Food Bioprocess Technol
DOI 10.1007/s11947-014-1257-2

ORIGINAL PAPER

Oxygen Incorporation and Dissolution During Industrial-Scale Red Wine Fermentations

M. Isabel Moenne · Pedro Saa · V. Felipe Laurie · J. Ricardo Pérez-Correa · Eduardo Agosin

Received: 3 September 2013 / Accepted: 6 January 2014

Abstract Oxygen management is critical to ensure the appropriate development of yeast and avoid its detrimental effects on sensory quality of wine. Oxygen additions during alcoholic fermentation are typically carried out through pump-over operations, which contributions have not been appropriately quantified. In this work, we designed a set of experiments with different pump-over modes (closed, open, and with Venturi) to evaluate oxygen dissolution and consumption during industrial-scale fermentations. Closed pump-overs incorporate negligible amounts of oxygen, while open pump-overs with Venturi incorporate the highest, i.e., 3 mg/L (approximately twice more oxygen than the conventional open pump-overs). A highly heterogeneous vertical distribution of dissolved oxygen was also found, with approx. 80 % of the total concentrated at the top of the tanks. When analyzing oxygen dissolution during the maceration, initial low oxygen levels were encountered in spite of the high free SO_2 concentrations (an inhibitor of enzymatic oxidation). We speculate that the latter is due to the high initial CO_2 content, which prevents oxygen dissolution during the initial period. In the case of the fermentation stage, the observed low oxygen concentrations are mainly due to elevated yeast activity. We also followed oxygen dissolution kinetics during fermentation by estimating the oxygen dissolution rate and a global consumption constant for different fermentation stages. Our results confirmed the negative impact of CO_2 on oxygen dissolution and the elevated yeast biological activity of the tumultuous fermentation, as the main causes for the observed low dissolved oxygen levels. Overall, the present work will help improve the management of oxygen during fermentation and winemaking.

Keywords Oxygen · Pump-over · Wine fermentation · Winemaking · *Saccharomyces cerevisiae*

M. I. Moenne · P. Saa · J. R. Pérez-Correa · E. Agosin
Department of Chemical and Bioprocess Engineering, College of Engineering, Pontificia Universidad Católica de Chile, Casilla 306 Correo 22, Santiago, Chile

V. F. Laurie
School of Agricultural Sciences, Universidad de Talca, 2 Norte 685, Talca, Chile

J. R. Pérez-Correa · E. Agosin (✉)
ASIS-UC Interdisciplinary Research Program on Tasty, Safe and Healthy Foods, Pontificia Universidad Católica de Chile, Casilla 306 Correo 22, Santiago, Chile
e-mail: agosin@ing.puc.cl

Introduction

Discrete oxygen addition during alcoholic fermentation is a common practice in most wineries, as it promotes yeast biomass synthesis and contributes to a sound fermentation (Fornairon-Bonnefond et al. 2003; Rosenfeld et al. 2003). Several studies have shown that the risk of stuck and sluggish fermentations is reduced after oxygen additions of 10 to 20 mg/L (Rosenfeld et al. 2004; Sablayrolles and Barre 1986), particularly when performed at the end of the yeast growth phase (Sablayrolles et al. 1996). Nevertheless, oxygen can also be detrimental when added in excess, enhancing wine oxidation, color degradation, and the synthesis of off-flavors (Salmon 2006). In spite of its importance, oxygen additions during wine fermentation are typically carried out heuristically through pump-over operations, which contribution to oxygen dissolution has not been appropriately characterized so far.

During red wine production, the fermenting must is pumped from the bottom of the tank and over onto the cap (i.e., pump-overs). The aims of pump-over operations are the following: incorporate of oxygen into the must, favoring the extraction of color and flavor compounds from skin and seeds, to avoid the spoilage of the must by keeping the cap in contact with the liquid, and to remove metabolic heat formed during fermentation (Boulton et al. 1996).

Published online: 25 January 2014

Depending on the desired level of air exposure of the must, the pump-over operations are implemented either as closed, open, or with an in-line Venturi. In closed pump-overs, the fermenting must is pumped out from the bottom of the tank and re-incorporated over the cap by means of flexible or fixed tubing (i.e., with almost no air contact). In open pump-overs, the juice or wine extracted from the racking valve of the tank is splashed over a vat (or a screened vat) connected to a pump that drives the aerated juice to the top of the tank. Finally, pump-overs with an in-line Venturi valve (a recently adopted technology) are also used for the incorporation of air into the circulating must. Surprisingly, there are only isolated reports on the amount of oxygen incorporated during these common winery operations (Bisson and Butzke 2000; Bosso et al. 2009; Vidal and Aagaard 2008), especially considering that the latter impacts the resulting wine quality, as it hinders oxygen addition management during the winemaking process.

The rate of dissolution within the liquid phase depends on its equilibrium concentration, which relates with the liquid temperature and composition, the amount of solids, and the mixing provided by the bubbles of CO_2 produced by the yeast cells (Saa et al. 2012; Singleton 1987). CO_2 has been shown to play opposite roles on the oxygen dissolution in wine fermentations. On one hand, it reduces the oxygen dissolution rate due to the dilution effect (Devatine et al. 2007; Saa et al. 2012, 2013), but on the other hand, CO_2 bubbling increases this rate due to enhanced mixing during the exponential growth phase (Garcia et al. 1994; Vlassides and Block 2000). Overall, in small fermentation tanks, CO_2 reduces the oxygen dissolution rate. Nevertheless, its impact at a larger scale remains to be assessed.

To date, several studies have characterized the oxygen concentration and distribution in pilot and industrial-scale winemaking during micro-oxygenation and aging (Adoua et al. 2010; Laurie et al. 2008; Nevares et al. 2008, 2010); however, to the best of our knowledge, the fate of the oxygen added through pump-overs during wine fermentations in industrial tanks has not been reported yet. Such studies should be essential to decide, on a quantitative basis, when and how much oxygen should be added.

In this work, the oxygen incorporation and dissolution during red wine fermentations were characterized at different positions within wine tanks and after the use of different pump-overs modes. In addition, oxygen dissolution and consumption evolution during wine maceration and fermentation was evaluated.

Materials and Methods

Wine Fermentations Incorporation and dissolution of oxygen was measured during pump-overs accomplished in several red wine fermentations. For this study, data from 41 commercial red wine fermentations carried out during 2009, 2010, and 2013 was employed. The studied fermentations comprise eight different wine varieties harvested in Chile: Cabernet Sauvignon, Carménère, Merlot, Petit verdot, Pinot noir, Cabernet franc, Carignan, and Alicante Henri Bouschet (Table 1). The wine varieties tested in each experiment were selected depending on their availability at the different wineries.

