Port Wine Oxidation Management: A Multiparametric Kinetic Approach

Rui Costa Martins,[†] Ana Rita Monforte,[‡] and António Silva Ferreira*,[‡,§]

[†]School of Health Sciences, Life and Health Sciences Research Institute (ICVS), ICVS/3B's, PT Government Associate Laboratory, University of Minho, Braga/Guimarães, Portugal

[‡]Escola Superior de Biotecnologia, Universidade Católica Portuguesa, Rua Dr. António Bernardino de Almeida, 4200-072 Porto, Portugal

[§]Institute for Wine Biotechnology, Department of Viticulture and Oenology, University of Stellenbosch, Private Bag XI, Matieland 7602, South Africa

ABSTRACT: Port wine is a flagship fortified wine of Portugal, which undergoes a particularly long aging period, developing a dynamic sensory profile over time, responsible for several wine categories, which is dependent upon the type of aging (bottle or barrel). Therefore, the quality of the product is dependent upon the chemical mechanisms occurring during the aging process, such as oxidation or Maillard reactions. To attain the desired quality management, it is necessary to understand how technological parameters, such as temperature or oxygen exposure, affect the kinetics of the formation of key odorants, such as sotolon. There is a lack of information about the impact of the storage conditions (oxygen and temperature) on Port wine quality. In this study, the effect of these two parameters were investigated to increase the knowledge database concerning aging management of Port wines. It was found that sotolon formation is highly dependent upon oxygen and temperature. There is however a synergistic effect between these two parameters that could significantly increase the concentration. The kinetic parameters of oxygen, sotolon, and other compounds related to Port aging (cis- and trans-5-hydroxy-2-methyl-1,3-dioxan, 2-furfural, 5-hydroxy-methyl-furfural, and 5-methyl-furfural) are also reported. Kinetic models with Monte Carlo simulations, where the oxygen permeability dispersion and temperature are the parameters under evaluation, were applied. On the basis of the modeling predictions, it would seem that the temperature of a cellar would have a more significant impact on the Port wines stored in containers where the oxygen intake is higher (barrels) when compared to containers with low oxygen permeability (bottles using cork stoppers).

KEYWORDS: Port wine, aging, oxygen, sotolon, kinetic parameters, storage management

INTRODUCTION

Port wine is a fortified wine with an ethanol content of about 20% and produced in a specific region in Portugal known as the Douro. These red wines typically undergo long periods of aging (>4 years), either through bottle aging (Vintage category) or barrel aging (Tawny category) for up to 60 years and even longer.

During this maturation period, the color and wine aroma undergo some significant changes, caused by the increasing and decreasing levels of some of the chemical constituents. These changes become more pronounced with extended aging. The age of the product is related to the value, leading to a popular Portuguese expression concerning Port wines of "the older, the better". The aromatic profile, which changes during aging, is the result of several underlying reactions. Therefore, if one wants to understand the sensory attributes of Port, it is important to understand the mechanisms involved as well as the interconnectivity among them. Several of these mechanisms are to a large extent already described, such as the Maillard reaction[1−5] and several oxidation reactions.[6−10]

In Port wines, sotolon [3-hydroxy-4,5-dimethyl-2(5H)-furanone] was recognized as the key molecule in the "perceived age" of barrel-stored Port wine and, consequently, in the aroma quality of the product. The odor threshold value in Port wine was estimated at 19 μg/L,[11] and the concentration can rise from a few dozen μg/L in a young wine to 1 mg/L in wines older than 50 years.

The presence of this compound in food was originally explained as the result of an aldol condensation reaction between 2-ketobutyric acid and pyruvic acid,[12] similar to the mechanism proposed for the formation of abhexon [5-ethyl-3-hydroxy-4-methyl-2(5H)-furanone] in protein hydrolisates.[12] The formation mechanism of this compound is not yet fully understood. The Maillard reaction was tested to be a potential pathway of formation,[3,11,13] while many authors connect the sotolon formation to oxidation[14] and some authors connect the sotolon formation to both mechanims.[15,16] Silva Ferreira et al.[17] demonstrated that the formation of sotolon in Port wine is clearly related to the temperature and the presence of oxygen. These two parameters are therefore critical concerning Port quality. The fact that both parameters influence this compound to a large extent would suggest that this molecule is a hybrid compound, meaning that it can originate in a connection between oxidation and the Maillard reaction. This still needs to be confirmed.
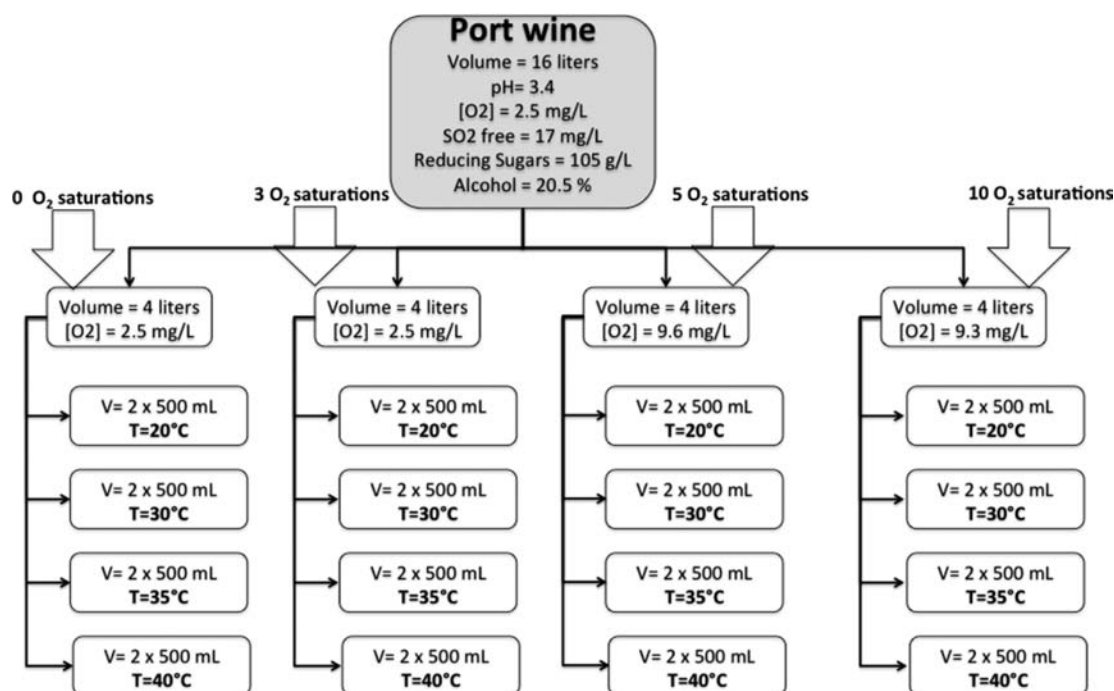
Figure 1. Experimental design.

This problem has an important consequence for the management of the storage of Port, especially in the case of "Vintage" (bottles) and "Tawny" (barrels) Port styles, where the correct temperature and O₂ permeability are needed to attain the desired sensory quality of the aged wine. Although sotolon is the key odorant in Port wine, the quality of the end product is also dependent upon other relevant chemicals.

In this study, a Port wine has been subjected to various degrees of oxygen and temperature exposure over time. The consumed oxygen was measured, and various compounds related to the known mechanisms (Maillard reaction and oxidation) were also quantified. These analyses were used to calculate the kinetic parameters, which, in turn, were used to determine how the compounds are affected by the treatments (oxygen and temperature). These parameters also aided in a simulation of the wine composition. This information was then used to superimpose the oxidized wines into an existing Port wine database. This model could possibly in the future be used as a predictive tool for the production of a specific wine style by adjusting oxygen and temperature control accordingly.

