On-line monitoring of oxygen as a method to qualify the oxygen consumption rate of wines

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ARTICLE INFO

ABSTRACT

Measuring the oxygen content during winemaking and bottle storage has become increasingly popular due to its impact on the sensory quality and longevity of wines. Nevertheless, only a few attempts to describe the kinetics of oxygen consumption based on the chemical composition of wines have been published. Therefore, this study proposes firstly a new fitting approach describing oxygen consuming kinetics and secondly the use of an Artificial Neural Network approach to describe and compare the oxygen avidity of wines according to their basic chemical composition (i.e. the content of ethanol, titratable acidity, total sulfur dioxide, total phenolics, iron and copper). The results showed no significant differences in the oxygen consumption rate between white and red wines, and allowed the sorting of the wines studied according to their oxygen consumption rate.

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1. Introduction

Oxygen is ever-present during all phases of the winemaking process (Calderón, del Alamo-Sanza, Nevares, & Laurie, 2014), contributing to important chemical changes that affect the sensorial characteristics and the aging capacity of wines (del Alamo-Sanza, Pando, & Nevares, 2014; Oliveira, Silva Ferreira, De Freitas, & Silva, 2011). Oxygen is required for a sound alcoholic fermentation, color stabilization, reduction of the astringent and bitter characters, etc. (Holm Hansen, Nissen, Sommer, Nielsen, & Arneborg, 2001; Schmidtke, Clark, & Scollary, 2011). However, an excess of oxygen could lead to microbiological spoilage and oxidation reactions that could negatively affect the wine's color, flavor and aging capacity (Escudero, Asensio, Cacho, & Ferreira, 2002; Laurie, Salazar, Campos, Cáceres-Mella, & Peña-Neira, 2014; Schmidtke et al., 2011).

The non-enzymatic oxidation process is thought to begin with the activation of oxygen by metal catalyzers, and the oxidation of phenols into quinones with the concomitant reduction of oxygen into hydrogen peroxide (H_2O_2). The resulting species can further react with other wine molecules and produce sensory changes associated with wine oxidation, such as the production of acetaldehyde (Elias, Andersen, Skibsted, & Waterhouse, 2009; Kreitman, Laurie, & Elias, 2013; Laurie et al., 2012). During this process, important chemical compounds are affected, including phenols (Danilewicz, 2003, 2007; du Toit, Marais, Pretorius, & du Toit, 2006; Kondrashov, Ševčík, Benáková, Koštířová, & Štípek, 2009; Laurie et al., 2014; Mulero, Pardo, & Zafrilla, 2010; Oliveira, Knapic, & Pereira, 2012; Vrček, Bojić, Žuntar, Mendaš, & Medić-Šarić, 2011; Zúñiga, Pérez-Roa, Olea-Azar, Laurie, & Agosin, 2014), sulfur dioxide (Danilewicz, 2003, 2007; Danilewicz, 2014; Zúñiga et al., 2014), ethanol (Danilewicz, 2014), ascorbic acid, glutathione. etc (Du Toit, Lisjak, Marais, & Du Toit, 2006)

Various authors have evaluated the influence of oxygen in wine via antioxidant capacity measurements (Kondrashov et al., 2009; Mulero et al., 2010; Vrček et al., 2011), which could serve as a rough estimate of the wine's affinity towards oxygen consumption (Mulero et al., 2010). Other studies have measured the redox potential of the wine as an indication of its oxidation state (Danilewicz, 2003; Del Alamo, Nevares, & Cárcel, 2006; Kilmartin

& Zou, 2001). In both cases, the wine matrix complexity and the errors associated have limited the chances of finding a good methodology to predict or classify wines according to their oxidation potential. Other studies evaluated the chemical changes in red wines subjected to saturation cycles with the result that the first cycle affects wine the most (Carrascon, Fernandez-Zurbano, Bueno, & Ferreira, 2015; Ferreira, Carrascon, Bueno, Ugliano, & Fernandez-Zurbano, 2015). The reactivity and consumption of oxygen during wine oxidation depends on chemical composition and the conservation temperature of the wine. Moutounet indicated that a red wine saturated with air consumed its dissolved oxygen in 25 h at 13 °C, while it only takes 3 h at a temperature of 30°°C (Moutounet & Mazauric, 2001).

This paper studied the oxygen consumption patterns of red and white real wines, and compared the accepted model of oxygen consumption with a new model fitting to approximate the kinetics of oxygen consumption. By means of an Artificial Neural Networks (ANNs) model, based on the basic chemical composition of these wines, we have defined an index capable of comparing wine oxidation rates.

2. Materials and methods

2.1. Wine samples

The alcohol degree (AD) was measured using a distillation method; titratable acidity (TA), pH, volatile acidity (VA) were measured using a potentiometric method; total sulfur dioxide (free and combined SO_2) according to an iodometric method and iron (Fe) and copper (Cu) using an FAAS external calibration with standards in hidroalcoholic solution (13%). These components were analyzed for a set of 32 real wines (RW) (16 reds, RRW + 16 whites, WRW) following European methods (CEE., 1990). Also, the content of total phenols (TP) was measured using the Folin-Ciocalteu method as described by Singleton and Rossi (Singleton & Rossi, 1965) (Table 1).