Oxygen Incorporation Due to Different Pump-Over Modes The amounts of oxygen incorporated by three types of pump-overs, either closed, open, or with an in-line Venturi injector (Mazzei® Injector Company, LLC, Bakersfield, USA) (Fig. 1a), were determined in-line using optical mini planar oxygen sensors (PSt3, PreSens®, Regensburg, Germany), glued to the inside of a sight glass fitting (stainless steel tubing with a glass section) with food-grade silicone. The oxygen level of the must could be followed from the outside by means of an oxoluminicense-based dissolved oxygen meter 3 LCD-trace Fibox v7 (measuring span of 0–20 mg/L and 15 μg/L of O_2 detection limit) (PreSens®, Regensburg, Germany). This optical sensor does not show the problems of the Clark's electrode, e.g., relatively long response times and oxygen consumption during the measurement (Fernández-Sánchez et al. 2007), and it is ideal for noninvasive measurements. Oxygen incorporation was calculated as the mean difference between the dissolved oxygen concentrations of the stream of must leaving and returning to the tank (measured after the point in which oxygen incorporation was achieved, i.e., vat or Venturi) in each trial. Oxygen concentration was recorded every 1 s at the two points as previously mentioned.

Depending on the pump-over mode, the glass fittings were coupled to one of racking valves of the tanks, the exit of the vat, the inlet and outlet of the Venturi, and the exit of the impelling pump (Fig. 1a). This experimental setup allowed assessing the contribution of each device (i.e., pump, vat, Venturi) to the total oxygen incorporation in the fermenting tanks.

The number of wines studied under each pump-over mode was as follows: 6 fermentations with closed pump-overs (during the early fermentation stages), 10 with open pump-overs, and 19 with an in-line Venturi during the tumultuous and stationary fermentation stages. The varying number of trials in each case depended on the number of fermentations available at the wineries under each different mixing procedure. Seven different wine varieties were included in these experiments (Table 1). The type of pump employed was a positive displacement pump (Maxi80 Liverani®, Lugo, Italy), with an average flow rate of 15 m^3/h. Finally, the temperatures of the fermenting musts ranged between 17 and 27 °C during the trials.

Dissolved Oxygen Gradients Inside Large Industrial Wine Tanks During Open Pump-Overs The dissolved oxygen concentration within industrial wine tanks during open pump-overs was measured using PSt3 optical dipping probes

Table 1 Experimental data sets used in this work

Experiment	Wine varieties[a]	Number of pump-overs
Oxygen incorporation by different pump-over modes: closed, open, and with Venturi	Cabernet Sauvignon (13), Carménère (7), Merlot (7), Petit verdot (2), Cabernet franc (2), Carignan (2), Alicante Henri Bouschet (2)	6 closed pump-overs, 10 open pump-overs, and 19 with an in-line Venturi: 35 total
Dissolved oxygen gradients inside large industrial wine tanks during open pump-overs	Cabernet Sauvignon (1), Carménère (1)	32
Oxygen dissolution and consumption kinetics during wine maceration and fermentation	Pinot noir (2), Cabernet Sauvignon (1), Carménère (1)	76

[a] Figures in parentheses represent the number of fermentations carried out using each grape variety

(PreSens®, Regensburg, Germany), at three different heights inside a 40,000-L industrial tank. For this purpose, a 316 stainless steel tubing (2.5 cm diameter, 0.8 cm thick, and 4.3 m long) with openings in the pipeline (7 cm width and 6 cm long) at three different heights (2.1, 3.2, and 4.3 m from the top of the tank) was introduced into the center of the tank (Fig. 1b). The latter allowed to safely insert the optical oxygen and temperature probes (PT1000, PreSens®, Regensburg, Germany) at a fix position within the tube, corresponding to the opening in the pipeline.

Cabernet Sauvignon and Carménère grapes were employed in this experiment (Table 1). Cabernet Sauvignon must (harvested with 26.3 °Bx and initial must density of 1,110 g/L), inoculated with *Saccharomyces cerevisiae* yeast Zymaflore F15® (Laffort, Bordeaux, France), was first fermented, and later, the Carménère (harvested with 29 °Bx and initial must

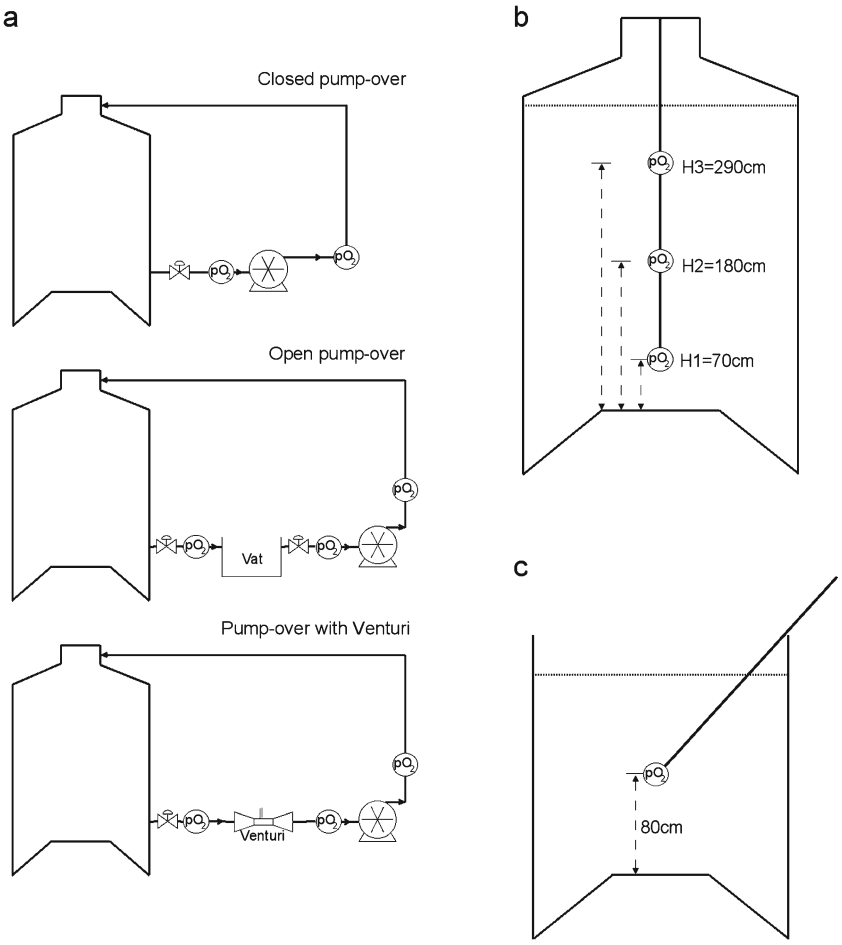

Fig. 1 Schematic experimental setups for studying oxygen incorporation and dissolution during pump-over operations in red wine industrial fermentations (pO_2 represent the points in which oxygen was measured). **a** Oxygen incorporation by different pump-over modes: closed, open, and with Venturi; **b** axial distribution of dissolved oxygen during open pump-overs at three heights in industrial wine tanks (40,000 L); **c** oxygen dissolution and consumption kinetics during winemaking in 5,000-L tanks (diagrams not to scale)

density of 1,120 g/L), inoculated with *S. cerevisiae* L2056® (Lalvin, Toulouse, France). Cabernet Sauvignon and Carménère musts were fermented at 22±1 and 24±3 °C, respectively. The type of yeast utilized in each case was chosen by the winemakers based on technical considerations and their winemaking protocols.