Therefore, the main objectives of this research were to (i) investigate and propose an empirical mathematical model that explains the effect of oxygen consumption and temperature on the formation of sotolon, cis- and trans-5-hydroxy-2-methyl-1,3-dioxane (cis- and trans-dioxane), 2-furfural, 5-hydroxy-methyl-furfural (5HMF), and 5-methyl-furfural (5MF) and (ii) calculate the kinetic parameters of these compounds and achieve a chemoinformatic model to predict the wine style based on the effect of the type of container, oxygen consumption, and temperature.

The information obtained from this study could potentially aid in developing desired wine styles.

■ MATERIALS AND METHODS

Reagents. All chemicals and standards used were obtained from Sigma-Aldrich, St. Louis, MO (high-purity grade, >99.0%). cis- and trans-dioxane were synthesized according to Maillard.[18] Dichloro-

methane and anhydrous sodium sulfate were obtained from Merck, Germany.

Port Wines. A total of 34 Port wines between the ages of 1 and 129 years (kindly donated by Symington Co., Porto, Portugal), were used for the construction of the database. Wines were made according to standard traditional Port winemaking procedures, and all of the wines used for the database creation were aged in "pipas" (550 L spent-oak barrels). For the kinetic study (isothermal protocol), 16 L of Port wine with pH 3.4, 2.5 mg/L dissolved oxygen, a free SO₂ level of 17 mg/L, 105 g/L of reducing sugars, and 20.5% alcohol and produced in the year of the experiment (without any oak contact) were used.

Experimental Setup. The determination of the kinetics of aging Port wine was performed under differing constant storage temperatures and oxygen concentrations (Figure 1). Wines were stored at 20, 30, 35, and 40 °C in temperature-controlled incubators. The oxygen treatments included 0 (F1), 3 (F2), 5 (F3), and 10 (F4) saturations. For each combination of oxygen/temperature, used glass vessels filled with 500 mL of wine were used. Oxygen saturation was obtained by stirring the sample vigorously for about 1 h until an oxygen concentration of about 8−9 mg/L was reached. This was performed in a laminar flow chamber under ultraviolet (UV) light to prevent microbial contamination.

The F1 group was never supplemented with O₂. F2 were saturated at sample days 14, 35, and 56. F3 were saturated in the beginning of the experiment and in sample days 14, 28, 42, and 56. F4 were saturated with O₂ at all sampling day points (0, 14, 21, 28, 35, 42, 49, 56, and 63).

This forced aging experimental protocol was performed in duplicate for practical reasons. Not all samples were analyzed by gas chromatography−mass spectrometry (GC−MS) on the replicate trial and were used as a cross-check procedure.

Dissolved Oxygen Measurements. The oxygen concentration was measured using a Fibox 3 LCD fiber optic oxygen transmitter, a polymer optical fiber, and planar oxygen-sensitive spots (5 mm sensor spots PSt3), from PreSens Precision Sensing GmbH (Germany). The sensor was positioned in the center of the 500 mL glass vessel and remained in contact with the wine at all times. Oxygen levels were measured at nine intervals (weekly) during the 63 day isothermal storage.

Chemical Analysis. Analyses of volatile compounds were performed at nine intervals (weekly) during the 63 days.

5372

dx.doi.org/10.1021/jf4005109 | J. Agric. Food Chem. 2013, 61, 5371−5379

The extraction procedure was based on the method described previously by Silva Ferreira.[11] To describe it briefly, a 50 mL sample of Port is spiked with 50 μL of 3-octanol in a hydroalcoholic solution (427 mg/L) as the internal standard. Anhydrous sodium sulfate (5 g) is added to increase ionic strength, after which the wine is extracted twice with 5 mL of dichloromethane. The two organic phases obtained are combined and dried over anhydrous sodium sulfate. A total of 2 mL of this organic extract is concentrated to 0.4 mL under a constant nitrogen stream.

The most common enological parameters were determined according to the Office International Vigne et Vin (OIV) methods [ethanol (%), free SO_2, and pH]. Reducing sugars were determined according to the methods as described in the "Portuguese Official Standards (NP) for Spirits and Alcoholic Beverages" (NP-1420).

GC−MS Analysis. Extracts were analyzed using a Varian 450 gas chromatograph, equipped with a mass spectral detector, Varian 240-MS, and the Saturn GC−MS workstation software version 5.51. The column used was Stabilwax-DA (60 m × 0.25 mm × 0.25 μm) fused silica (Restek, Bellefonte, PA). The injector port was heated to 220 °C. The injection volume was 1 μL in splitless mode, and the split vent was opened after 30 s. The carrier gas was helium C-60 (Gasin, Portugal), at a constant flow of 1 mL/min. The oven temperature was 40 °C (for 1 min), then increased at 2 °C/min intervals to 220 °C, and held there for 20 min. All mass spectra were acquired in the electron impact (EI) mode (ionization energy, 70 eV; source temperature, 180 °C). The ion trap temperatures were 230, 45, and 170 °C, respectively. The mass range was m/z 33−350, with a scan rate of 6 scans/s in full-scan mode. The emission current was 50 μA, and the electron multiplier was set in relative mode to the autotune procedure. The maximum ionization time was 25 000 μs, with an ionization storage level of m/z 35.

Compound identification was achieved by comparing retention times and mass spectra obtained from a sample containing pure, authentic standards. Kovats indices were calculated and also used as validation when compared to mass spectra, as reported in the National Institute of Standards and Technology (NIST) 05 MS library database. Two injections of dichloromethane extracts was carried out.

Compound quantification was performed on the basis of standard calibration curves.

Regression Analysis. Nonlinear regression for model fitting was performed on all data, using the one-step methodology[19] by maximizing the likelihood function for all temperatures and using bootstrap sampling to estimate the prediction residuals sum of squares (PRESS) criterion.[20,21] The semi-studentized residuals were also inspected for outliers and randomness and tested for normality. The lack of fit test was performed to determine the adequacy of the regression model, and the studentized effect (t ratio) $(\beta_i/s\beta_i)$ was studied at a 5% confidence level.[22,23]

Kinetic Modeling. The general rate law for compound formation is

$$r = \frac{d[\text{compound}]}{dt} = k_{\text{obs}}[\text{compound}] \tag{1}$$

The most common kinetic models reported in the literature to describe the kinetics of compound formation are zero-, first-, or second-order reaction models

$$C = C_0 + kt \qquad \text{(zero order)} \tag{2}$$

$$C = C_0 \exp(kt) \qquad \text{(first order)} \tag{3}$$

$$1/C = 1/C_0 + kt \qquad \text{(second order)} \tag{4}$$

where C_0 is the initial compound concentration.

Oxygen-related degradation occurs via multiple pathways and/or reactions, but it can be considered that O_2 is depleted by the first-order reaction kinetics, where its apparent kinetic rate is an average value of all oxidation reactions that occurs in Port wine during storage at any given temperature

$$[O_2](t) = [O_2]_0 \exp(-k_{\text{app}}t) \tag{5}$$

where $[O_2]_0$ is the initial oxygen concentration (mg/L) and k_{app} (day^{-1}) is the apparent kinetic rate at the given time t in days.

Therefore, k_{app} may follow the Arrhenius behavior with temperature

$$k_{\text{app}} = k_{\text{ref}} \exp\left[-\frac{E_a}{T}\left(\frac{1}{T} - \frac{1}{T_{\text{ref}}}\right)t\right] \tag{6}$$

where k_{ref} (day^{-1}) is the kinetic rate at the reference temperature T_{ref} (K), E_a represents the Arrhenius activation energy (J mol^{-1}), and R is the universal gas constant (J mol^{-1} K^{-1}).