Another set of model wines or training wines (TW) (54 reds, RTW and 54 whites, WTW) were prepared with ultrapure water and varying concentrations of six substances closely linked with the wine's redox chemistry (Danilewicz, Seccombe, & Whelan, 2008; Zúñiga et al., 2014) and easily measureable in a winery setting (i.e. alcohol content, titratable acidity, sulfur dioxide, total phenolics, iron and copper, as further explained below). Ethanol (96% v/v), L(+)-Tartaric acid (99.7–100.5%) and sodium hydrogen sulfite (40% w/v) were acquired from Panreac (Barcelona, Spain), whilst iron standard solution (iron III nitrate nonahydrate in nitric acid 0.5 mol/L, 1000 mg/L), and copper standard solution (copper II nitrate in nitric acid 0.5 mol/L, 1000 mg/L) were purchased from Scharlab (Barcelona, Spain). Gallic acid (97.5–102.5%), and (+) Catechin hydrate (98%) were obtained from Sigma-Aldrich (San Luis, Missouri, USA). The concentrations of the compounds present in the training wines were as follows: Red training wines, AD (% vol): 13, 14, and 15; TA (gTH_2/L): 4.5, 5.25 and 6; SO_2T (mg/L): 30, 50 and 70; Fe (mg/L): 0.5, 4 and 8; Cu (mg/L): 0.03, 0.4 and 0.8; and TP (mg GAE/L): 2000, 3000 and 4000. For white training wines, the concentrations chosen were as follows: Alcohol (% vol): 11.5, 12.5 and 13.5; TA (gTH_2/L): 4.5, 5.25 and 6; SO_2T (mg/L): 30, 75 and 100; Fe (mg/L): 0.5, 3 and 6; Cu (mg/L): 0.03, 0.4 and 0.8; and TP (mg GAE/L): 200, 300 and 400. In order to reduce the number of possible combinations in a statistically reliable fashion, the Box-Behnken factorial design (Box & Behnken, 1960) was used, resulting in 54 combinations for each type of training or model wine (red and white) (Table 1, Supplementary material).

The chemical parameters used were chosen based on some of the following considerations: Ethanol is the second most abundant compound in wines which, in addition to assisting in the dissolution of many compounds, including most of the wine phenolics, is one of the main substrates of oxidation after the phenolics (Danilewicz, 2014; Elias & Waterhouse, 2010; Elias et al., 2009). Organic acids such as tartaric acid are known substrates of oxidation (Clark, Prenzler, & Scollary, 2003; Danilewicz, 2014) which could be easily estimated by measuring total acidity, and contribute to the wine's pH and redox potential. In general, low pH imposes restrictions on oxidation (Danilewicz, 2007; Del Alamo et al., 2006; Oliveira et al., 2011), whilst a higher pH favors oxidation reactions (Danilewicz, 2003). Sulfur dioxide acts as one of the major antioxidant agents of wine by reacting with the H_2O_2 formed during phenol oxidation (Danilewicz, 2007), thus contributing significantly to the antioxidant capacity of wines, particularly whites (Abramovič, Košmerl, Poklar Ulrih, & Cigić, 2015; Danilewicz, 2007). Iron and copper stand out due to their influence as catalysers of oxidation reactions and their contribution to the wine's antioxidant capacity (Danilewicz, 2007; Oliveira et al., 2011; Zúñiga et al., 2014). Finally, wine phenolic compounds have a direct influence on wine oxidation, being the main reactants of activated oxygen in solution. As such they are an important contributor to antioxidant capacity (del Alamo-Sanza et al., 2014; Escudero et al., 2002; Holm Hansen et al., 2001; Oliveira et al., 2011).

2.2. Air saturation of wines

Each of the wines (32 RW and 108 TW) was saturated with atmospheric oxygen using an air press pump (Selecta, Spain) that allowed the sparging of air into the liquid. Air dissolution stopped when the partial pressure of oxygen in the wine was the same as that of the atmosphere (i.e. 100% air saturation, equivalent to a concentration of approximately 7 mg/L of oxygen in water at P_{atm} 1013 hPa and 35 °C). The atmospheric pressure was measured using a digital barometer with an accuracy of ±0.3 hPa at 20 °C (PTB110, Vaisala Oyj, Finland). To prevent oversaturation of oxygen in solution, high-speed air flow (i.e. air flow rates >1 mL/min) and very small bubbles were avoided, using a procedure described elsewhere (Näykki, Jaluske, Helm, & Leito, 2013). After saturating the wines, the dissolved oxygen content (DO) of the samples were measured every 100 s during 60 h, as explained below. The data with values above 100% (associated with an oversaturation) were discarded at the time of the statistical treatment. Then, the oxygen depletion from 100% of air sat. until a value of 5% of air saturation was evaluated.

2.3. Measurement of DO and kinetics of oxygen consumption

The oxygen-saturated wines were transferred onto 20 mL respiration vials with integrated optical oxygen sensors for DO measurements (OXVIAL20, PyroScience GmbH, Aachen, Germany) which are airtight. The sensors are stripes of oxygen sensitive redflash-indicators glued to the inner wall of the vials [resolution: 0.01% O_2 (0.005 mg/L) at 1% O_2, 0.05% O_2 (0.025 mg/L) at 20% O_2; Accuracy: ±0.02% O_2 (0.01 mg/L) at 1% O_2 or ±0.2% O_2 (0.1 mg/L) at 20% O_2], which allow DO readings by means of eight optical fibers connected to a two FireStingO_2 4-channels optical oxygen meters (PyroScience GmbH, Aachen, Germany). All of the assays were performed in groups of eight vials simultaneously. The samples were kept in a thermostatic bath at a constant temperature of 35 ± 0.01 °C (Julabo FP40-ME, Seelbach, Germany). The oxygen sensors of each vial were calibrated according to the manufacturers' protocol, with measurements performed at two calibration points: oxygen-free water (0% air saturation) and air-saturated water (100% air saturation). Measurements were performed in ultrapure water in saturation conditions according to ISO

Table 1
Chemical composition of the wines tested (i.e. 16 white and 16 red wines) and correlation results between OCRI and given chemical parameter.

