Untargeted voltammetric approaches for characterization of oxidation patterns in white wines

Asael Gonzalez, Stephane Vidal, Maurizio Ugliano*

Nomacorc France, 7 Av. Yves Cazeaux, 30230 Rodilhan, France

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ABSTRACT

Chemical and electrochemical changes associated with controlled oxidation were measured in thirteen commercial white wines, in order to evaluate the potential of linear sweep voltammetry to provide relevant information on the oxidative behavior of individual wines. For a given amount of oxygen consumed, substantial diversity of oxidative behaviors was observed. A good correlation ($R^2 = 0.69$) was observed between the rate of O_2 consumption of individual wines and the total charged passed during linear sweep voltammetry, but not with their Folin-Ciocalteu values. Onset potential of anodic oxidation was also related to oxygen consumption capacity of wine, indicating an important contribution of easily oxidizable substrates. Subtraction of voltammograms of oxidized wines from their corresponding non-oxidized controls generated new voltammograms representative of the global changes induced by oxidation. These new voltammograms contained several features related to oxygen consumption rates of each wine, and could be considered as a 'wine oxidation signature'.

1. Introduction

During the storage of white wine in bottles, tanks, or barrels, undesirable changes can occur, due to reaction between atmospheric oxygen and wine components. These modifications, generally referred to as oxidative spoilage, result in browning and loss of fresh, fruity, and varietal aroma characters (Ugliano, 2013; Ugliano et al., 2011). Certain protective agents such as SO_2 are also typically lost with the onset of oxidative spoilage, further exposing the wine to quality depreciation. Recent studies have shown that the ability of a wine to react with oxygen and the outcomes of oxidation in terms of degree of oxidative spoilage are strongly wine specific (Danilewicz, 2016; Ferreira et al., 2015; Waterhouse et al., 2016) so that certain wines appear to be more resistant against oxidative spoilage. Wine phenolics, in particular those having an ortho-diphenol structure, are supposed to be the first substrates of oxidation, generating quinones and hydrogen peroxide that are able to propagate oxidative reactions to aroma and phenolic components. Nucleophilic addition is one major reaction mechanisms associated with wine oxidative spoilage, having quinones as key substrate (Danilewicz, 2016; Ugliano, 2013). Therefore, competition among different wine nucleophiles for oxidation-derived quinones could play a central role in the specificity of wine response to oxidation. Several strong nucleophiles able to react with quinones are present in wine, including SO_2, phenolics, amino acids, ascorbic acid, and volatile and non-volatile thiols (Danilewicz, 2003, 2016; Nikolantonaki, Chichuc,

Teissedre & Darriet, 2010; Nikolantonaki & Waterhouse, 2012; Nikolantonaki, Magiatis & Waterhouse, 2014; Oliveira et al., 2017). However, due to the remarkable complexity of wine composition, the actual pool of reactions competing for central oxidation intermediates such as quinones is difficult to evaluate, with multiple competing reactions taking place at the same time. In a recent study on the oxidation of red wines, the unusual behavior of certain wines was described in which reaction with oxygen did not result in SO_2 loss, suggesting that wine could contain other more reactive antioxidants (Ferreira et al., 2015). Application of advanced 'omic' approaches to the study of wine oxidation also allowed to describe for the first time consumption of SO_2 via sulfonation reactions (Arapitsas et al., 2016). In spite of these advances, the wine-dependent nature of oxidative response of wine remains difficult to rationalize, and the practical management of oxidation in the winery remains challenging, especially in the case of white wines.