At any temperature, it is possible to assume that sotolon formation is proportional to the oxygen consumption

$$\frac{d[\text{Sot}]}{dt} \propto \frac{d[O_2]}{dt} \tag{7}$$

where [Sot] (μg/L) is the sotolon concentration, $[O_2]$ (mg/L) is the oxygen concentration, and t is the storage time (days).

Taking this into consideration, it is possible to derive the following mass balance:

$$[\text{Sot}]_0 + b[O_2]_0[\text{Sot}]_t + b[O_2]_t \tag{8}$$

where b is the proportional coefficient for O_2 consumption versus sotolon formation.

This balance makes it possible to derive the following sotolon formation kinetics:

$$[\text{Sot}]_t = [\text{Sot}]_0 + b[O_2]_0[1 - \exp(-k_{\text{app}}t)] \tag{9}$$

However, for practical reaons, in Port wine production, it is easier to rather compute the consumed oxygen. Using the same mass balance presented in eq 8, it is possible to find a direct theoretical relationship between the consumed oxygen and the compound formation, which can express its concentration as a function of the consumed O_2.

$$[\text{Sot}]_t = [\text{Sot}]_0 + f(\text{consumed } O_2) \tag{10}$$

Such behavior is possible to be traduced empirically into first-order kinetics, such as

$$[\text{Sot}]_t = [\text{Sot}]_0 + [O_2]_{\text{consumed}} k_{\text{ref}} \exp\left[-\frac{E_a}{R}\left(\frac{1}{T} - \frac{1}{T_{\text{ref}}}\right)t\right] \tag{11}$$

where the time−temperature integral must be computed during regression analysis.[19,20,24]

Therefore, when the oxygen consumption and temperature are monitored during storage (aging), it is possible to estimate the compound concentration and predict it under new storage conditions.

The remainder of the studied compounds (cis- and trans-dioxanes, 2-furfural, and 5MF) and 5HMF also tend to follow first-order reversible kinetics[25]

$$C(t) = C_{\text{eq}} - (C_{\text{eq}} - C_0)\exp\left[-\frac{E_a}{R}\left(\frac{1}{T} - \frac{1}{T_{\text{ref}}}\right)t\right] \tag{12}$$

where C_{eq} is the final concentration of these compound found in Port wines older than 25 years.

Feature Space Analysis. For the construction of the Port wine database, 37 Port wines (ages ranging from 0 to 129 years) were analyzed according to the method as explained above. A total of 90 volatile compounds (alcohols, esters, acids, sulfur compounds, norisoprenoides, and acetals) were quantified and used for the construction of the database.

The discriminant feature space was determined by performing singular value decomposition on scaled compositional data. Relevant compounds, capable of discriminating the wines, were obtained through the singular values above the first singular value of the randomized by sample compositional table.[26] Furthermore, Q statistics (Q_α at 5% significance)[27,28] and T-hotling statistics (TH,α at 5% significance)[29,30] were used to measure the distance to the average composition. The accelerated aging of Port wines in this study was

then projected into the feature space and analyzed for feature proximity by the K-NN (*k*'s nearest neighbor) algorithm, and the classification probability of each accelerated wine was assessed by bootstrapping.[31]

Monte Carlo (MC) Simulation. The MC method is widely used to determine the extent of a deterministic effect, given a number of stochastic inputs.[32] We used the MC simulation to estimate the uncertainty effect on the amount of oxygen that permeates through a cork stopper or barrel at a given period of time to estimate its effect on sotolon concentrations during simulated storage. The following combinations were considered for the simulated wines: (i) temperatures of 5, 10, 15, and 20 °C [typical wine cellars temperatures, including two extreme values of a low (5 °C) and a high (20 °C) temperature], (ii) a barrel permeability of around 10 ± 2 mg/L per year,[33] and (iii) a cork O_2 permeability of approximately 1.5 ± 0.75 mg/L per year.[34,35] Therefore, the distribution of the chemical composition computed assumes that all permeated O_2 are consumed through chemical reactions in the wine. The MC results were afterward compared to the Port wine database.

The MC algorithm uses the derived kinetics to estimate the chemical composition in terms of (i) oxygen, (ii) sotolon, (iii) 2-furfural, (iv) 5MF, (v) 5HMF, and (vi) *cis-* and *trans*-dioxanes. Because sotolon formation is dependent upon the O_2 permeability and consumption, the MC method was implemented as follows.

Step 1: Generate MC samples based on the statistical distribution of O_2 permeability and kinetic parameters.

Step 2: For each MC case study, (a) calculate $[O_2]$, (b) calculate the sotolon formation based on the temperature and content of O_2; in this case, we consider that, inside barrels or bottles, the daily O_2 intake is well below the saturation point of 9 mg/L and, therefore, all O_2 taken in (IO_2) is either dissolved or consumed and computed by $dO_2/dt + k[O_2] - IO_2 = 0$ (pseudo-function determines O_2 in algorithm 1), and (c) estimate the remainder of the studied chemical parameters (2-furfural, 5HMF, 5MF, and *cis-* and *trans*-dioxane).

Step 3: Study the MC results by projecting the results into the Port wine database feature space and classify them by hierarchical cluster analysis (HCA).

Statistical Analysis. Statistical analysis and the developed methodology involving the experimental data were performed with R 2.12.1 for MAC, using the following packages: (i) classical multivariate analysis library (MVA), (ii) main library of Venables and Ripley's (MASS), (iii) Harrell miscellaneous (Hmisc), and (iv) R-base packages (R-Project).

RESULTS AND DISCUSSION

Oxygen Consumption. The oxygen consumption in the Port wines over time follows the first-order rate law, as shown in Figure 2. In fact, in this study and on the basis of previous works,[17] as well as the experimental observations, we assumed that, for the O_2 consumption and the formation of sotolon and other molecules, the reactions obey a first-order rate law. Only treatments with 0 and 10 saturations are shown in Figure 2 to prevent clutter. The fact that the oxygen consumption is of first-order reaction kinetics has important implications regarding the effects on the oxygen consumption rate (Figure 2). At 10 saturations, significant O_2 consumption rates are observed, whereas 0 saturation leads to extremely low rates of O_2 consumption. The 3 and 5 saturations (not shown) follow the same behavior and are located in order between 0 and 10 saturations. The oxygen consumption was more rapid at the beginning of the trial up to 21 days, after which the consumption slowed. The dissolved oxygen reacts with compounds that are present at high concentrations at the onset of oxygenation, which will cause the faster consumption rate. The consumption rate for the F4 samples (supplemented every week) slowed because there was a lack of substrate (compounds capable of reacting with oxygen) in the later
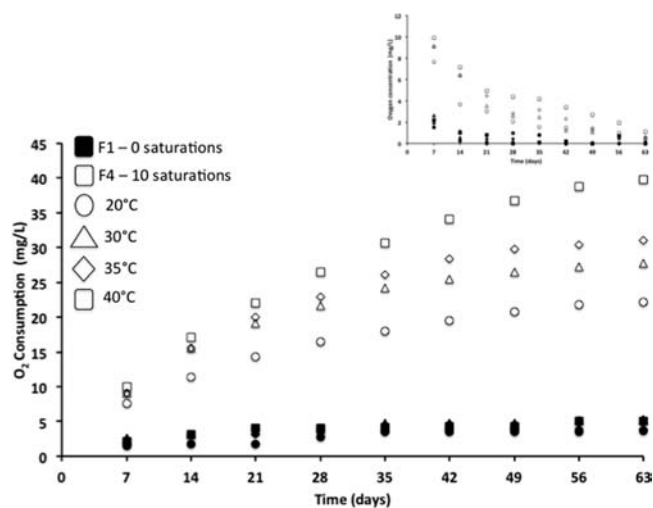
Figure 2. Oxygen consumption as a function of time at different temperatures and O_2 saturations and oxygen uptake in each week.

period of the trial. The oxygen consumption rates were high at all of the studied temperatures, with kinetic rates of 0.010, 0.012, 0.012, and 0.018 per day at 20, 30, 35, and 40 °C, respectively.