During the Cabernet Sauvignon fermentation, a total of 18 pump-overs were performed during the 10 days of fermentation (the first 6 days corresponded to tumultuous fermentation), while for the Carménère, 14 pump-overs were conducted during the 6 days of fermentation (the first 4 days corresponded to tumultuous fermentation). Normally, two pump-overs per day were carried out for both fermentations. In both cases, the evolution of the fermentation was followed by the decrease of must density using appropriate hydrometers (Alla France®, Chemillé, France). As a reference, fermentations were considered completed when the residual sugar content of the wine was lower than 2 g/L. The flow rate of the pump used during these experiences was approx 15 m^3/h, as in the previous experiment, and the duration of pump-overs ranged between 20 and 30 min, according to the winemaking protocol of the winery.

Oxygen Dissolution and Consumption Kinetics During Wine Maceration and Fermentation Oxygen dissolution and consumption kinetics of four red wine fermentations were followed in closed and open pump-over modes during pre-fermentative maceration and fermentation stages. The pre-fermentative maceration was performed by placing dry ice (solid CO_2) in the surface of the cap during the first days of the treatment, and adding 35 and 20 mg/L of SO_2 during the first and the second day of maceration, respectively. Again, fermentation evolution was periodically checked by the decrease of must density using appropriate hydrometers (Alla France®, Chemillé, France). The must density measurements were employed as an indicator of the fermentation evolution, which enabled the subsequent analysis of dissolved oxygen content during the different phases of wine fermentation.

Measurements were taken in two 5,000-L open stainless steel tanks (Fig. 1c). First, the fermentation of two Pinot noir wines (harvested with 24.2 °Bx and initial must density of 1,107 g/L) from Casablanca valley was followed, and later, one Carménère and one Cabernet Sauvignon from the same valley (harvested with 23.9 and 24.2 °Bx and initial must densities of 1,104 and 1,106 g/L, respectively) (Table 1). All these fermentations were carried out with native yeasts, i.e., without inoculation of commercial wine yeast strains. Pinot noir, Cabernet Sauvignon, and Carménère musts were fermented at 22±2.6, 25±2.6, and 25±1.4 °C, respectively. SO_2 concentrations were also traced during the maceration and fermentation processes, employing the reference Ripper method (Buechsenstein and Ough 1978).

Dissolved oxygen concentration was measured inside the tanks at 1.3 m from the top (approx the geometric center of the tank), by placing a 316 stainless steel tubing (2.5 cm diameter and 0.8 cm thick) containing both the optical oxygen (PreSens®, Regensburg, Germany) and temperature probes (PT1000, PreSens®, Regensburg, Germany) as previously explained. A total of 18 pump-overs were performed for each Pinot noir (6 in maceration and 12 fermentation), while in the case of Cabernet Sauvignon and Carménère fermentations, 22 (6 in maceration and 16 fermentation) and 18 (4 in maceration and 14 fermentation) pump-overs were conducted, respectively (usually two pump-overs per day during the fermentation). The flow rate employed during the pump-overs was approximately 5 m^3/h and they lasted approximately 10 min, according to the winemaking protocol of the winery.

Kinetics of Oxygen Consumption During Pump-Overs Oxygen consumption kinetics during industrial wine fermentations was described using the following mass balance equation (Silva and Lambri 2006),

$$\frac{dO_2}{dt} = -K_{global} \cdot O_2 \tag{1}$$

where K_{global} represents a kinetic constant which includes the biological, enzymatic, and chemical consumption. The latter expression can be analytically solved as follows:

$$O_2(t) = O_2(0) \cdot \exp\left(-K_{global} \cdot t\right) \tag{2}$$

where $O_2(0)$ represents the initial oxygen concentration.

Regression and Statistics Estimated consumption parameters were fitted by minimizing the sum of squared residual errors between predicted and experimental dissolved oxygen data,

$$\underset{K_{global}}{\text{Min}} \sum_{i=1}^{N} \left(O_2^{model} - O_2^{meas}\right)^2 \tag{3}$$

where O_2^{model} represents predicted dissolved oxygen concentration, O_2^{meas} denotes the measured dissolved oxygen concentration, K_{global} is the consumption kinetic constant, and N represents the number of measurements. We also developed an empirical correlation between the oxygen dissolution rate ($\Delta O_2/\Delta t$) and the must's density (ρ), in which case the estimated parameters were fitted using the same strategy (minimizing the sum of squared residual errors between the model and experimental data). In both cases, regressions were carried out using the nonlinear optimization routine *fminsearch* of MATLAB®. To assess the confidence of the estimated

parameters after the regressions, the respective 95 % confidence interval was calculated employing the MATLAB® functions *nlparci* and *nlpredci*. Finally, to compare the different treatments or group means, Student's *t* test and one-way ANOVA analysis were employed (depending if two or more groups were compared), to determine whether the observed by differences were statistically significant with 95 and 99 % confidence levels. The MATLAB® Statistics Toolbox was used for these analyses.

Results and Discussion

Oxygen Incorporation by Different Pump-Over Modes The amount of oxygen incorporated using the different pump-over modes (closed, open, and with Venturi) was assessed by calculating the mean of the difference between the dissolved oxygen concentrations of the stream of must leaving and returning to the tank. According to the pump-over mode, different amounts of oxygen were incorporated into the fermenting must (Table 2). On average, closed, open, and pump-overs with Venturi added 0.05±0.02, 1.4±0.52, and 3.0±1.3 mg/L of oxygen, respectively (mean plus 1 standard deviation). One-way ANOVA test at 99 % confidence level yielded a p value <0.01 ($5.7 \cdot 10^{-6}$) which statistically support the observed difference upon the different modes of operation. Thus, closed pump-overs incorporate, in average, almost no oxygen, as would be expected due to the limited contact with air. In fact, this operation mode is mainly used for must circulation and homogenization purposes (Boulton et al. 1996). On the other hand, open pump-overs with Venturi were the most efficient configuration for oxygen addition, incorporating twice more oxygen than the traditional open one (t test, p value <0.05).

When it comes to comparing the oxygen incorporation during the fermentation of different red wine varieties (Fig. 2), no statistical differences were obtained at each operation mode (One-way ANOVA, p value <0.05). This trend is conserved among the grape varieties studied, suggesting that this tendency might be valid for most, if not all, red grape varieties. The latter is relevant, as it suggests that the pump-over mode must be chosen fundamentally on the oxygen dose to be added, independent of the grape variety to ferment.