Electrochemical methods, in particular linear sweep and cyclic voltammetry using either glassy carbon or carbon paste electrodes, have been applied to the analysis of wine phenolics (Kilmartin et al., 2001, 2002; Makhotkina & Kilmartin, 2012; Ugliano, 2016). In addition, some studies have shown that voltammetry can be used to study the reactions of quinones in the presence of different nucleophiles (Makhotkina & Kilmartin, 2009; Oliveira, Barros, Silva Ferreira, & Silva, 2016), therefore providing an interesting approach to the study of wine oxidation. However, due to the complexity of wine composition,

* Corresponding author at: Department of Biotechnology, University of Verona, Villa Lebrecht, Via della Pieve – 37020 S. Floriano di S. Pietro in Cariano (VR), Italy.
E-mail addresses: s.vidal@nomacorc.be (S. Vidal), maurizio.ugliano@univr.it (M. Ugliano).

the voltammetric signal obtained during wine analysis appears rather convoluted so that it is difficult to associate specific voltammetric features to oxidation of individual compounds or reaction products. Deconvolution strategies have been proposed to resolve voltammetric peaks, but they have rarely been applied to wine analysis. Chemometric approaches involving multivariate statistical methods have been used in order to maximize the relevant chemical information obtained by analyzing voltammetric data (Palys, Orba, Bos, & van der Linden, 1991; Myung-Hoon et al., 1993), especially in conjunction with multi-array systems such as electronic noses and tongues (Gay et al., 2010; Rodriguez-Mendez et al., 2014). In recent years, the introduction of disposable electrochemical sensors has opened novel possibilities in electrochemical analyses, increasing specificity towards single analytes and facilitating analytical procedure (Apetrei, Apetrei, De Saja & Rodriguez-Mendez, 2011; Ugliano, 2016).

In the present study, linear sweep voltammetry using disposable carbon paste electrodes was applied to the study of the behavior of 13 commercial white wines under conditions of controlled oxidation. Voltammetric signals of oxidized and non-oxidized wines were treated by means of different strategies, and raw and treated voltammograms were compared with chemical parameters related to wine oxidation, in order to assess the potential of linear sweep voltammetry to provide relevant characterization of white wine oxidative behaviors.

2. Materials and methods

2.1. Wines

The wines used in the study are reported in Supplementary Table 1, along with some of the main compositional parameters. All wines were purchased in retail stores or directly at the winery, and were from either 2012 or 2013 vintages. Four bottles (750 mL) of the same production batch were obtained for each wine.

2.2. Oxidation experiments

Bottles of each wine were pooled together by carefully transferring the content of each bottle in a 3 L vessel under a stream of N_2. Dissolved oxygen was measured using a Nomasense P6000 oxygen analyzer fitted with a dipping probe (Nomacorc, Thimister, Belgium) ensuring that dissolved oxygen was below 200 µg/L. Where required, wines were deoxygenated by bubbling N_2 until a value lower than 200 µg/L was obtained. For each wine two modalities were then prepared, one non-oxygenated and one oxygenated. For the non-oxygenated samples, 375 mL transparent glass bottles fitted with Pst3 oxygen sensors (Nomacorc, Thimister, Belgium) for non-destructive measurement of dissolved oxygen were flushed with N_2 and filled under a N_2 stream with samples of each wine. Headspace was then flushed with N_2, and each bottle was sealed with synthetic co-extruded closures (Nomacorc, Thimister, Belgium). Fill level was adjusted so that after closure insertion no ullage was present. After 40 min from filling, dissolved oxygen was measured, confirming that in all cases it was lower than 200 µg/L. The bottles prepared under these conditions represented the non-oxygenated control, accounting for all transformation occurring during the timeframe of the experiment and not associated with oxygen. The remaining portion of the original 3 L blend of each wine was vigorously shaken in air until a final concentration of 6.5 ± 0.2 mg/L of dissolved oxygen was obtained. Each wine was then transferred into 375 mL transparent glass bottles previously fitted with the same sensors as above, which were sealed as described. Oxygen measurement confirmed that in all cases wines contained 6.5 ± 0.2 mg/L of dissolved oxygen. All bottles were incubated at 22 ± 0.5 °C. Oxygen consumption in the bottles was assessed daily by non-destructive measurement of dissolved oxygen. Upon consumption of 5 ± 0.1 mg/L of oxygen, bottles of oxygenated wines as well as non-oxygenated controls were opened and analyzed, so that all wines consumed an equal amount of oxygen within a reasonably short and similar timeframe. All experiments were carried out in duplicate.