We are aware that, by changing the composition of the matrix, deviations in the kinetic values will occur. The behavior of the compound will however be similar, and these deviations will not affect the main purpose of the paper, which is to know how certain parameters (oxygen and temperature) will influence the formation of sotolon.

The activation energy (E_a), which constitutes a measure of sensitivity toward the temperature, is in addition very low, $E_a = 3.5$ kJ/mol, but is close to values that have been previously reported for free radicals (3 kJ/mol).[36]

Because the O_2 consumption tends to lower values, the time needed to consume all of the oxygen increases at low concentrations, therefore having a significant effect on the Port wine aging process and, consequently, the formation of molecules dependent upon the presence of oxygen.

Sotolon Formation. The formation of sotolon in the Port wines is presented in Figure 3.

As a first-order kinetics that is dependent upon the consumed oxygen, model eqs 8 and 9 for the isothermal experiment were fitted to the experimental data. The low PRESS, correlation coefficient, *t* values, and the lack of fit test
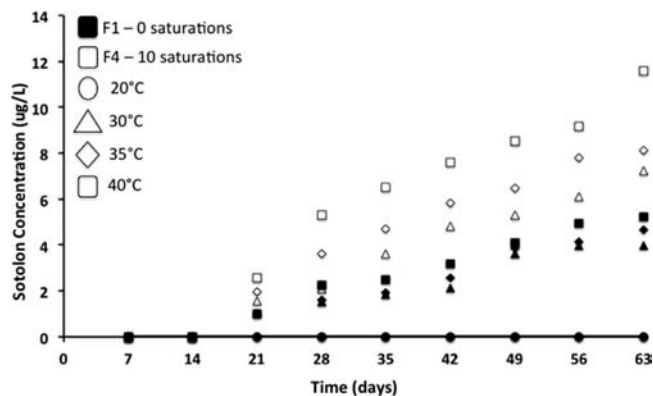
Figure 3. Sotolon concentration as a function of time at different temperatures and O_2 saturations.

results ($p < 0.01$) confirm the validity of the model for prediction and simulation.

There was an increase in the sotolon concentration over time. It is clear that consumed oxygen had a significant impact on the formation of sotolon. Samples that have been submitted to oxygenation had a higher rate of sotolon increase when compared to the samples that did not undergo forced oxidation. These samples also had a higher sotolon concentration at the end of the experiment.

The temperature also played a critical role in the formation of this compound because more sotolon was formed at higher temperatures. As an example, at 10 saturations, sotolon formation was higher at 40 °C when compared to 30 °C. This effect was also observed at 0 saturation.

In terms of kinetics, the sotolon formation rate at 40 °C was high when compared to the other temperatures ($k = 0.0157$ μg of Sot/mg of O_2 per day); this rate however changed as a function of the oxygen consumption (Figure 3).

The rate of formation values obtained is in agreement with the values previously obtained by Silva Ferreira et al.[17]

Following an increased number of O_2 saturations, the rate of formation increased linearly according to the consumed O_2. The temperature, as stated before, had a significant impact on the sotolon formation, which is reflected in the E_a value (92 ± 5 kJ/mol). In fact, this value is higher than in most oxidative processes, where the E_a value is in the range of 20 kJ/mol.[36] An increase in the storage temperature and the consumed oxygen concentration would thus lead to an increase in sotolon formation. For example, at 20 °C, the ratio between the sotolon formation rate and O_2 consumption is 0.08, whereas it is 0.2 at 40 °C (a 247% increase).

This effect is visually portrayed in a surface plot (Figure 4), where the sotolon formation rate (RSot) is plotted against
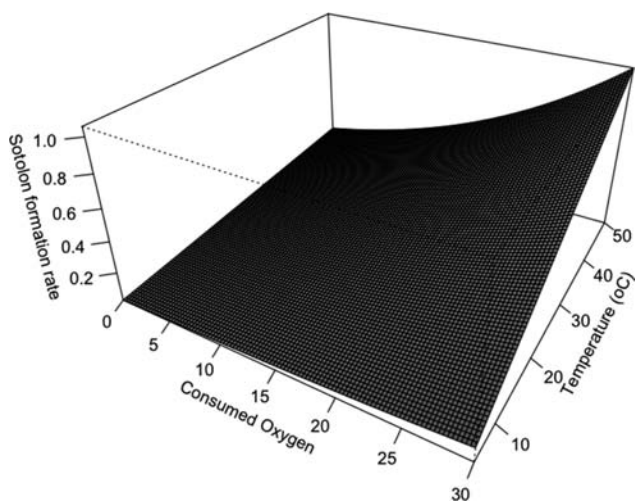
Figure 4. Surface plot of the sotolon formation rate as function of the consumed O_2 and storage temperature.

oxygen and temperature. RSot is given by RSot = $CO_2 k_{app}$, which states that RSot is directly proportional to the O_2 consumption rate and dependent upon the storage temperature.

Other Molecules. Additional compounds known to be connected to oxygen (i.e., *cis*- and *trans*-dioxane) and temperature (5HMF, 2-furfural, and 5MF) were also investigated.

The influence of the oxygen, temperature, and time on *cis*-dioxane and 2-furfural are presented in panels a and b of Figure 5, respectively. Kinetic rates and model parameters are presented in Tables 3 and 4.

These compounds were fitted to a first-order reversible kinetic, as discussed in the Materials and Methods. Statistical significance of this empirical model is also assured by the same parameters as for sotolon and O_2 consumptions (see Tables 1 and 2). These compounds present considerably lower kinetic rates when compared to sotolon, such as 2-furfural at $k_{20\ °C} = 6.7 \times 10^{-6}$ per day, 5HMF at $k_{20\ °C} = 2.1 \times 10^{-5}$ per day, and *cis*-dioxane at $k_{20\ °C} = 7.2 \times 10^{-4}$ per day (Table 1). Two groups can therefore be considered in terms of temperature sensitivity: (i) high E_a, 2-furfural (169 kJ/mol), 5MF (169 kJ/mol), and HMF (230 kJ/mol) and (ii) low E_a, *cis*-dioxane (76 kJ/mol) and *trans*-dioxane (86 kJ/mol). 2-Furfural, 5MF, and 5HMF are well-known compounds, with their formation being linked to high temperatures, such as thermal processing or the Maillard reaction. Their high E_a and low kinetic rates in Port wines indicate that these compounds are not formed in a significant concentration during aging. The E_a of *cis*- and *trans*-dioxanes are within the range of most oxidation reactions.[36] Dioxanes are formed by the reaction of acetaldehyde and glycerol. Acetaldehyde is known to be an oxidation marker and an important marker of the age of Port wine.[37] Thus, it would make sense that the activation E_a for dioxanes is lower, because the precursor is present in high concentrations in oxidative conditions and therefore not dependent upon the temperature.