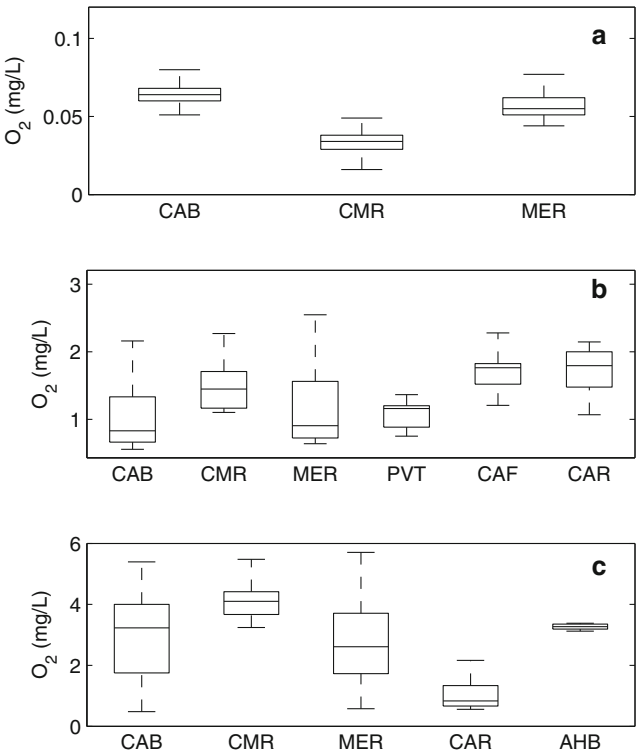

Fig. 2 Boxplots of oxygen incorporation into industrial red wine fermentations by different types of pump-overs. **a** Closed pump-over; **b** conventional open pump-over; **c** open pump-over with Venturi. The abbreviations for grape varieties are: Cabernet Sauvignon (*CAB*), Carménère (*CMR*), Merlot (*MER*), Petit verdot (*PVT*), Cabernet franc (*CAF*), Carignan (*CAR*), and Alicante Henri Bouschet (*AHB*)

The partial contribution of each pump-over apparatus to the concentration of dissolved oxygen was as follows: in open pump-overs, the vat and the pump contribute similar amounts (48.6±9.8 % for the vat, and 51.4±29.5 % for the pump; equivalent to approximately 0.7 mg/L of dissolved oxygen each). For pump-overs with Venturi, the estimated contributions of the Venturi and the pump were 20.1±9.8 and 79.9±

Table 2 Oxygen incorporated in fermenting grape musts using different pump-over modes

Pump-over mode	Min (mg/L)	Max (mg/L)	Average (mg/L)	Standard deviation (mg/L)	Coefficient of variation (%)
Closed	0.02	0.11	0.05	$1.7 \cdot 10^{-2}$	38.4
Open	0.6	2.5	1.36	0.52	35.4
With Venturi	0.5	5.6	3.03	1.38	43
ANOVA	Sum of squares	Degrees of freedom	Mean square	F	p value
Between groups	41.3	2	20.7	18.3	$5.7 \cdot 10^{-6}$
Within groups	35	31	1.13		
Total	76.3	33			

14.9 % respectively, equivalent to 0.6±0.4 and 2.5±0.4 mg/L of dissolved oxygen. These findings seem contradictory at first sight, as we previously determined that closed pump-overs, incorporated almost no oxygen (approx 0.05 mg/L). However, it has to be considered that the oxygen is dissolved in the liquid phase in two steps. First, the inlet stream (oxygen-poor) comes in contact with the surrounding air, incorporating oxygen in the form of bubbles. Then, the oxygen from the air bubbles is transferred through the gas–liquid interface into the liquid phase, where it dissolves. The rate upon which the oxygen gas is transferred to the liquid phase depends mainly on the hydrodynamic condition, among other factors (Gagnon et al. 1998). At this point, the pump plays a key role dissolving the air bubbles. When the liquid stream approaches the pump, the liquid turbulence is greatly enhanced, which improves the oxygen mass transfer by increasing the liquid velocity and the breakup of gas bubbles (Wang and Zhong 1996a, b). The latter can be used to explain the differences in the dissolved oxygen contributions of the vat, Venturi, and pump in the different studied configurations. In conventional open pump-overs, the vat incorporates more oxygen bubbles than the Venturi, as the fermenting must is exposed to air for a longer period of time. On the other hand, the Venturi injector dissolves more oxygen than the vat, which explains the higher dissolved oxygen concentration of the leaving stream. Overall, the open pump-over with Venturi is more efficient at dissolving oxygen due to the combined effect of the Venturi injector and the pump.

Finally, another relevant factor during the operation of the different types of pump overs was the variability in the amount of oxygen dissolved in the must. Indeed, it is not only important the average amount of oxygen added, but also its reproducibility in repeated trials. As shown in Table 2, oxygen additions with pump-overs are, in general, heterogeneous. The latter strengthens the importance of characterizing these modes of operation, to estimate, at least roughly, the amount of oxygen expected to be added in each operation.

Dissolved Oxygen Gradients Inside Large Industrial Wine Tanks During Open Pump-Overs We found significant differences in the oxygen content of fermenting musts between the top and the bottom of 40,000-L tanks following a pump-over (Fig. 3). Most of the oxygen added through pump-overs (approx 80 %) reaches only the upper portion of the must within the wine tank (2.9 m from the bottom of the tank). The other two points (heights) measured at 0.7 and 1.8 m from the bottom of the tank, especially the lowest, received almost no oxygen during the course of the fermentation (<1 %). The latter can be explained by the form in which open-pump overs are performed (i.e., must exposed to air is added to the top) and the mixing regime present in wine fermentations. García et al. (1993) reported important gradients in pH, dry weight, sugars, and ethanol concentrations, among others between

