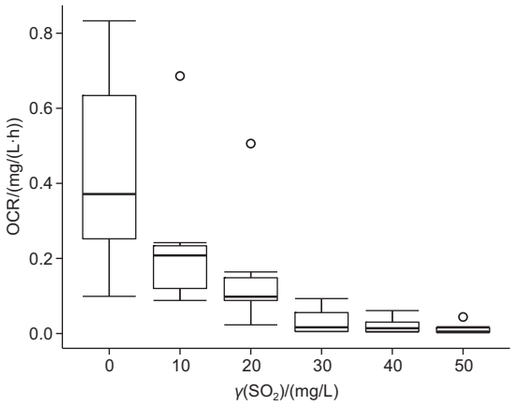

Fig. 2. Effect of free SO_2 on oxygen consumption rate (OCR) of yeast strains in model solution at 20 °C. Yeasts were harvested within two weeks after the end of the alcoholic fermentation and adjusted to 200 nephelometric turbidity units (NTU). Boxplots represent two repeated measurements of six different yeast strains

Effect of wine matrix on OCR

The effect of the wine matrix (white *vs*. red wine) was investigated using three yeast strains (EC 1118, CY 3079 and Fermicru VB 1). Defined amounts of yeast harvested after alcoholic fermentation were added to a sterile filtered white wine and three different red wines (A, B and C) without any free sulfur dioxide. After one month of storage under anaerobic conditions, the wines were saturated with oxygen and the DO decrease was registered. It followed an almost linear pattern comparable to that of the model solution. To calculate the net OCR of the yeasts, the overall DO decrease was corrected for the chemical oxygen binding by the respective wines without added yeast, which served as a blank. Results are given in Table 2.

Again, the OCR is not enhanced by increasing yeast lees concentrations in a linear way, thus confirming the results obtained in model solution. Furthermore, for comparable yeast concentrations, OCR figures are of the order of those found in model solution (Fig. 1). However, there is a clear tendency towards a decrease of yeast OCR in red wines as compared to the white wine. Testing the hypothesis that red wine matrix results in a lower OCR than white wine matrix, paired *t*-tests were used for the three series of experiments. The hypotheses were confirmed for

Table 2. Effect of wine matrix on the oxygen consumption rate (OCR) of suspended yeast in real wines at 20 °C as affected by yeast concentration

Yeast strain	Wine sample	Turbidity/NTU					C.L.
		50	100	200	300	400	
		OCR/(mg/(L·h))					
EC 1118	white wine	0.53	0.86	1.21	1.48	1.94	
	red wine A	0.50	0.73	1.14	1.46	1.62	*
CY 3079	white wine	0.65	1.12	1.14	1.14	1.14	
	red wine B	0.47	0.64	0.63	0.64	0.66	***
Fermicru VB 1	white wine	0.14	0.18	0.28	0.36	0.39	
	red wine C	0.10	0.14	0.26	0.35	0.38	**

Data corrected for chemical binding of dissolved oxygen by the wines. Yeasts were harvested one month after alcoholic fermentation and aged for another month in the respective assay wines before OCR measurements. C.L.=confidence level, NTU=nephelometric turbidity units. *90 % C.L., **95 % C.L. and ***99 % C.L.; *N*=2

EC 1118, CY 3079 and Fermicru VB 1 at a 90, 99 and 95 % confidence level, respectively.

This matrix effect has been explained by a partial adsorption of red wine polyphenols by yeasts. As a consequence, these phenols strongly interact with components of yeast cell membrane responsible for the oxygen consumption. It is assumed that they elicit a collapse of the cytoplasmic intermembrane space, which lowers the accessibility and reactivity of oxygen towards the sterols and unsaturated fatty acids of the cell membrane. Major losses of overall reactivity of oxygen occur during the first ten days of yeast contact with red wine polyphenols. These losses are irreversible and also observed after soluble polyphenols had been separated from the yeast cells (*8*,*23*). Contrary to these findings obtained on model solutions spiked with 3, 6 and 9 g/L of phenolic material extracted from red wine, the impact of red wine phenols on yeast OCR under more realistic conditions of standard red wines (approx. 2 g/L of total phenols) seems rather negligible.