Wine	OCRI	Fe (mg/L)	Cu (mg/L)	Fe/Cu	AD (%)	TA (mg/L)	SO2 T (mg/L)	SO2F (mg/L)	SO2C (mg/L)	TP (mg/L)	pH	Vol Ac (g/L)
White real wines												
8	41.41	0.70	0.03	23.33	13.20	5.10	83	39	44	350	3.36	0.30
4	42.99	0.30	0.05	6.00	12.10	5.20	64	32	32	228	3.31	0.24
16	49.17	1.00	0.45	2.22	13.70	6.00	107	24	83	250	3.12	0.42
9	50.73	0.80	0.48	1.67	13.40	5.80	117	27	90	249	3.20	0.28
12	51.57	1.40	0.08	17.50	12.80	5.60	96	32	64	234	3.11	0.31
2	51.69	0.80	0.11	7.27	12.60	5.50	119	20	99	245	3.48	0.16
7	51.70	0.80	0.28	2.86	13.20	5.70	112	40	72	288	3.29	0.29
14	51.90	1.30	0.08	16.25	12.80	5.50	80	31	49	253	3.11	0.40
15	52.09	1.40	0.08	17.50	12.80	5.50	84	32	52	223	3.12	0.28
3	53.15	1.20	0.05	24.00	12.50	5.30	104	32	72	288	3.13	0.38
1	55.28	0.70	0.06	11.67	13.80	6.10	98	19	79	363	3.14	0.51
5	56.10	1.50	0.05	30.00	12.70	5.30	98	24	74	258	3.11	0.29
11	60.00	4.10	0.11	37.27	12.60	5.50	79	29	50	236	3.21	0.23
6	60.21	0.80	0.14	5.71	13.40	5.40	113	37	76	333	3.31	0.27
10	62.33	0.90	0.58	1.55	13.60	5.40	85	26	59	278	3.01	0.47
13	62.53	1.10	0.09	12.22	13.30	5.30	101	36	65	218	3.34	0.39
Average	53.30	1.18	0.17	13.56	13.03	5.51	96.25	30.00	66.25	268	3.21	0.33
Correlation		0.3969	0.1911	0.0764	0.2989	0.0692	0.2211	−0.1390	0.2445	−0.0677	−0.2517	0.2755
p level		0.1279	0.4783	0.7786	0.2608	0.7990	0.4105	0.6077	0.3615	0.8031	0.3471	0.3018
Wine	Red Real Wines											
8	39.05	0.70	0.06	11.67	14.70	5.30	36	30	6	2933	3.46	0.37
16	39.99	1.40	0.06	23.33	14.10	5.20	37	16	21	2906	3.63	0.47
13	40.97	1.40	0.05	28.00	14.00	5.20	32	13	19	2579	3.63	0.47
4	43.25	1.80	0.17	10.59	14.50	4.70	64	42	22	3456	3.76	0.41
1	44.72	2.40	0.17	14.12	13.90	5.20	53	27	26	3497	3.65	0.35
11	45.22	1.80	0.13	13.85	13.30	5.00	24	21	3	2383	3.59	0.47
9	47.00	0.80	0.22	3.64	13.70	4.60	45	19	26	2624	3.89	0.45
10	49.40	1.80	0.13	13.85	13.20	4.70	18	10	8	2056	3.73	0.41
7	49.73	2.50	0.15	16.67	14.00	4.80	70	40	30	3483	3.72	0.37
6	50.90	2.80	0.14	20.00	14.10	4.70	64	37	27	3288	3.80	0.40
15	53.57	1.20	0.11	10.91	13.10	3.90	19	8	11	1865	3.65	0.46
14	58.39	1.60	0.38	4.21	14.10	4.10	31	16	15	2410	3.89	0.48
12	61.19	1.80	0.11	16.36	12.30	4.40	59	33	26	2401	3.85	0.63
3	63.50	2.50	0.17	14.71	13.90	4.60	69	31	38	2920	3.56	0.70
5	64.04	2.50	0.17	14.71	13.90	4.60	69	39	30	2892	3.68	0.59
2	67.32	1.60	0.62	2.58	14.00	4.70	29	18	11	2460	3.74	0.46
Average	51.14	1.79	0.18	13.70	13.80	4.73	44.94	25.00	19.94	2760	3.70	0.47
Correlation		0.3771	0.6134	−0.3992	−0.3380	−0.6344	0.2231	0.1161	0.2090	−0.2706	0.3271	0.6498
p level		0.1499	0.0115[*]	0.1256	0.2005	0.0083[**]	0.406	0.6685	0.274	0.3108	0.2126	0.0064[**]

[*] p-level < 0.05, [**] p-level < 0.01.

5814:2012 (International Standard Organization, 2012) and in an oxygen-free water at a concentration of 0 mg/L. The 0% calibration standard was prepared based on a strong reductant; in this case, sodium dithionite ($Na_2S_2O_4$) (Panreac, Barcelona, Spain) at a concentration of 30 g/L.

All oxygen-measuring equipment had a temperature probe, pressure transducer, and humidity sensors used for temperature, pressure and humidity compensation. The corresponding temperature probes were introduced in the thermostatic bath, independently from the luminescence equipment as to have other means of correcting the measured values and ensuring the quality of the measurements. Similarly, the atmospheric pressure of the oxygen meter was checked with the digital barometer during every assay.

As suggested before, the testing of wines was carried out under the same temperature conditions, by keeping the samples in a high accuracy thermostatic bath at 35 °C in darkness. All of the wines (140 real and model samples) were exposed to air until saturation, and the kinetics of oxygen depletion was measured following the concentration of DO.

2.4. Artificial Neural networks

Finally, an ANN model was built for the categorization of the wines according to their oxygen consumption rate. This model was trained by correlating the chemical data and the oxygen con-sumption curves of the 108 model wines, and tested using the 32 real white and red wines, as further explained below. Due to the wine matrix complexity and the multiplicity of chemical compounds linked with the wine's antioxidant capacity (Zúñiga et al., 2014), is that several ANN were proposed in order to categorize the oxygen consumption rate of the wine on the basis of its basic chemical composition. The multilayer perceptron ANN used in this work presents 7 input variables (input layer, Fig. 1 supplementary) of each network: a binary variable indicating whether the wine is red or white and the six chosen chemical parameters of the wines (i.e. A%, TA, SO2T, Fe, Cu, and TP). The output variable (output layer, Fig. 1 supplementary) of each ANN was a numeric-type variable that represents the wine's oxygen consumption rate and was used to compare wines with different rates. Both the input and output variables were normalized so that the mean of each variable is zero and the standard deviation is one.