MC Simulations. For the simulations of wine composition, the container was chosen on the basis of the O_2 permeability of 1.5 ± 0.75 mg/L per year for cork stoppers and 10 ± 2 mg/L per year for barrels. Storage temperatures were chosen as 5, 10, 15, or 20 °C. The time of storage was also selected as 2, 5, 10, 15, or 20 years. The MC algorithm uses the above-derived kinetics to estimate the chemical composition in terms of (i) oxygen, (ii) sotolon, (iii) 2-furfural, (iv) 5MF, (v) 5HMF, and (vi) *cis*- and *trans*-dioxane. We assume that, inside barrels or bottles, the daily O_2 intake is well below the saturation point (8−9 mg/L) and, therefore, all of the O_2 intake is either dissolved or consumed.

Tables 3 and 4 are simulated Port wine composition according to the temperature, time aged, and the containers used (barrel or bottle) obtained with MC simulations. Figure 6 shows an example of a simulated Port wine stored at 15 °C for 10 years (Table 3).

Figure 7 is a dendogram of the Port database, which included Port samples of various ages (indicated in black and the age reported in the sample name). MC predictions for wines stored for 10 years at 10 and 20 °C were then included in the dendogram. Samples indicated in color are the simulated aged Port wines. These wines have been included in the dendogram according to all of the parameters measured. This would allow for the positioning of the wines within the database and, thus, make predictions as to what age the samples would be associated.

Wines in the database are mostly clustered by age (Figure 7). This classification is mainly influenced by the presence of sotolon and *cis*- and *trans*-dioxanes. It is possible to observe three very distinct groups of wines according to age: (i) extremely old (around 129 years), (ii) 10−76 years old, and (iii) young (0−5 years).

When the simulated wines aged in bottles and barrel are compared, it is evident that the barrel-aged wines are associated
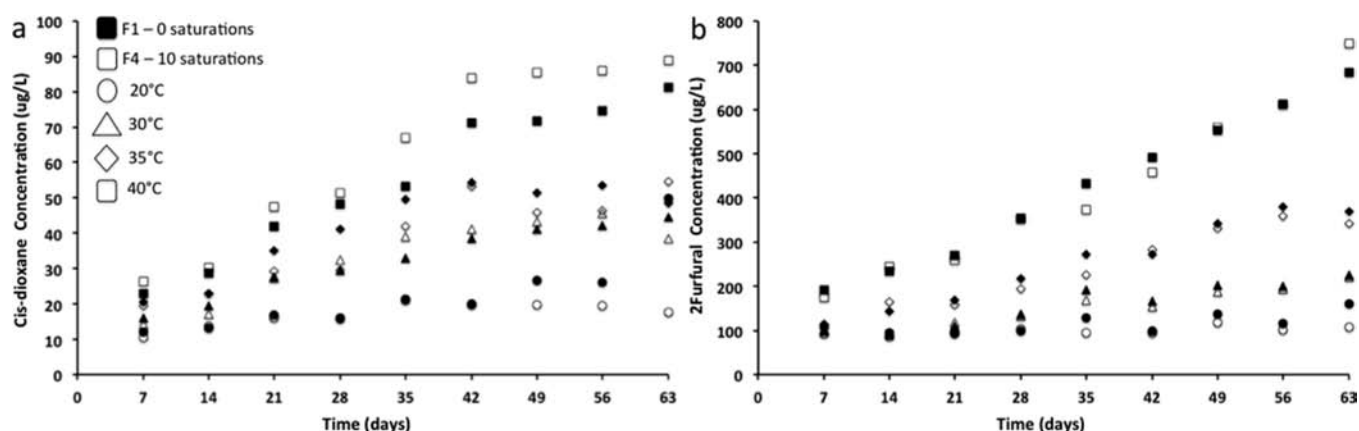
Figure 5. (a) *cis*-Dioxane and (b) 2-furfural concentrations as a function of time at different temperatures and O₂ saturations.

Table 1. Isothermal Kinetic Rates for Sotolon, 2-Furfural, 5MF, 5HMF, *cis*-Dioxane, and *trans*-Dioxane

temperature (°C)	kinetic rate (day⁻¹)			
	20	30	35	40
sotolon	0.004 ± 0.0004	0.005 ± 0.0004	0.008 ± 0.0006	0.016 ± 0.0006
2-furfural	$(6.7 \pm 0.5) \times 10^{-6}$	$(5.6 \pm 1.0) \times 10^{-5}$	$(8.7 \pm 2.0) \times 10^{-5}$	$(1.9 \pm 0.3) \times 10^{-4}$
5MF	$(9.4 \pm 0.6) \times 10{-5}$	0.0014 ± 0.0005	0.0022 ± 0.0005	0.0043 ± 0.0008
5HMF	$(2.1 \pm 0.1) \times 10^{-5}$	$(3.4 \pm 0.2) \times 10^{-4}$	$(3.9 \pm 2.4) \times 10^{-4}$	$(1.7 \pm 0.4) \times 10^{-3}$
cis-dioxane	$(7.2 \pm 0.7) \times 10^{-4}$	0.0016 ± 0.0001	0.0021 ± 0.0002	0.004 ± 0.0002
trans-dioxane	$(2.2 \pm 0.4) \times 10^{-5}$	$(6.5 \pm 0.7) \times 10^{-4}$	0.001 ± 0.0001	0.002 ± 0.0001

Table 2. Global Optimization Models for Isothermal for 2-Furfural, 5MF, 5HMF, *cis*-Dioxane, and *trans*-Dioxane

	kinetic parameters				
	2-furfural	5MF	5HMF	*cis*-dioxane	*trans*-dioxane
C_0 (μg/L)	164 ± 9.7	7.25 ± 0.27	1563 ± 123	3.7 ± 0.19	1.6 ± 0.001
k_{ref} (day⁻¹)	$(6.7 \pm 0.5) \times 10^{-6}$	$(9.4 \pm 0.6) \times 10^{-5}$	$(2.1 \pm 0.1) \times 10^{-5}$	$(7.2 \pm 0.7) \times 10^{-4}$	$(2.2 \pm 0.4) \times 10^{-5}$
E_a (kJ/mol)	169 ± 27	169 ± 32	230 ± 58	76 ± 13	86 ± 13

Table 3. MC Simulation Results for Isothermal Storage of Port Wine Inside Barrels