Total oxygen consumption capacity

Three yeast strains (EC 1118, CY 3079 and Fermicru VB 1) suspended in model solution at a concentration of 300 NTU each were used to assess their total oxygen uptake capacity and consumption kinetics using the manometric method at 20 °C. As shown in Fig. 3, plotting oxygen uptake against time yielded concave-like curves displaying an almost linear increase of oxygen consumed at the beginning, followed by a transition to a steady state indicating depletion of the yeast oxygen uptake resources after 45 to 55 h and 40 to 60 mg/L of consumed DO. These results indicate that even minor concentrations of suspended yeast cells corresponding to approx. 50 NTU ($3.15 \cdot 10^6$ cells/mL) would suffice to consume one saturation concentration of DO. Under industrial winemaking conditions, total oxygen consumption capacity of yeast lees is not a limiting factor to protect wine against oxidation.

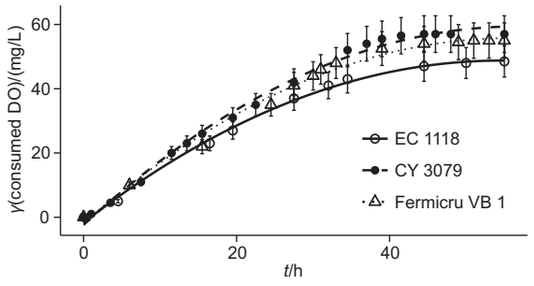

Fig. 3. Total oxygen consumption capacity and consumption kinetics shown through dissolved oxygen (DO) consumed by suspended yeast cells in model solution of 300 nephelometric turbidity units (NTU) one month after alcoholic fermentation. Error bars indicate the standard deviation of the measurement (10 %). Lines are based on second-degree polynomial fitting

Combined effects of lees concentration, age and free SO_2 in real wines

In an unstructured assay, samples were obtained from 25 unfiltered white wines produced under commercial conditions and fermented with the yeast strains used in this study. The samples were split into two aliquots, one of them containing the original yeast and the other one was filtered to serve as a blank. After oxygen supply by aeration, the DO decrease in both aliquots at 20 °C was plotted against time. Yeast concentration in the wines ranged from 8 to 310 NTU, initial free sulfur dioxide from 0 to 57 mg/L, and the age of the wines from 2 to 26 weeks after alcoholic fermentation.

The DO decrease plots obtained were either close to linear or exponential functions depending on the individual wine. Both patterns have been reported by other authors (*24*,*25*). In most of the filtered wines, however, the plots took the form of a more or less convex curve fitting a negative exponential function. They correspond to a first--order reaction kinetics indicating a change of the OCR over time and representing the exclusive chemical binding of DO. In the unfiltered wines containing yeast, both DO consumption by the yeast and DO consumption due to chemical binding accumulated to a total, which still displayed an overall reaction order of close to one that could be represented best by an exponential equation. Fig. 4 gives an example. Under these conditions, no workable OCR values were obtained since they change throughout

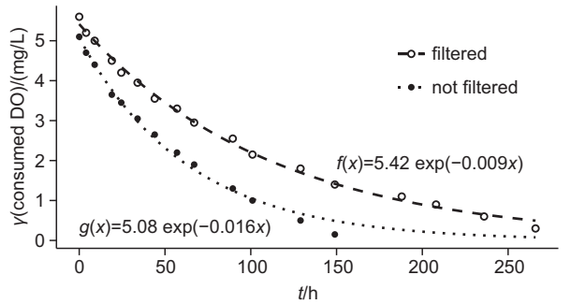

Fig. 4. Exponential dissolved oxygen (DO) consumption in a white wine (43 mg/L of free SO_2) with suspended yeast (Fermicru VB 1, 50 NTU) and after filtration, 8 weeks postfermentation

the period of the test according to the instantaneous DO concentration (*10*). Instead, mathematical treatment of the equations of both curves as described in the Materials and Methods section provided the percentage and the absolute amount of DO consumed by yeast, with the remaining part corresponding to the DO consumed by chemical oxidation of the wine matrix.