The training of each ANN, employed to adjust the weight and offset values of the neurons, was done using the data acquired from the 108 model wines. Here, the back propagation algorithm based on the *Levenberg-Marquardt* method implemented with the *trainlm* function of Matlab® was employed (Hagan & Menhaj, 1994). The minimum value of the gradient was adjusted to 10^{-15}, and 20% of the samples were used for model validation. The process of training was conducted by varying the number of neurons included in the hidden layer of the ANN between two

and 20, and replicating each of them 200 times in order to minimize the influence of the random neural network initialization of the ANN in the final outcome of the training.

2.5. Statistical analysis

MATLAB software was employed to implement the consumption kinetic models and the ANNs, both MATLAB® and Microsoft Excel (2016), were used to perform the correlation and error analyses. STATGRAPHICS was employed for the ANalysis Of VAriance (ANOVA) tests shown in this work.

3. Results and discussion

3.1. Kinetics of oxygen consumption

All of the wines (140 real and model samples) were exposed to air until saturation, and the kinetics of oxygen depletion was measured following the concentration of DO. Fig. 1 is a graph showing the results of monitoring the oxygen consumption of the training white wines together with the two real white wines which presented limit tendencies (Fig. 1A); the results of monitoring the oxygen consumption of the real white wines together with the two

training ones which presented limit tendencies are also shown (Fig. 1B). The results indicate that only three (WRW4, WRW9 and WRW10) real white wines fall outside the tendencies of the training wines. In the case of the red wines Fig. 1C shows the training red wines together with the two reds which presented the most extreme tendencies, and the tendencies of the red wines together with the two model reds presenting the most different tendencies are shown in Fig. 1D, indicating that only one of the real wines studied (RRW14) follows a different kinetic to the training ones. To date, it has been established that red wines can consume more oxygen than whites (Danilewicz, 2015; Kreitman, Danilewicz, Jeffery, & Elias, 2016; Moutounet & Mazauric, 2001; Singleton, 1987), however, the kinetics of oxygen consumption between the two have not been well established. In this case, the analyses of the model and white and red real wines show that both types do not have significantly different kinetics of oxygen consumption, and that they were different to those reported by Moutounet and Mazauric (2001) and widely accepted in the sector. These differences may be due to the measuring conditions, such as temperature, the measurement system and the fact that this was not done in a closed environment (Moutounet & Mazauric, 2001). Dr. Danilewicz used a Clarke-type electrode to perform readings at ambient temperature (20–25 °C) and the system was not completely closed during the measurements. In our case, continuous
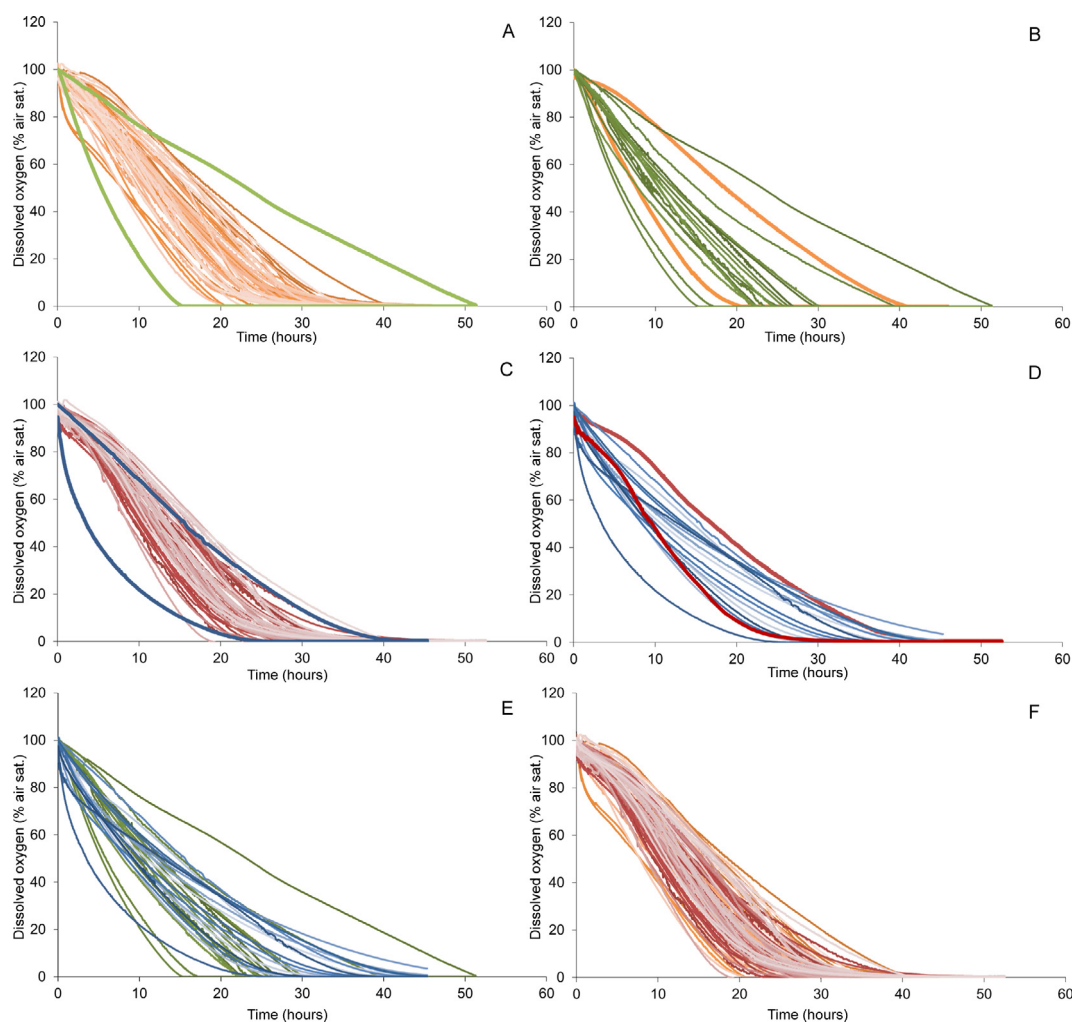