temperature (°C)	age (year)	barrel					
		sotolon (μg/L)	*cis*-dioxane (mg/L)	*trans*-dioxane (mg/L)	5MF (μg/L)	2-furfural (mg/L)	5HMF (mg/L)
5	2	0.98 ± 0.11	6.1 ± 0.3	1.0 ± 0.05	8.0 ± 0.4	11 ± 0.6	1.0 ± 0.5
5	5	6.1 ± 0.7	12 ± 0.6	1.6 ± 0.08	9.3 ± 0.5	29 ± 1.3	24 ± 0.1
5	10	24 ± 2.4	21 ± 1.0	2.6 ± 0.1	11 ± 0.6	58 ± 2.7	50 ± 2.5
5	15	55 ± 5.8	27 ± 1.2	3.6 ± 0.2	$13. \pm 0.7$	88 ± 4.3	72 ± 3.6
5	20	98 ± 10	32 ± 1.6	4.5 ± 0.2	15 ± 0.8	117 ± 6.1	97 ± 4.8
10	2	2.0 ± 0.2	9.4 ± 0.5	1.3 ± 0.07	10 ± 0.6	42 ± 2.0	5.6 ± 2.7
10	5	13 ± 1.2	19 ± 1.0	2.5 ± 0.1	14 ± 0.7	105 ± 5.2	139 ± 6.3
10	10	50 ± 5.1	30 ± 1.5	4.3 ± 0.2	22 ± 1.1	211 ± 11	278 ± 14
10	15	115 ± 5.1	38 ± 1.8	5.9 ± 0.3	29 ± 1.5	317 ± 15	415 ± 21
10	20	203 ± 21	43 ± 2.1	7.4 ± 0.4	36 ± 1.9	424 ± 20	556 ± 27
15	2	3.0 ± 0.2	14 ± 0.73	2.0 ± 0.1	36 ± 1.9	147 ± 6.4	302 ± 15
15	5	26 ± 2.7	27 ± 1.3	4.1 ± 02	32 ± 1.6	367 ± 19	759 ± 37
15	10	102 ± 10	40 ± 2.0	7.0 ± 0.3	56 ± 2.7	728 ± 37	1509 ± 82
15	15	233 ± 23	46 ± 2.2	9.4 ± 0.5	$78. \pm 4.1$	1096 ± 55	2230 ± 114
15	20	412 ± 46	49 ± 2.3	11 ± 0.6	99 ± 4.8	1459 ± 76	2983 ± 157
20	2	8.2 ± 0.8	22 ± 1.0	3.2 ± 0.2	40 ± 2.0	489 ± 26	1543 ± 68
20	5	51 ± 5.0	38 ± 1.9	6.6 ± 0.3	85 ± 4.2	1218 ± 59	3829 ± 191
20	10	202 ± 20	47 ± 2.3	11 ± 0.6	150 ± 7.0	2411 ± 124	7462 ± 387
20	15	462 ± 47	50 ± 2.5	14 ± 0.7	206 ± 10	3608 ± 185	11050 ± 566
20	20	806 ± 85	51 ± 2.4	15 ± 0.9	253 ± 13	4737 ± 215	14300 ± 784

with the older Port wines (between 20 and 35 years). On the other hand, the bottle-aged wines are associated with much younger Port wines (between 0 and 10 years). This separation is most likely due to the oxygen availability in the different

5376

dx.doi.org/10.1021/jf4005109 | *J. Agric. Food Chem.* 2013, 61, 5371–5379

Table 4. MC Simulation Results for Isothermal Storage of Port Wine Inside Bottles

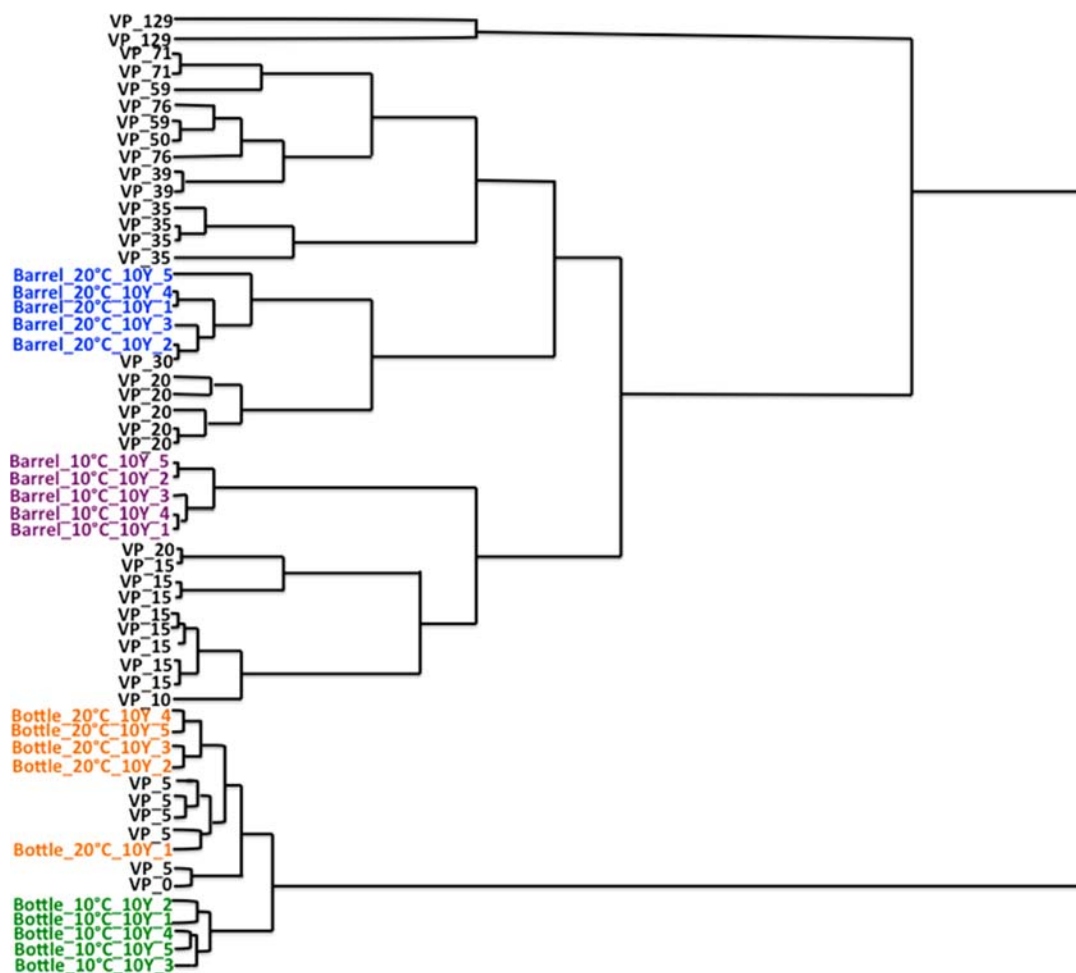|		bottle					
temperature (°C)	age (year)	sotolon (μg/L)	cis-dioxane (mg/L)	trans-dioxane (mg/L)	5MF (μg/L)	2-furfural (mg/L)	5HMF (mg/L)
5	2	0.1 ± 0.08	6.1 ± 0.3	0.9 ± 0.05	8.1 ± 0.4	11 ± 0.06	9.8 ± 0.5
5	5	0.9 ± 0.5	12 ± 0.6	1.6 ± 0.08	9.2 ± 0.5	29 ± 0.1	24 ± 1.1
5	10	3.6 ± 1.8	21 ± 1.1	2.6 ± 0.1	11 ± 0.6	58 ± 0.3	48 ± 2.3
5	15	8.0 ± 3.9	27 ± 1.3	3.6 ± 0.2	13 ± 0.7	88 ± 4.4	72 ± 3.43
5	20	16 ± 7.2	32 ± 1.6	4.5 ± 0.2	15 ± 0.8	117 ± 5.5	97 ± 4.7
10	2	0.3 ± 0.1	9.5 ± 0.5	1.2 ± 0.07	10 ± 0.5	43 ± 2.4	55 ± 3.0
10	5	1.8 ± 0.9	19 ± 1.0	2.2 ± 0.1	15 ± 0.7	106 ± 5.4	139 ± 6.9
10	10	7.5 ± 3.9	30 ± 1.5	4.3 ± 0.2	22 ± 1.1	212 ± 11	279 ± 14
10	15	18 ± 9.1	38 ± 2.0	5.9 ± 0.3	29 ± 1.5	316 ± 17	417 ± 22
10	20	29 ± 16	43 ± 2.0	7.3 ± 0.3	36 ± 1.6	424 ± 22	556 ± 30
15	2	0.6 ± 0.3	15 ± 0.7	2.1 ± 0.1	17 ± 1.0	147 ± 6.9	303 ± 15
15	5	4.0 ± 2.0	28 ± 1.4	4.1 ± 0.2	32 ± 1.5	369 ± 17	751 ± 36
15	10	15 ± 6.7	40 ± 1.7	7.0 ± 0.3	55 ± 2.6	734 ± 38	1507 ± 77
15	15	32 ± 17	46 ± 2.7	9.4 ± 0.5	78 ± 4.1	1097 ± 534	2232 ± 111
15	20	60 ± 33	49 ± 2.4	11 ± 0.5	100 ± 4.8	1464 ± 844	2996 ± 158
20	2	1.2 ± 0.6	22 ± 1.2	3.2 ± 0.2	40 ± 2.1	469 ± 24	1536 ± 76
20	5	7.4 ± 3.9	38 ± 1.9	6.6 ± 0.3	85 ± 4.5	1218 ± 60	3811 ± 193
20	10	31 ± 14	48 ± 2.4	11 ± 0.6	150 ± 7.2	2410 ± 110	7465 ± 359
20	15	67 ± 37	50 ± 2.4	13 ± 0.7	206 ± 10	3599 ± 184	10950 ± 508
20	20	119 ± 64	51 ± 2.4	15 ± 0.8	250 ± 13	4782 ± 252	14370 ± 652
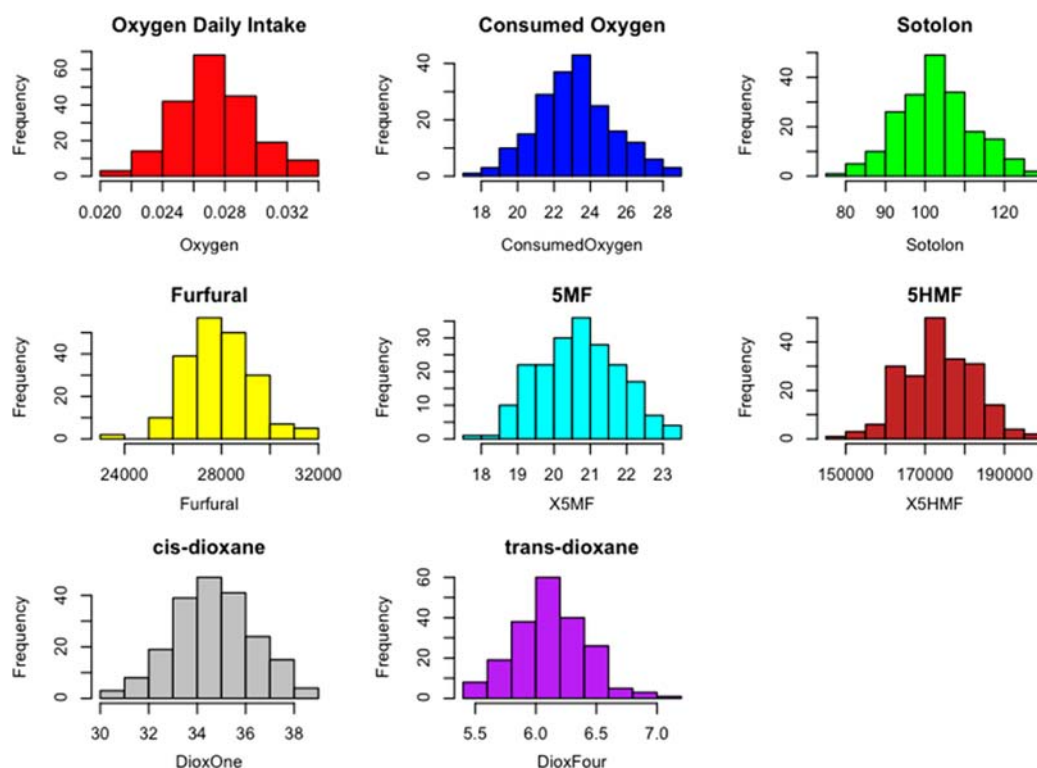
Figure 6. Port wine kinetic rate chart and expected scenario for T = 15 °C, t = 10 years, and O_2 permeability = 10 ± 2 mg/L per year (barrel).