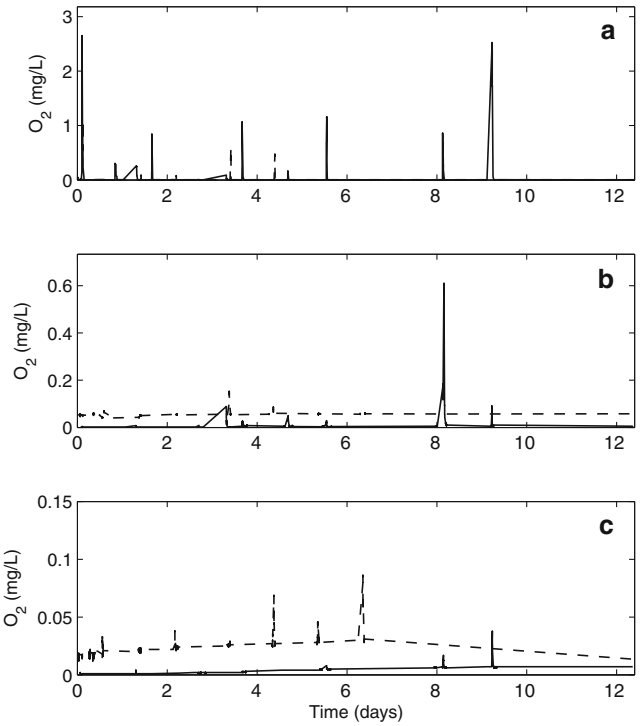

Fig. 3 Dissolved oxygen (DO) levels inside 40,000-L industrial wine tanks during pump-overs in fermentations of Cabernet Sauvignon (*solid lines*) and Carménère musts (*dashed lines*). **a**–**c** Panels show DO at the top, middle, and bottom of the tanks, respectively

different levels of unagitated beer fermentations at pilot scale, inoculated at the top of the reactor. According to the authors, these results are due to the presence of poor mixing in the downwards direction, which is a direct result of the liquid recirculation patterns owed to the rise of CO_2 bubbles produced by the yeasts. Furthermore, it has been reported that during the high-CO_2 production stages (exponential growth phase), cell distribution is uniform in unagitated wine fermentors (Vlassides and Block 2000), which discards any major influence of the yeast cells distribution in the observed dissolved oxygen distribution. Thus, the latter suggests that the mixing patterns resulting from the gaseous CO_2 bubbling are the main factor responsible of the observed dissolved oxygen distribution. This has been also observed in a recent work (Saa et al. 2013), where the CO_2 was shown to play a major role on the oxygen dissolution profile observed in an experimental bubble column, mimicking enological CO_2 generation.

Therefore, the efficiency of oxygen addition by pump-overs inside large wine tanks is limited. The presence of large oxygen gradients during pump-overs indicate that tank zones will be exposed longer to different dissolved oxygen concentrations. The latter could impact the fermentation evolution, as the yeast cells will be exposed to different amounts of oxygen during this process. Considering that wine yeasts require determined amounts of oxygen for successfully completing the fermentation (Sablayrolles and Barre 1986), it is important to take into account this large heterogeneity in dissolved

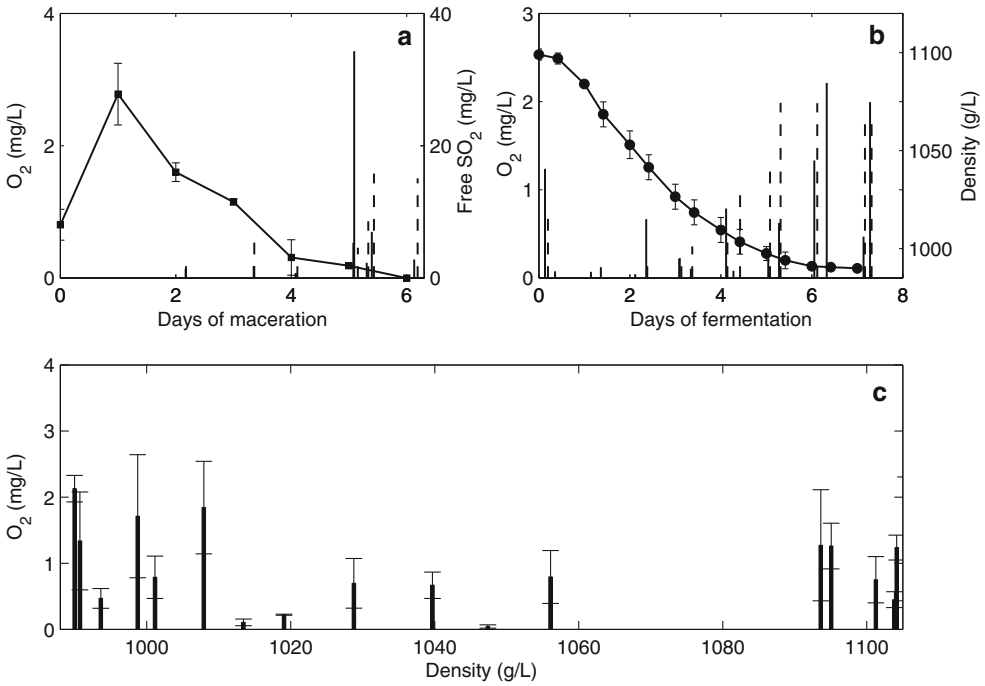

Fig. 4 Dissolved oxygen (DO) concentrations during maceration and fermentation of two Pinot noir. **a** Evolution of DO (*solid* and *dashed lines*) and the free SO_2 concentration (*filled squares*) during the maceration process; **b** evolution of DO (*solid* and *dashed lines*) during alcoholic fermentation. The fermentation evolution is illustrated by the decrease of must's density (*filled circles*); **c** DO levels reached during open pump-overs in Pinot noir fermentations as a function of must densities

oxygen distribution when performing pump-overs at industrial scale.

Finally, there is a notorious difference in the dissolved oxygen concentrations reached in the top of the tank for the Cabernet Sauvignon and Carménère fermentations (Fig. 3a). For Cabernet Sauvignon, the highest dissolved oxygen concentration reached was 2.6 mg/L, while for Carménère, only 0.6 mg/L was achieved. The latter can result from the high dependency of this process on external features not fully controllable, such as operators, oxygen dissolution in the vat, and time of exposure, among others. In fact, just in terms of oxygen incorporation, this operation possesses a variability of around 35 % (Table 2) which, combined with external factors and the high working volumes, might be responsible for the large difference observed.

Oxygen Dissolution and Consumption Kinetics During Wine Maceration and Fermentation The evolution of dissolved oxygen of grape musts from two commercial Pinot noir, a Cabernet Sauvignon, and a Carménère was followed during the maceration and fermentation processes. Figure 4 illustrates the dissolved oxygen evolution in Pinot noir musts.