Fig. 1. Oxygen kinetics of: A) model white wines (orange) and extremely real white wines (green), B) real white wines and extremely model white wines, C) model red wines (red) and extremely real red wines (blue), D) real red wines and extremely model red wines, E) real red and white wines, and F) all studied model wines. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

oxygen readings with a photoluminescence detector at 35 °C were collected, and this was carried out in a perfectly airtight environment.

Oxygen consumption occurs via multiple pathways and/or reactions, but mathematically, some authors consider that O_2 is consumed following a first-order reaction kinetics $C = C_0 \cdot \exp(kt)$, where its apparent kinetic rate is an average value of all oxidation reactions occurring in wine during storage at any given temperature (Costa Martins, Monforte, & Silva Ferreira, 2013; Oliveira, Barros, Silva Ferreira, & Silva, 2015), therefore the following equation could be used:

$$[O_2](t) = [O_2]_0 \cdot \exp(-k_{app} \cdot t) \qquad (1)$$

where $[O_2]_0$ is the initial oxygen concentration (mg/L) and k_{app} (1/day) is the apparent kinetic rate at the given time (t) in days. Considering that k_{app} may follow the Arrhenius behavior with temperature (Costa Martins et al., 2013; Oliveira et al., 2015; Palacios Macías, Caro Pina, & Pérez Rodríguez, 2001), the reactions were conducted at 35 °C allowing the samples to consume the oxygen in shorter periods of 60 h instead of much longer times reported elsewhere (Singleton, 1987). In order to ensure a common oxygen interval for the 140 wines tested, that was representative of each kinetic pattern, the data was shortened between 85% and 5% of air sat., allowing an equation to be established that represents that specific interval for all the wines studied. The results presented in Table 2, obtained with eq. 1, show that the best fit was obtained for the real red wines (adjusted R-Sq = 0.976). Similarly, the correlation between Kapp and the chemical parameters that differentiate the real wines presented in Table 1 were evaluated. More specifically, Kapp was significantly and positively correlated only with the copper content of real white (Pearson correlation coefficient r = 0.7816, p = 0.0003) and red wines (r = 0.7185, p = 0.0017) (Table 2 supplementary material), indicating that at higher copper content there would be a greater initial speed of oxygen consumption.

A study of the other adjustments to represent the oxygen consumption rate indicates that oxygen consumption in the 140 wines studied has a better fit with the phenomenological trend described in Eq. (2).

$$O2(t) = \frac{a}{1 + b \cdot e^{c \cdot t}} \text{ with } a > 0, \ b > 0 \text{ and } c > 0 \qquad (2)$$

These results with values of parameter a between 88,347 and 143,651 showed a positive and statistically significant correlation with the copper content of the real red wines (r = 0.7544, p = 0.0007) (Table 2, supplementary material). In a similar manner, the **b** values, between 0.036 and 0.528, showed a statistically significant and positive correlation with the copper content of the real red wines (r = 0.7542, p = 0.0003). Similarly, the **c** values between 0.093 and 0.273 showed a statistically significant and positive correlation with the copper content of the real white wines (r = 0.5922, p = 0.0157). In all cases, a good fit was found for all wines studied with adjusted R-Sq values between 0.989 and 0.999. Therefore, as with the proposed model, the copper content of the wine is the parameter most closely related to oxygen consumption.

The comparative study of both adjustments for all the wines studied indicates that the phenomenological method fits the real data more significantly than the exponential equation. As an example the wines best adjusted to the exponential model (WRW9 and RRW14) were chosen and the consumption kinetics and adjustments made with both methods have been shown (Fig. 2A and B). It can be seen that the exponential method is only better adjusted at the start of the kinetics, with the proposed method being better adjusted to all the kinetic, as shown by the residuals graph (Fig. 2C and D). The analysis of variance of the statistics of both adjustments (R², RSMS, mean absolute error, max absolute error Table 2) performed for all the wines and for each of the 4 types of wine separately (training and real white and red wines) indicates that the R² of the proposed adjustment in Eq. (2) is significantly higher than the R² of the exponential model. It has also been found that the error made is significantly greater in all the studied wines with the exponential method (Fig. 2E and H).

Oxygen consumption kinetics shown in Fig. 1 can be represented with a function like that presented in Fig. 3. There are several statistical parameters extracted from this function or from its derivative function that can describe it, and therefore describe the

Table 2
Summary of the fit of the equation to the different kinetics of the 140 wines and error results. (A) exponential equation and (B) phenomenological equation.