vessels. When considering the barrel-aged wines, there is a clear separation between the two temperatures. A higher temperature (20 °C) was associated with wines of 30–35 years of age, while lower storage temperatures were associated with wines of about 10–20 years of age. For the bottle-aged wines, the wines stored at 10 °C were associated with young Port wines (not aged at all). The wines stored at 20 °C were associated with Port wines in the 5-year-old cluster.

Results further show that, in bottles (O_2 ingress through cork), an O_2 intake of 1.5 ± 0.75 mg/L per year led to a bigger range (larger error) of the sotolon concentration at the end of the 10 year storage period when compared to wines aged in barrels. In Tables 3 and 4, it is possible to observe that the wines stored in bottles have a sotolon concentration of 0.1–118 μg/L as usually found in Port wines of about 10 years of age, whereas during storage in the barrel, an increase in the sotolon concentration was observed to the amount of 1–800 μg/L.

Figure 7. Dendogram of MC predictions for 10-year-old wines stored in bottles and barrels at 10 and 20 °C with the normal aged wines.

It would seem as if the presence of oxygen was the more important parameter in the aging character of Port wines. The separation between temperatures (within a specific oxygen management, e.g., barrel or bottle) was higher for the oxygen-exposed treatment (barrel) when compared to the low oxygen exposure (bottle). It could be concluded that these two parameters work in a synergistic mechanism and amplify the aging effect.

This study showed the kinetics of oxygen consumption. In a wine with a high O_2 concentration, the rate of consumption was higher than when compared to a wine with a low O_2 concentration. At lower concentrations, the consumption rate was much slower, resulting in a longer consumption time. Higher initial O_2 concentrations also resulted in wines with a more pronounced aged bouquet.

Sotolon is mainly responsible for this aged bouquet, and this study shows the importance of oxygen consumption in the formation of this compound, as also reported in other studies.[17,38] It would seem as if the temperature combined with the presence of O_2 could have a synergistic effect, causing a significant increase in the formation of this compound. This study also confirmed the dependency of the formation of other molecules upon the temperature.

The amount of oxygen introduced in wine strictly depends upon the winemaking practices. Consequently, the right oxygen management allows for the modulation of the chemical and sensorial profiles of Port wines. In fact, it allows us to know in which manner the oxygen and temperature in cellars should be controlled and how both the oxygen and temperature affect the quality of the Port wine. It is indeed much more important to control these factors for barrel-aged wines (especially temperature) than for bottle-aged wines because of the high levels of dissolved oxygen in the former.

■ AUTHOR INFORMATION

Corresponding Author
*Telephone: +351-22-558-0001. Fax: +351-22-509-0351. E-mail: asferreira@porto.ucp.pt.

Notes
The authors declare no competing financial interest.

■ REFERENCES

(1) Maillard, L. C. Action des acides aminés sur les sucres: Formation des mélanoidines par voie méthodique. *C. R. Acad. Sci.* **1912**, *154*, 66–68.

(2) Hodge, J. E. Dehydrated foods, chemistry of browning reactions in model systems. *J. Agric. Food Chem.* **1953**, *1* (15), 928–943.

(3) Hoffman, T.; Schieberle, P. Evaluation of the key odorants in a thermally treated solution of ribose and cysteine by aroma extract dilution techniques. *J. Agric. Food Chem.* **1995**, *43*, 2187–2194.

(4) Pipris-Nicolau, L.; Revel, G.; Bertrand, A.; Maujean, A. Formation of flavor components by the reaction of amino acid and carbonyl compounds in mild conditions. *J. Agric. Food Chem.* **2000**, *48*, 3761–3766.