DO consumed by yeast ranged from 0 to 47 % (data not shown). Trends of the large impact of the concentration of yeast and free SO_2 on DO consumption by yeast could be observed, confirming results obtained on model solution. However, using multivariate analyses, not one of the tested parameters (yeast strain, yeast concentration, wine, age and free SO_2 concentration) showed any significant influence on the DO consumption rate by yeast, expressed as the reaction rate coefficient of first order kinetics. The combination and interaction of the various parameters do not allow for a reliable prediction of yeast reactivity towards DO in a given wine.

Synthesis of acetaldehyde

Total acetaldehyde was measured after complete depletion of DO in the 25 wines referred to above. Differences in acetaldehyde concentration between the filtered and the unfiltered aliquots of each wine were not significant and did not exceed ±3 mg/L (data not shown). These results indicate that the consumption of up to 7.5 mg/L of DO by postfermentation yeast lees of the *S. cerevisiae* strains assayed in this study does not elicit any increase of acetaldehyde content. They suggest the absence of any respiratory metabolism, thus supporting earlier findings (*7–10*) that DO is mainly consumed by lipid oxidation. On the other hand, since yeast viability may vary considerably in practice, one cannot exclude that respiration contributes to DO consumption under different oenological conditions.

Discussion

The oxygen consumption capacity of postfermentation yeast cells has been known in both the wine and the beer industry for considerable time. However, it has been unclear how it is affected by oenological parameters, and how much DO is indeed consumed by yeast lees instead of reacting with intrinsic wine constituents by chemical binding, leading necessarily to wine oxidation.

Suspended wine yeast lees are able to consume up to several saturation concentrations of DO, with the exact extent depending primarily on their amount. Their DO consumption kinetics varies between a linear pattern and first-order kinetics. Despite their considerable total oxygen consumption capacity relying mainly on lipid oxidation (*7–10*), they are not able to consume all oxygen picked up by wine. Our results indicate a concomitant chemical oxygen binding by oxidizable wine constituents.

The rate at which yeast cells consume oxygen, reported as OCR, is an essential parameter for assessing the balance between the amounts of DO consumed by the yeast biomass and by the wine matrix, respectively. It can be assumed that the higher the OCR of the yeast lees, the less DO is available for the chemical oxidation of the wine. However, it is impossible to predict the extent to which

yeast contributes to total DO consumption under commercial winemaking conditions for two reasons: first, the combinations and interactions of variable oenological parameters such as yeast strain, concentration, age, wine matrix and free SO_2 make it difficult to forecast the rate at which DO is consumed by yeast as such; second, the rate of the chemical binding of DO to the wine phenolics is an unknown variable, thus rendering it impossible to deduce the percentage of DO consumed by yeast from its OCR.

It has been shown for the first time that sulfur dioxide strongly inhibits the reactions involved in oxygen consumption by yeast lees, with levels of more than 20 mg/L of free SO_2 at wine pH making it almost meaningless. Thus, the antioxidant effects of both yeast lees and sulfur dioxide cannot be used in a complementary way. In wines stored with relatively high concentrations of free SO_2, the protection against oxidation by yeast lees is less beneficial than in wines with low sulfite concentrations. Since the antioxidant effect of yeast lees is most expressed when free SO_2 is low or absent, they are a valuable winemaking tool when wines without the added SO_2 are to be produced.

When wines are stored in stainless steel tanks, inadvertent exposure to atmospheric oxygen occurs during winery operations such as pumping, centrifugation, filtration, fining, cold stabilization and bulk wine transportation. Under these conditions, the magnitude of oxygen uptake is highly variable and ranging from 0.1 to 3 mg/L in each one of these operations (*26–29*). Storage under headspace may provide further amounts of oxygen. Therefore, delayed filtration has been proposed to make use of the oxygen uptake by yeast lees and mitigate the detrimental effect of uncontrolled oxygen uptake by oxygen-sensitive, fruity white wines (*30*). However, the antioxidant property of yeast lees is largely diminished by the presence of free SO_2.