			WTW	WRW	RTW	RRW	Average
Parameters (A)		Max	3.3781E−05	4.3873E−05	3.453E−05	3.983E−05	3.8003E−05
	k	Mean	2.2557E−05	2.476E−05	2.3386E−05	2.0528E−05	2.2808E−05
		Min	2.1406E−05	1.0712E−05	1.6961E−05	1.4319E−05	1.585E−05
		Max	0.976	0.971	0.983	0.993	0.981
	R²	Mean	0.938	0.963	0.939	0.976	0.954
		Min	0.875	0.943	0.889	0.935	0.911
		RMSE	5.9495	4.3501	6.062	3.3789	5.5163
	Error	MAE	5.2484	3.6965	5.4318	2.9032	4.8738
		MxAE	10.9709	10.26	10.1228	6.9526	10.1033
Parameters (B)		Max	129.9	135.1	150.6	151.328	143.651
	a	Mean	99.63	111.829	103.978	126.185	110.406
		Min	83.4	97.272	87.269	90.393	88.347
		Max	0.261	0.398	0.605	0.689	0.528
	b	Mean	0.095	0.214	0.135	0.35	0.199
		Min	0.307	0.085	0.022	0.063	0.036
		Max	0.307	0.276	0.298	0.063	0.273
	c	Mean	0.2	0.178	0.201	0.178	0.176
		Min	0.124	0.091	0.094	0.124	0.093
		Max	0.999	0.997	1	0.999	0.999
	R²	Mean	0.997	0.996	0.998	0.997	0.997
		Min	0.983	0.994	0.991	0.991	0.989
		RMSE	1.2870	1.4530	0.9919	1.1288	1.1741
	Error	MAE	1.0799	1.1839	0.8406	0.9442	0.9839
		MxAE	3.4586	4.3788	2.5435	3.6019	3.2195

RMSE (Root mean square error), MAE (mean absolute error), MxAE (maximum absolute error).
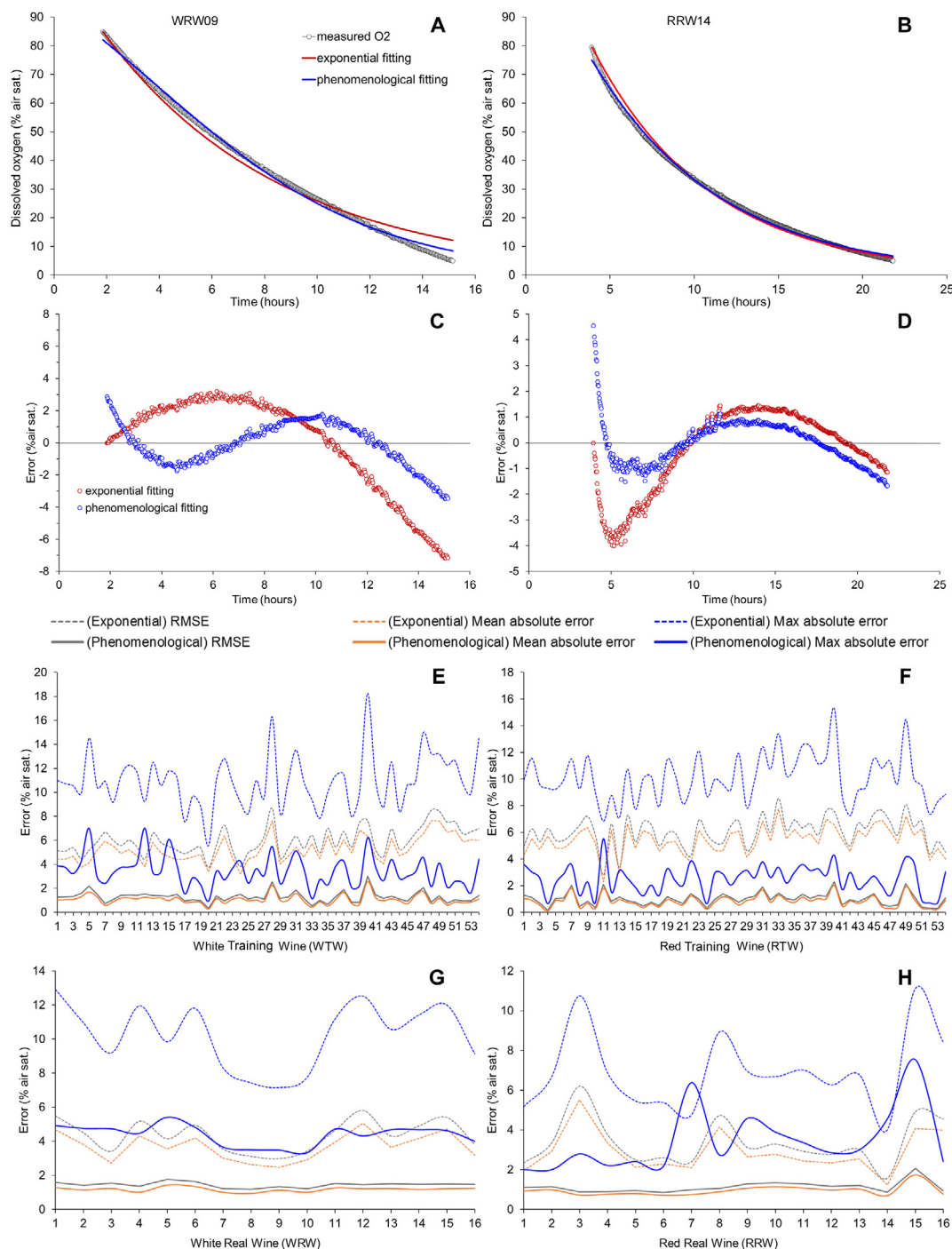p-level < 0.01 for every error.