Low oxygen dissolution was observed during the maceration process (Fig. 4a). In musts, the main form of oxidation that takes place is enzymatic oxidation, which occurs at a much faster rate than chemical oxidation (Dubernet and Ribéreau-Gayon 1973, 1974). This oxidation is conducted by polyphenol oxydases (PPO), using phenolic compounds (e.g., hydroxycinnamic acids, trans-caftaric, and coutaric acid, among others) and oxygen as substrates (Macheix et al. 1991). Previously, it has been reported that SO_2 can inhibit PPO activity by 75 to 90 % when 50 mg/L SO_2 are added to musts (Dubernet and Ribéreau-Gayon 1974), which seems to disagree with the observed data (Fig. 4a). Indeed, lower oxygen levels were observed when SO_2 concentration was at its highest (beginning of the maceration). This contradictory result might be explained due to the "protective" effect of CO_2 against oxygen dissolution during the first days of the maceration. Devatine et al. (2011) have demonstrated that complete protection of wines and musts is obtained when CO_2 content is high. Moreover, the authors showed that micro-oxygenation is totally inefficient in the presence of initial high CO_2 concentrations, even if there is no production of CO_2. At this point, we speculate that because of the high additions of CO_2 during the first days of maceration, oxygen dissolution is very ineffective during this period (approx until the second day, Fig. 4a). In fact, no oxygen dissolution is observed during the first 2 days of the maceration, despite the pump-overs carried out. Then, as the CO_2 escapes from the liquid to the gas phase due to the successive pump-overs and the SO_2 levels decrease, oxygen is able to dissolve in the must and to be consumed by enzymatic oxidation reactions. The latter is observed after the second day, in which oxygen dissolves into the must and is quickly consumed (Fig. 4a). Indeed, this hypothesis has to be properly validated and is by no means definitive; however it might explain the lower oxygen levels encountered at the beginning of the maceration. Finally, in terms of the dissolved oxygen evolution, overall heterogeneous levels were achieved during this stage in the different fermentations. Dissolved oxygen concentrations obtained during pump-overs ranged between 0.2 and 3.6 mg/L, which reinforces the high variability of the open pump-over processes.

During the enological fermentation, the dissolved oxygen concentration was also very variable. Dissolved oxygen levels for Pinot noir ranged between 0.02 and 1.0 mg/L (Fig. 4b), while for Cabernet Sauvignon and Carménère, oxygen values varied between 0.03 and 2.1 mg/L (data not shown). Nevertheless, it is noteworthy that for all four fermentations, higher dissolved oxygen concentrations were observed at the end of the fermentation, as indicated by the density plateau reached. This correlates well with the low yeasts activity, the major contributor of oxygen consumption during alcoholic fermentation (Salmon 2006). Figure 4c shows the dissolved oxygen levels reached in all the fermentations as a function of the must's density. Dissolved oxygen concentrations achieved are generally higher at the onset and at the offset of the alcoholic fermentation, i.e., when yeast concentration and activity are at their lowest, respectively. Therefore, must density might be regarded as a useful winemaking indicator of the capacity of the fermenting must to dissolve oxygen (see below).

Kinetics of Oxygen Dissolution and Consumption During Alcoholic Fermentations During the application of open pump-overs, the dissolved oxygen response followed the kinetics illustrated in Fig. 5a. Similar response curves were recently reported for unagitated alcoholic fermentations under enological conditions, on a laboratory scale (Saa et al. 2012). As shown in this figure, the first part of the response curve corresponds to the oxygen addition, in which the dissolved oxygen concentration increases as the pump-over advances. Then, when the pump-over is finished, the dissolved oxygen concentration decreases as the yeast cells consume the available oxygen. There are also other sinks for oxygen, such as chemical consumption and physical desorption, which are, however, less important than the yeast activity during alcoholic fermentation (Aceituno et al. 2012; Saa et al. 2012; Salmon 2006).

To better understand the dissolved oxygen dissolution and consumption kinetics, we examined both parts of the curve in detail for all the fermentations (Pinot noir, Cabernet Sauvignon, and Carménère). First, we computed the average oxygen dissolution rate ($\Delta O_2/\Delta t$) for all the pump-overs analyzed from the addition curve (Fig. 5a). This parameter was calculated by fitting a linear curve from the moment of the start of the oxygen addition until the end of the pump over (beginning of oxygen consumption). The slope of the fitted curve represents the oxygen dissolution rate and represents the average amount of oxygen that dissolves per unit of volume and time.

The main advantages of this parameter are that it does not solely rely on the particular oxygen concentration level determined at a particular point during the pump-overs (as shown before, pump-overs are quite variable in terms of oxygen incorporation), but it rather depends on the rate of change of the oxygen concentration, and secondly, it is very useful for

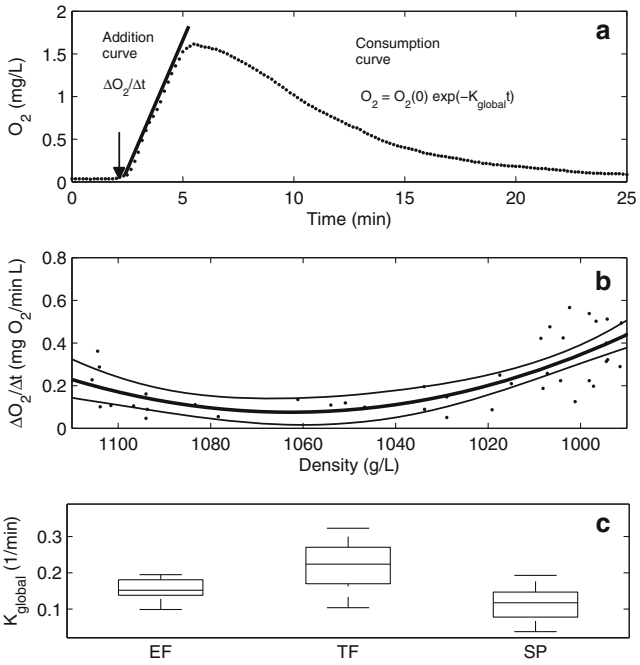

Fig. 5 Oxygen dissolution and consumption kinetics during alcoholic fermentation. **a** A typical dissolution curve occurring during an open pump-over in a 5,000-L wine tank during alcoholic fermentation (*black dots*). The *arrow* indicates the pump-over start and the onset of oxygen dissolution. The first part of the curve corresponds to the oxygen addition, in which the oxygen dissolution rate ($\Delta O_2/\Delta t$) can be estimated as the slope of the curve, while the second part describes the global oxygen consumption by the fermenting must which is modeled using Eq. (3); **b** oxygen dissolution rate as a function of must's density in Pinot noir, Cabernet Sauvignon, and Carménère wines. The *thicker line* represents a parabolic relation of the form $\Delta O_2/\Delta t = a \cdot \rho^2 + b \cdot \rho + c$ between both variables (R=0.74), while the *thinner lines* denote the 95 % confidence interval of the trend of the fitted values (*black dots*); **c** oxygen consumption constant for different fermentation stages: early fermentation (EF), tumultuous fermentation (TF), and stationary phase (SP)

managing oxygen doses as it indicates the amount of dissolved O_2 per unit of volume and time in a particular stage of the fermentation. A parabolic relationship between the must's density and the oxygen dissolution rate of the form $\Delta O_2/\Delta t = a \cdot \rho^2 + b \cdot \rho + c$ ($a = 7.76 \cdot 10^{-5} \pm 3.37 \cdot 10^{-5}$, $b = -1.65 \cdot 10^{-1} \pm 7.07 \cdot 10^{-2}$, $c = 87.5 \cdot 10 \pm 3.7 \cdot 10$, R=0.74) was found (Fig. 5b). At the fermentation's onset and offset (around 1,100 and 990 g/L density, respectively), the oxygen dissolution rates are higher than during the tumultuous fermentation phase (between 1,090 and 1,020 g/L densities). The latter could be explained by the higher yeast activity and CO_2 production rates, typical of this phase (Casalta et al. 2010).