When wines are stored in standard barrels of 225 L, the amount of oxygen they receive varies between 10 and 45 mg/L per year with an average of 20 mg/L per year, decreasing with the age of the barrels (*31,32*). Assuming an average oxygen uptake of 0.05 mg/L per day in the barrel, a yeast OCR of 0.002 mg/(L·h) would be required to remove that oxygen at the rate it dissolves in the wine if yeast cells were the only oxygen receptor. However, a simultaneous and variable chemical binding of oxygen is unavoidable. It increases with increasing concentrations of free SO_2 and lowers the reductive effect exerted by yeast lees. As a practical consequence, traditional white wine ageing under semi-oxidative conditions in barrels is carried out with low concentrations of free SO_2 and high amounts of yeast lees periodically resuspended by stirring. The redox behaviour during traditional barrel ageing does not apply to fruity white wines usually stored in tanks with higher concentrations of free SO_2.

Continuous oxygen supply comparable to barrel ageing also takes place when red wines are submitted to micro-oxygenation in tanks. Yeast lees compete for oxygen with red wine phenols and hinder the wine ageing process (*8*). This finding explains why young red wines withstand more oxygen uptake than aged ones after clarification. However, these relationships are further complicated by a partial adsorption of polyphenols on yeast cell membranes, which lowers the overall reactivity of both yeast and polyphenols towards oxygen (*23*). Our results indicate that a typical yeast concentration of 100 NTU in young red wines is able to consume DO at a rate of 0.1 to 1 mg/(L·h) during the first month after primary fermentation (Table 2). Thereby, they were only slightly less effective than in white wines where they consumed up to 47 % of total DO. These findings indicate that yeast lees can consume a substantial amount of DO provided during micro-oxygenation and withdraw it from reactions with red wine phenols as intended by this treatment.

The quantification of yeast biomass is essential for properly working with yeast lees. Suspended yeast cells cause turbidity that varies as some function of the number of yeast cells per volume unit. In contrast to cell counting, turbidity measurement is an easily manageable procedure implemented in many wineries. However, despite its feasibility for evaluating yeast concentrations in beer (*33*) or after fermentation of filtered grape juices, it has some limitations when applied to real wines. It is not feasible after malolactic fermentation or in wines obtained from poorly clarified juices due to sources of turbidity other than yeast. For white wines, a stringent juice clarification using pectolytic enzymes is considered a major factor determining wine quality. A residual juice turbidity of between 50 and 100 NTU has been recommended (*34,35*). This residual turbidity consists predominantly of fruit tissue particles tending to settle by the end of primary fermentation. Under these conditions, the use of nephelometric turbidimeters may allow for an assessment of suspended yeast cell density in white wines. However, proper calibration procedures must account for the non-linearity of the calibration curve and for different measuring principles (*36,37*).

Conclusions

The novel concept of monitoring DO consumption in wine containing yeast lees in comparison with that of a filtered aliquot used as a blank allowed to differentiate precisely between the amounts of DO consumed by yeast cells and by the wine matrix, respectively. Postfermentation yeast cells significantly accelerate the DO consumption rate in white and red wines by consuming variable amounts of DO. Thereby, they protect the wine against reactions of chemical oxidation to a certain extent without producing acetaldehyde. The rate and extent of this oxygen consumption depend on the amount of suspended yeast lees, free sulfur dioxide, wine matrix, temperature, age and yeast strain. The various combinations and interactions of these parameters do not allow for predicting the DO consumption and the antioxidant effect of yeast lees under commercial winemaking conditions. At best, it can be stated that this effect increases with increasing concentrations of suspended yeast cells, although not always in a linear way. On the other hand, the level of free sulfur dioxide is of major importance, decreasing drastically the oxygen consumption rate of yeast lees and their ability to protect wine against oxidation. Thus, sulfur dioxide and yeast lees do not act as antioxidants in a complementary way. The use of yeast lees to prevent oxidation is most efficient in wines with low or no free sulfites. Their presence during micro-oxygenation of red wines is able to quench a substantial percentage of the added oxygen and requires more consideration.

Acknowledgements

The authors gratefully acknowledge technical support provided by Vino Kupljen, Jeruzalem, Slovenia, to confirm conclusions of this study under commercial winemaking conditions.

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