Fig. 2. Decrease in oxygen consumption in two real wines (WRW09 and RRW14) and decrease according to exponential adjustment (A) and the proposed method (B). Graphical representation of the errors MSE (Root mean square error), MAE (mean absolute error), MxAE (maximun absolute error) of each real and training wine studied, error comparison between both models for WRW09 (C) and for RRW14 (D), error comparison for all white training wines (E), for all red training wines (F), for all white real wines (G) and for all red real wines (H).

oxygen consumption kinetics. In this article, three parameters were analyzed to describe this function because of their relationship with the wines' oxygen consumption capacity. The first of these parameters, hereinafter referred to as feature 1, is defined as the time in which the DO of the wine falls from full saturation to 50% air saturation, and its value in hours was named t_1 (Eq. (3)). The second parameter, which will be referred as feature 2, corresponds to the time in which the mathematical integral of the

function contained half of the total area under the function, and its value in hours will be named t_2 (Eq. (4)). Finally, the third parameter calculated, hereinafter referred as feature 3, represents the time in which the integral of the rate of oxygen consumption curve contained half of its total area, and its value in hours will be named t_3 (Eq. (5)). Thus, a wine with high avidity to consume oxygen will show low times t_1, t_2 and t_3. These three parameters (t_1, t_2, and t_3) are represented in Fig. 3, where I_2 and I_3 represent
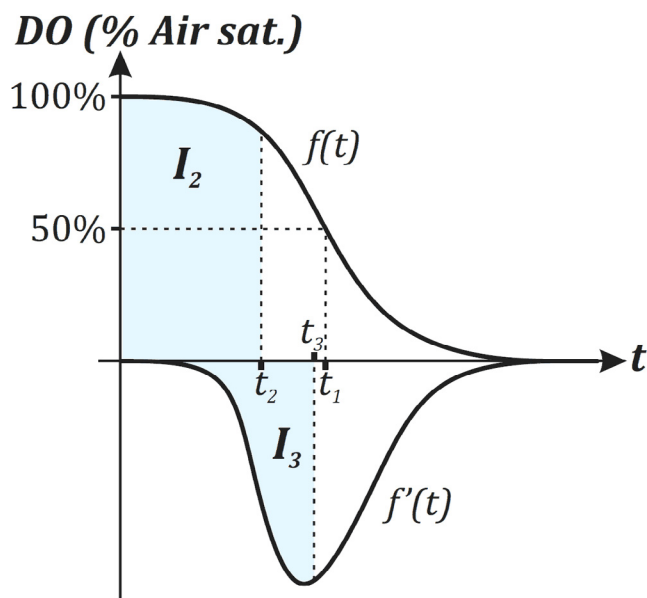
Fig. 3. Graphical representation of the oxygen consumption curve $f(t)$, its derivative function $f'(t)$, and the three features used to describe the wines' oxygen consumption capacity (t_1, t_2, and t_3), where I_2 and I_3 represent half of the total area under the oxygen consumption curve and its derivative function, respectively.

half of the total area under the oxygen consumption curve and its derivative function, respectively. The mathematical representations of these three parameters are as follows:

$$t_1 \text{ so that } f(t_1) = 50\% \tag{3}$$

$$t_2 \text{ so that } I_2 = \int_0^{t_2} f(\tau)d\tau = \frac{1}{2} \cdot \int_0^{\infty} f(\tau)d\tau \tag{4}$$

$$t_3 \text{ so that } I_3 = \int_0^{t_3} f'(\tau)d\tau = \frac{1}{2} \int_0^{\infty} f'(\tau)d\tau \tag{5}$$

where $f(t)$ is the evolution curve of DO over time and $f'(t)$ its derivative function.

As seen in Fig. 1, not all of the wines were fully capable of consuming the oxygen provided during saturation (particularly reds), showing an asymptotic pattern at DO levels below 5% air sat. The prior serves as an indication of the importance of analyzing the data through the calculation of different parameters. For instance, feature 1 gives greater weight to the oxygen that remains in the wine, while feature 3 offers an indication of the total oxygen consumed, with feature 2 being a descriptive value of the dissolved oxygen evolution curve.

The linear correlation between each the chemical characteristics of the wine (%AD, TA, SO_2, Fe, Cu, TP, and a variable indicating whether the wine is white or red (W/R)) and the three features used to describe the wines' oxygen consumption velocity (t_1, t_2 and t_3) were calculated. The Pearson correlation coefficient results were −01604[+], 00636, −0.0478, −0.2677[**], −0.3077[***], −0.2099[*], and −0.2022[*], respectively, for feature 1, −0.0811, −0.1008, −01189, −0.3211[***], −0.3863[***], −0.0551, and −0.0265, respectively, for feature 2, and −0.0475, 0.0287, −0.1703[*], −0.2500[**], −0.2869[***], −0.0620, and −0.0363, respectively, for feature 3, where [+], [*], [**], [***] indicate $p < 0.1$, $p < 0.05$, $p < 0.01$ and $p < 0.001$, respectively. In all cases, the individual significant correlations between these wine characteristics and the features chosen to describe the wine's oxygen consumption rate were, in absolute value, between 0.20 and 0.39 for feature 2, with Cu and Fe being

the highest. These correlation results expose the relationship between the chemical characteristics of the wine analyzed and the oxygen consumption features proposed in the article. Because of these correlation results, it would be desirable to build a black-box model to relate these seven characteristics and the three features chosen in order to improve the predicting capabilities by combining the information contained on each characteristic. To this end, an ANN-based model was created to estimate the selected features and to qualify wines by their oxygen consumption rate, using simple chemical compositional parameters commonly used in wineries.

3.2. Results of the Neural networks

Three ANNs were created to estimate each one of the three oxygen consumption features (t_1, t_2, t_3) considered based on simple chemical compositional parameters commonly used in wineries. Initially, the training process of the ANNs was carried out in order to adjust the weights of each neuron of the network, and to choose the optimal number of hidden neurons. The number of neurons in the hidden layer for the ANN associated with each feature was chosen as the one with the lowest validation error, being eight hidden neurons for feature 1, and four hidden neurons for features 2 and 3. Once trained, the three neural networks were tested with the optimal number of neurons in each case, using the data obtained from the real wines.