(5) Oliveira e Silva, H.; Guedes de Pinho, P.; Machado, B. P.; Hogg, T.; Marques, J. C.; Câmara, J. S.; Albuquerque, F.; Silva Ferreira, A. C. Impact of forced-aging process on Madeira wine flavor. *J. Agric. Food Chem.* **2008**, *56*, 11989–11996.

(6) Singleton, V. L. Oxygen with phenols and related reactions in musts, wines and model systems: Observations and pratical implications. *Am. J. Enol. Vitic.* **1987**, *38*, 69–77.

(7) Danilewicz, J. C. Review of reaction mechanims of oxygen and proposed intermediates reduction products in wine: Central role of iron and copper. *Am. J. Enol. Vitic.* **2003**, *2003*, 73–85.

(8) Waterhouse, A. L.; Laurie, V. F. Oxidation of wine phenolics: A critical evaluation and hypotheses. *Am. J. Enol. Vitic.* **2006**, *57* (3), 306–313.

(9) du Toit, W.; Marais, J.; Pretorius, I.; Du Toit, M. Oxygen in must and wine: A review. *S. Afr. J. Enol. Vitic.* **2006**, *27*, 76–94.

(10) Kilmartin, P. A. The oxidation of red and white wines and its impact on wine aroma. *Chem. N. Z.* **2009**, *73*, 18–22.

(11) Silva Ferreira, A. C.; Barbe, J.-C.; Bertrand, A. 3-Hydroxy-4,5-dimethyl-2(5*H*)-furanone: A key odorant of the typical aroma of oxidative aged Port wine. *J. Agric. Food Chem.* **2003**, *51*, 4356–4363.

(12) Dubois, P.; Rigaud, J.; Dekimpe, J. Identification of 4,5-dimethyltetrahydrofuran-2,3-dione in a Flor sherry wine. *Lebensm.-Wiss. Technol.* **1976**, *9*, 366–368.

(13) Câmara, J. S.; Marques, J. C.; Alves, M. A.; Silva Ferreira, A. C. 3-Hydroxy-4,5-dimethyl-2(5*H*)-furanone levels in fortified Madeira wines: Relationship to sugar content. *J. Agric. Food Chem.* **2004**, *52*, 6765–6769.

(14) Escudero, A.; Cacho, J.; Ferreira, V. Isolation and identification of odorants generated in wine during its oxidation: A gas chromatography−olfactometric study. *Eur. Food Res. Technol.* **2011**, *2011*, 105–110.

(15) Cutzach, I.; Chatonnet, P.; Dubourdieu, D. Influence of storage conditions on the formation of some volatile compounds in white fortified wines (Vins doux naturels) during the aging process. *J. Agric. Food Chem.* **2000**, *48*, 2340–2345.

(16) Silva Ferreira, A. C.; Hogg, T.; Guedes de Pinho, P. Identification of key odorants related to the typical aroma of oxidation-spoiled white wines. *J. Agric. Food Chem.* **2003**, *51*, 1377–1381.

(17) Ferreira, A. C. S.; Avila, I. M. L. B.; de Pinho, P. G. Sensorial impact of sotolon as the "perceived age" of aged Port wine. In *Natural Flavors and Fragrances*; Frey, C., Rouseff, R., Eds.; American Chemical Society: Washington, D.C., 2005; ACS Symposium Series, Vol. *908*, Chapter 10, pp 141–159.

(18) Maillard, B. Additions radicalaires de diols et de leurs dérivés: Diesters et acétals cycliques. Ph.D. Thesis, University Bordeaux 1, Bordeaux, France, 1971; Number 325.

(19) Arabshahi, A.; Lund, D. B. Considerations in calculating kinetic parameters from experimental data. *J. Food Process. Eng.* **1985**, *7*, 239–251.

(20) Hastie, T.; Tibshirami, R. Discriminant adaptative nearest neighbour classification. *IEEE Trans. Pattern Anal. Mach. Int.* **1996**, *18*, 607–616.

(21) Bates, D. M.; Watts, D. G. *Non-linear Regression Analysis and Its Applications*; John Wiley and Sons: New York, 1998.

(22) Neter, J.; Kutner, M. H.; Nachsteine, C. J.; Wassermen, W. *Applied Linear Statistical Models*; Irwin: Chicago, IL, 1996.

(23) Martins, R. C.; Silva, C. L. M. Computational design of accelerated life testing to frozen foods. *J. Food Eng.* **2004**, *64*, 455–464.

(24) Ricardo, V.; James, H. Reaction kinetics in food systems. *Handbook of Food Engineering*, 2nd ed.; CRC Press: Boca Raton, FL, 2006; pp 125–286.

(25) Baig, S.; Rehman, F. Signal modeling using singular value decomposition. In *Advances in Computer, Information, and Systems Sciences, and Engineering*; Elleithy, K., Sobh, T., Mahmood, A., Iskander, M., Karim, M., Eds.; Springer-Verlag: Berlin, Germany, 2006; pp 31–37.

(26) Nomikos, P.; MacGregor, J. Monitoring batch process using multiway principal component analysis. *AIChE J.* **1994**, *40*, 1361–1375.

(27) Westerhuis, J. A.; Gurden, S. P.; Smilde, A. K. Generalized contribution plots in multivariate statistical process monitoring. *Chem. Intell. Lab. Syst.* **2000**, *51* (1), 95–114.

(28) Conlin, A. K.; Martin, E. B.; Morris, A. J. Confidence limits for contribution plots. *J. Chemom.* **2000**, *14*, 725–736.

(29) Qin, S. J. Statistical process monitoring: Basics and beyond. *J. Chemom.* **2003**, *17*, 480–502.

(30) Kindratenko, V. V.; Treiger, B. A.; Van-Espen, P. J. M. Classification of silver microcrystals via K-NN clustering of their shape descriptors. *J. Chemom.* **1997**, *11*, 131–139.

(31) Hahn, G. J.; Shapiro, S. S. *Statistical Models in Engineering*; John Wiley and Sons: Hoboken, NJ, 1997.

(32) Gardiner, C. W. *Handbook of Stochastic Methods for Physics, Chemistry and the Natural Sciences*; Springer-Verlag: Berlin, Germany, 1997.

(33) Australian Wine Research Institute (AWRI). *The Australian Wine Research Institute Annual Report 2004*; AWRI: Adelaide, South Australia, Australia, 2004.

(34) Lopes, P.; Saucier, C.; Teissedre, P. L.; Glories, Y. Impact of storage position on oxygen ingress through different closures into wine bottles. *J. Agric. Food Chem.* **2006**, *54*, 6741–6746.

(35) Lopes, P.; Silva, M. A.; Pons, A.; Tominaga, T.; Lavigne, V.; Saucier, C.; Darriet, P.; Teissedre, P. L.; Dubourdieu, D. Impact of oxygen dissolved at bottling and transmitted through closures on the composition and sensory properties of a Sauvignon Blanc wine during bottle storage. *J. Agric. Food Chem.* **2009**, *57* (21), 10261–10270.

(36) Formosinho, S. J. *Fundamentos de Cinética Química*; Fundação Calouste Gulbenkian: Lisbon, Portugal, 1983.

(37) Silva Ferreira, A. C.; Barbe, J.-C.; Bertrand, A. Heterocyclic acetals from glycerol and acetaldehyde in Port wines: Evolution with aging. *J. Agric. Food Chem.* **2002**, *50*, 2560–2564.

(38) Lavigne, V.; Pons, A.; Darriet, P.; Dubourdieu, D. Changes in the sotolon content of dry white wines during barrel and bottle aging. *J. Agric. Food Chem.* **2008**, *56* (8), 2688–2693.