During tumultuous fermentation, yeast cells require oxygen to synthetize essential membrane components such as ergosterol, which allow them to thrive the harsh conditions of alcoholic fermentations (Fornairon-Bonnefond et al. 2002, 2003; Rosenfeld et al. 2002). This high demand can be illustrated by the higher consumption constants (K_{global}) fitted to the consumption curves during this fermentation phase (Fig. 5c, TF). Nevertheless, this sole factor is not the only one

responsible for the lower oxygen dissolution rate observed. The other key factor that impacts oxygen dissolution is the CO_2 production (Chiciuc et al. 2010; Devatine et al. 2007, 2011; Devatine and Mietton-Peuchot 2009; Saa et al. 2013). Saa et al. (2013) studied in a laboratory column the oxygen dissolution rate in water under CO_2 bubbling conditions similar to the ones encountered during the tumultuous phase of alcoholic fermentations. Under these conditions, the authors reported an average oxygen dissolution rate of approximately 0.3 mg O_2/(L·min), which is similar to the one estimated in this work under real conditions during the tumultuous phase—around 0.2 mg O_2/(L·min). The negative impact of the CO_2 production is related to its high concentration, and not to its specific production rate, i.e., stripping (Saa et al. 2012), as it favors the dilution of the dissolved oxygen added in the gas phase upon its transfer to the liquid phase (Devatine et al. 2011; Devatine and Mietton-Peuchot 2009; Saa et al. 2013). As previously mentioned, CO_2 acts as a resistance to oxygen dissolution and not as a "physical sink" of consumption (Devatine et al. 2011). Combining the negative impact of CO_2 on the oxygen dissolution and the high biological uptake by the yeast cells during the exponential growth phase, one might be able to explain the significant contrast in the oxygen dissolution rates among the different fermentation phases.

Finally, the dissolved oxygen consumption during the different phases of the fermentation—early fermentation (EF), tumultuous fermentation (TF), and stationary phase (SP)—were also evaluated (Fig. 5c). Significant differences in the consumption constants for the different fermentation stages were determined (one-way ANOVA analysis; p value <0.01). On average, during the EF, TF, and SP, the oxygen consumption constants were 0.17±0.08, 0.22±0.06, and 0.12±0.06 mg O_2/(L·min), respectively. These results agree with the evolution of the yeast's activity during the alcoholic fermentation. The differences between the EF and SP values could be explained by the free SO_2 remaining from the maceration process (Fig. 4a, b). These results suggest that the main source of oxygen consumption during alcoholic fermentation is the yeast metabolic activity.

Conclusion

The present work comprises an integral study aiming to measure and better understand oxygen incorporation and dissolution through pump-overs during winemaking, at industrial scale. Regarding the operation mode of pump-overs, those with Venturi injectors incorporate approximately twice more oxygen than open pump-overs. Particular analysis of the contributions of the vat, the injector, and the pump to the oxygen dissolution during open pump-overs and with Venturi suggests that the pump plays a key role in dissolving the oxygen incorporated by the former. Closed pump-overs, as expected, incorporated negligible amounts of oxygen, which make them ideal for homogenization purposes without oxygenating the must. In large wine tanks, a highly heterogeneous distribution of dissolved oxygen after pump-over was determined. We hypothesize that this results mainly from the yeast CO_2 bubbling during the fermentation, which generates poor mixing in the downwards direction, although this explanation should be validated under appropriate conditions. Finally, when analyzing the course of oxygen dissolution and consumption, several findings stand out. During the maceration process, initial low levels of dissolved oxygen were found despite the presence of high concentrations of free SO_2, which is an effective oxidase inhibitor. We speculate that the latter is due to the high initial CO_2 content, which prevents oxygen dissolution during this period. Then, as the CO_2 escapes from the liquid and the SO_2 levels decrease, oxygen is able to dissolve in the must and to be consumed through enzymatic oxidation reactions. During the alcoholic fermentation, higher dissolved oxygen concentrations were observed at the end of the fermentation, which agrees well with the lower yeast activity in this stage. The kinetics of dissolved oxygen observed during this stage strongly suggests that both, the negative impact of CO_2 on the oxygen dissolution and the high biological uptake by the yeasts during the exponential growth phase, are the main variables responsible for the low dissolved oxygen levels achieved during the tumultuous fermentation phase. Overall, the present work will help improve the management of oxygen additions through pump-overs during winemaking at industrial scale.

Acknowledgments We are grateful to Felipe Ibáñez from Viña Carmen, and to Rodrigo Soto and Sofía Araya from Viña Veramonte, who allowed us to carry out these studies in the cited wineries. We are particularly grateful to the reviewers for their thorough and constructive comments that contributed significantly to improve the final manuscript.

Funding This work was carried out with the support of FONDECYT Project No. 1090520 and FONDEF grant no. D11I1139. M. Isabel Moenne was supported by a doctoral fellowship from the Chilean National Council of Scientific and Technological Research (CONICYT).

References

Aceituno, F. F., Orellana, M., Torres, J., Mendoza, S., Slater, A. W., Melo, F., & Agosin, E. (2012). Oxygen response of the wine yeast *Saccharomyces cerevisiae* EC1118 grown under carbon-sufficient, nitrogen-limited enological conditions. *Applied and Environmental Microbiology, 78*(23), 8340–52.

Adoua, R., Mietton-Peuchot, M., & Milisic, V. (2010). Modelling of oxygen transfer in wines. *Chemical Engineering Science, 65*(20), 5455–5463.

Bisson, L., & Butzke, C. (2000). Diagnosis and rectification of stuck and sluggish fermentation. *American Journal of Enology and Viticulture, 51*(2), 168–177.

Bosso, A., Guaita, M., Panero, L., Borsa, D., & Follis, R. (2009). Influence of two winemaking techniques on polyphenolic composition and color of wines. *American Journal of Enology and Viticulture, 60*(3), 379–385.

Boulton, R., Singleton, V., Bisson, L., & Kunkee, R. (1996). *Principles and practices of winemaking*. New York: Chapman Hall.

Buechsenstein, J. W., & Ough, C. S. (1978). SO2 Determination by aeration–oxidation: a comparison with Ripper. *American Journal of Enology and Viticulture, 29*(3), 10–13.

Casalta, E., Aguera, E., Picou, C., Rodriguez-Bencomo, J.-J., Salmon, J.-M., & Sablayrolles, J.-M. (2010). A comparison of laboratory and pilot-scale fermentations in winemaking conditions. *Applied Microbiology and Biotechnology, 87*(5), 1665–1673.

Chiciuc, I., Farines, V., Mietton-Peuchot, M., & Devatine, A. (2010). Effect of wine properties and operating mode upon mass transfer in micro-oxygenation. *International Journal of Food Engineering, 6*(6).