2.3. Chemical analyses

Voltammetric experiments were performed with a commercial Nomasense Polyscan electrochemical analyzer (Nomacorc, Belgium) using disposable screen printed sensors in a three-electrode arrangement. The working electrode (WE) was a screen-printed carbon paste electrode operating in conjunction with a screen-printed carbon paste counter electrode and a silver/silver-chloride (Ag/AgCl) reference electrode. A drop of sample was loaded onto a sensor, and linear sweep voltammograms were acquired between 0 and 1200 mV at a scan rate of 100 mV/s. After each measurement, the sensor was discarded and a new sensor was used. All measurements were carried out in duplicate. All potentials are reported against Ag/AgCl reference electrode. Derivative voltammograms were obtained with The Unscrambler (Camo, Norway), applying a 10 points Savitzky-Golay smoothing. Principal component Analysis (PCA) of raw and derivative voltammograms was carried out using The Unscrambler (Camo, Norway).

Free SO_2 measurements were carried out using a multi-channel flow injection analyzer FIAStar 5000 (FOSS Analytical, Rellingen, Germany). Calibration curves were prepared daily using solutions with known free SO_2 concentration (0, 10, 20, 30, 50, 100, 120, 150, 180 and 200 mg/L of free SO_2). Analyses were made in duplicate, with samples randomly placed in the sampler wheel. Total phenolics were determined by the Folin-Ciocalteu method. Falvan-3-ols and phenolic acids were analyzed by HPLC using a Jasco HPLC system (Jasco, Oklahoma City, USA) comprising a PU 2089+ ternary pump, a AS 2057 autosampler, a MD 2010+ 330 photodiode array detector and. The column was thermostated at 20 ± 1 °C. After injecting 10 µL of sample, separation was performed in an Aces 5 C_{18} 250 × 4.6 mm column (Advanced Chromatography Technologies, Aberdeen, Scotland). For detection and quantification of compounds, the chromatograms were recorded at 280, 320, 360 nm.

3. Results

Oxidation experiments lasted between 5 and 16 days, namely the time required to consume an oxygen dose of approximately 5 mg/L. Across all wines and replicates, total consumed oxygen averaged at 4.9 ± 0.4 mg/L for the oxidized samples. In the case of the non-oxidized controls, average total consumed oxygen was 0.12 mg/L ± 0.03. Considering the short time period, it can be assumed that oxygen ingress from or through the closure was negligible, so that only evolution of dissolved oxygen should be taken into account. Oxygen consumption parameters of the different white wines studied are summarized in Table 1. The wines displayed rather large differences in average O_2 consumption rates, which ranged from 0.26 mg O_2/L/h for the slowest sample to 1.26 mg O_2/L/h for the fastest sample. Table 1 also provides values for total free SO_2 lost as well as free SO_2 lost per mg of O_2 consumed. The amount of free SO_2 lost with oxidation ranged from 5.7 mg/L to 10.5 mg/L, while values of the ratio SO_2 lost/mg of O_2 consumed ranged from 1.1 to 2.3.

Linear sweep voltammograms of the different wines are shown in Fig. 1, highlighting large diversity of electrochemical properties and therefore of concentration and type of oxidizable compounds present. Readily oxidizable compounds such as caffeic acid, catechin, epicatechin, epigallocatechin gallate, and epicatechin gallate, which oxidize at the surface of the screen printed sensors, provide an electrochemical signal in the 335–390 mV range. Anodic current measured in this potential range varied greatly across different wines, with samples W2, W4, and W7 exhibiting the highest signal. Conversely, samples W5 and W12 exhibited the lowest anodic current in this region of the voltammogram. Catechin, epicatechin, epigallocatechin and epigallocatechin gallate are also expected to exhibit a second peak at approximately