Considering that the results obtained for each of the three features are not comparable (t_1, t_2, and t_3 variedor all the 140 wines between 2.89 and 23.19 h for t_1, between 3.69 and 14.31 h for t_2, and between 3.82 and 22.54 h for t_3), we have proposed an index to describe the oxygen consumption rate, called Oxygen Consumption Rate Index (OCRI). OCRI is a dimensionless variable that ranges between 0 and 100, taking values close to 0 when the oxygen consumption rate of the wine is low and values close to 100 when the oxygen consumption rate is high. Equation 6 shows the mathematical procedure to transform each feature into its corresponding OCRI:

$$OCRI_i(\%) = 100 \cdot \left(1 - \frac{t_i - T_{i,min}}{T_{i,max} - T_{i,min}}\right), i \in \{1, 2, 3\} \tag{6}$$

where: $T_{i,min}$ is 0 for the three characteristics considered ($i \in \{1, 2, 3\}$), describing the extreme case of a wine with the highest oxygen consumption rate, and $T_{i,max}$ is the maximum value of t_i for each characteristic ($i \in \{1, 2, 3\}$).

The mean absolute error, that is, the mean value of the absolute value of the difference between the value estimated by the ANN and the expected value, was employed to analyze the performance of the proposed ANN. Thus, the mean absolute error results for OCRI obtained using the first feature were 9.30% for the training wines, and 17.34% for the real wines, respectively. The second feature showed OCRIs with a mean absolute error of 7.04% for the training wines, and 17.29% for the real wines. The mean error results of OCRI obtained with the third feature were 7.55% for the training wines and 18.72% for the real ones. From these results, it can be observed that the lower average absolute error was obtained for the second feature, for both training and real wines. Additionally, given the results obtained for the training samples and the learning ability of neural networks, it is possible to think that the data would improve if the ANNs are trained with a larger database that includes more real wines.

The graphical representation of the results obtained with the ANN of feature 2 is shown in Fig. 4. This plot represent the OCRI measured against the OCRI estimated for feature 2, distinguishing red and white wines. Feature 2 showed the cloud of points with the best distribution along the reference line, suggesting that it is pos-
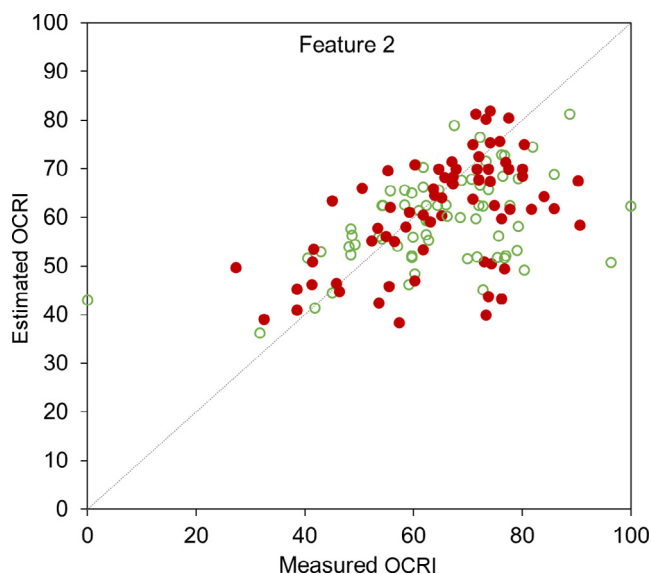
Fig. 4. Representation of the measured OCRI against the estimated OCRI of the neural network proposed for the feature 2, differentiating the white (open green circle) and red wines (solid red circle). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

sible that the data would be a good indicator of the avidity of these wines to consume oxygen. In the case of the white wines the correlation between measured and estimated OCRI was found to be 0.4807 and was 0.5177 for red wines, showing high significance in both cases (p < 0.001). In addition to that, the results presented previously about the correlation between oxygen consumption features and wine composition show that the highest correlation between some of the chemical compounds of the wines (particularly Fe and Cu) and the ANNs created were observed for feature 2. According to these results, t_2 was chosen as the best feature for the classification of the wines studied, based on its smaller error of estimation considering the OCRI, presented in the previous paragraph. As indicated previously, t_2 is defined as the time in which the area under the oxygen consumption curve from $t = 0$ to $t = t_2$ reaches 50% of the total area under the curve of air-saturated wines conserved at 35 °C (equation (4). It is reasonable to think that this parameter (t_2) should be small for wines with a great avidity for oxygen (i.e. wines with substances of greater reducing capacity or higher antioxidant capacity).

The results of the ANOVA comparing the chemical composition of white wines with OCRI values show a non-significant correlation (Table 1). However, the red wines in this study show a positive and statistically significant correlation between the OCRI and Cu content (0.6134, p = 0.0115) but no significant correlation with Fe; this result coincides with that reported by Ferreira et al., 2015 (Ferreira et al., 2015); and also with volatile acidity (0.6498, p = 0.0064), while there is a statistically significant negative correlation with total acidity (−0.6344, p = 0.0083). For instance, the red wine with the highest OCRI was a red sample (RRW2) with the highest Cu content (0.62 mg/L), but relatively low iron content (1.6 mg/L) and intermediate content total acidity (4.7 g/L) and volatile acidity (0.46 g/L). The red wine with the lowest OCRI was a red sample (RRW8) with the lowest Fe content (0.7 mg/L) and highest total acidity (5.3 g/L) and a low Cu content (0.05 mg/L) and relatively low volatile acidity (0.37 g/L). Therefore, it is not enough to discover the chemical properties to define the oxygen consumption rate of the studied wines, but rather the chemical properties of the wines need to be evaluated as a whole.

4. Conclusions

In this work, we propose a phenomenological fitting for oxygen consuming kinetics which improves on the widely used exponential method. To our knowledge, this is the first time real white and red wines are shown to have similar kinetics of oxygen consumption. The use of Artificial Neural Networks has turned out to be a valid tool for predicting oxygen consumption rates. Six chemical parameters selected among those commonly used in wineries have been used as inputs, allowing the estimation of the Oxygen Consumption Rate Index. A larger set of real wine samples should be considered in order to offer a practical tool to wineries.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.foodchem.2017.02.105.

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