Devatine, A., & Mietton-Peuchot, M. (2009). A mathematical approach for oxygenation using micro bubbles: application to the micro-oxygenation of wine. *Chemical Engineering Science, 64*(9), 1909–1917.

Devatine, A., Chiciuc, I., Poupot, C., & Mietton-Peuchot, M. (2007). Micro-oxygenation of wine in presence of dissolved carbon dioxide. *Chemical Engineering Science, 62*(17), 4579–4588.

Devatine, A., Chiciuc, I., & Mietton-Peuchot, M. (2011). The protective role of dissolved carbon dioxide against wine oxidation: a simple and rational approach. *Journal International des Sciences de la Vigne et du vin, 45*(3), 189–197.

Dubernet, M., & Ribéreau-Gayon, P. (1973). Presence et significance dans les mouts et les vins de la tyrosinase du raisin. *Connaissance Vigne Vin, 7*, 283–302.

Dubernet, M., & Ribéreau-Gayon, P. (1974). Causes et consequences de la consummation de l'oxygene par les mouts des raisin. *Vitis, 13*, 233–244.

Fernández-Sánchez, J. F., Roth, T., Cannas, R., Nazeeruddin, M. K., Spichiger, S., Graetzel, M., & Spichiger-Keller, U. E. (2007). Novel oxygen sensitive complexes for optical oxygen sensing. *Talanta, 71*(1), 242–250.

Fornairon-Bonnefond, C., Demaretz, V., Rosenfeld, E., & Salmon, J. (2002). Oxygen addition and sterol synthesis in *Saccharomyces cerevisiae* during enological fermentation. *Journal of Bioscience and Bioengineering, 93*(2), 176–182.

Fornairon-Bonnefond, C., Aguera, E., Deytieux, C., Sablayrolles, J.-M., & Salmon, J.-M. (2003). Impact of oxygen addition during enological fermentation on sterol contents in yeast lees and their reactivity towards oxygen. *Journal of Bioscience and Bioengineering, 95*(5), 496–503.

Gagnon, H., Lounes, M., & Thibault, J. (1998). Power consumption and mass transfer in agitated gas–liquid columns: a comparative study. *The Canadian Journal of Chemical Engineering, 76*, 379–389.

Garcia, A. I., Garcia, L. A., & Diaz, M. (1993). Mixing in unstirred batch fermenters. *Chemical Engineering Journal, 51*(3), B57–B61.

Garcia, A. I., Pandiella, S. S., Garcia, L. A., & Diaz, M. (1994). Mechanism for mixing and homogenization in beer fermentation. *Bioprocess Engineering, 10*(4), 179–184.

Laurie, V. F., Law, R., Joslin, W. S., & Waterhouse, A. L. (2008). In situ measurements of dissolved oxygen during low-level oxygenation in red wines. *American Journal of Enology and Viticulture, 59*(2), 215–219.

Macheix, J., Sapis, J., Fleuriet, A., & Lee, C. (1991). Phenolic compounds and polyphenoloxidase in relation to browning in grapes and wines. *Critical Reviews in Food Science and Nutrition, 30*(4), 441–486.

Nevares, I., Del Alamo, M., Cárcel, L. M., Crespo, R., Martin, C., & Gallego, L. (2008). Measure the dissolved oxygen consumed by red wines in aging tanks. *Food and Bioprocess Technology, 2*(3), 328–336.

Nevares, I., del Alamo, M., & Gonzalez-Muñoz, C. (2010). Dissolved oxygen distribution during micro-oxygenation. Determination of representative measurement points in hydroalcoholic solution and wines. *Analytica Chimica acta, 660*, 232–239.

Rosenfeld, E., Beauvoit, B., Rigoulet, M., & Salmon, J.-M. (2002). Non-respiratory oxygen consumption pathways in anaerobically-grown *Saccharomyces cerevisiae*: evidence and partial characterization. *Yeast, 19*(15), 1299–1321.

Rosenfeld, E., Beauvoit, B., Blondin, B., & Salmon, J. (2003). Oxygen consumption by anaerobic *Saccharomyces cerevisiae* under enological conditions: effect on fermentation kinetics. *Applied and Environmental Microbiology, 69*(1), 113–121.

Rosenfeld, E., Schaeffer, J., Beauvoit, B., & Salmon, J.-M. (2004). Isolation and properties of promitochondria from anaerobic stationary-phase yeast cells. *Antonie van Leeuwenhoek, 85*, 9–21.

Saa, P. A., Moenne, M. I., Pérez-Correa, J. R., & Agosin, E. (2012). Modeling oxygen dissolution and biological uptake during pulse oxygen additions in oenological fermentations. *Bioprocess and Biosystems Engineering, 35*(7), 1167–1178.

Saa, P. A., Perez-Correa, J. R., Celentano, D., & Agosin, E. (2013). Impact of carbon dioxide injection on oxygen dissolution rate during oxygen additions in a bubble column. *Chemical Engineering Journal, 232*, 157–166.

Sablayrolles, J., & Barre, P. (1986). Evaluation des besoins en oxygene de fermentations alcooliques en conditions oenologiques simulees. *Sciences des Aliments, 6*, 373–383.

Sablayrolles, J., Dubois, C., Manginot, C., Roustan, J.-L., & Barre, P. (1996). Effectiveness of combined ammoniacal nitrogen and oxygen additions for completion of sluggish and stuck wine fermentations. *Journal of Fermentation and Bioengineering, 82*(4), 377–381.

Salmon, J. (2006). Interactions between yeast, oxygen and polyphenols during alcoholic fermentations: practical implications. *LWT - Food Science and Technology, 39*(9), 959–965.

Silva, A., & Lambri, M. (2006). Oxygen measures and consumption in must and wine. *Analytica Chimica Acta, 563*, 391–395.

Singleton, V. L. (1987). Oxygen with phenols and related reactions in musts, wines and model systems: observations and practical implications. *American Journal of Enology and Viticulture, 38*(1), 69–77.

Vidal, B. S., & Aagaard, O. (2008). Oxygen management during vinifi cation and storage of Shiraz wine. *The Australian and New Zealand Wine Inudstry Journal, 23*, 56–63.

Vlassides, S., & Block, D. (2000). Evaluation of cell concentration profiles and mixing in unagitated wine fermentors. *American Journal of Enology and Viticulture, 51*(1), 73–80.

Wang, S. J., & Zhong, J. J. (1996a). A novel centrifugal impeller bioreactor. II. Oxygen transfer and power consumption. *Biotechnology and Bioengineering, 51*(5), 520–527.

Wang, S. J., & Zhong, J. J. (1996b). A novel centrifugal impeller bioreactor. I. Fluid circulation, mixing, and liquid velocity profiles. *Biotechnology and Bioengineering, 51*(5), 511–519.