Electrochemical triggering of the Chardonnay wine metabolome

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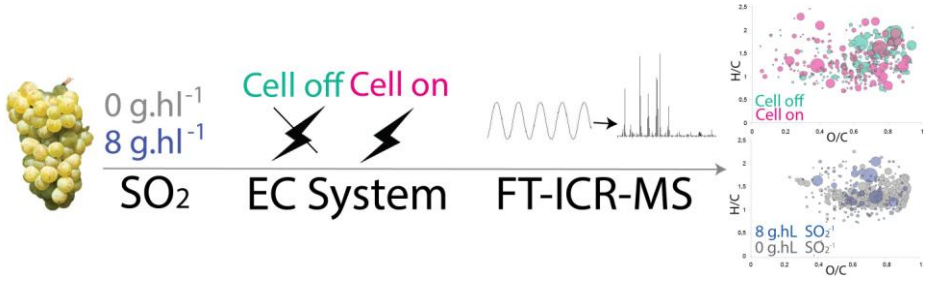
TOC graph: The electrochemical oxidation of two bottle-aged wines, which only differ by the SO_2 dose added at pressing, was shown to induce different reactions related to this pre-fermentation winemaking process. Ultrahigh resolution mass spectrometry revealed the corresponding oxidation fingerprints and the impact of the SO_2 initially added to the must.



Abstract

Oxidation of wine upon bottle ageing is a crucial matter of concern for the qualitative long-term storage of white wines. However, understanding the various molecular mechanisms potentially involved, which can impact the wine composition, requires that top-down analytical strategies are implemented. Here, we report the analysis of bottle aged Chardonnay wines made from the same must, but differing by the amount of SO_2 initially added to the must at pressing (0 and 8 $g.hL^{-1}$). Metabolomics fingerprints obtained from electrochemical simulation of oxidative reactions were obtained by the coupling of either on-line or off-line electrochemical oxidation to FT-ICR-MS detection. We reveal that, whatever the electrochemical DC voltage, wines with initial SO_2 addition displayed molecular fingerprints, which remained more similar to the non-oxidized wine without initial SO_2 addition. We further show that a diversity of sulfur-containing compounds appeared to be the most sensitive to oxidation, whereas nitrogen-containing compounds were mostly formed.

Keywords: Chardonnay wine; oxidation; SO2; FT-ICR-MS; electrochemistry (EC).

1. Introduction

In contrast with many other food products, quality wines are designed to age for durations that can vary from weeks after bottling to potentially decades. If this intuitively applies for red wines, it is also the case for white wines, and in particular for well-known dry Chardonnay wines from Burgundy. During its bottle ageing time, where various chemical changes occur (Hernanz et al., 2009; Kallithraka, Salacha, & Tzourou, 2009; Maury, Clark, & Scollary, 2010), the wine experiences three consecutive stages, corresponding to maturation and improved stability, organoleptic optimum, and decline (Arapitsas, Speri, Angeli, Perenzoni, & Mattivi, 2014). Through its antioxidant, antioxidasic and antiseptic properties, SO_2 is considered as an essential tool for promoting wine ageing, in particular for white wines, where it is used to prevent from browning (Li, Guo, & Wang, 2008), to slow down the decrease of desired aromas (Escudero, Asensio, Cacho, & Ferreira, 2002; Nikolantonaki, Magiatis, & Waterhouse, 2014; Roussis, Lambropoulos, & Tzimas, 2007), or more

generally to protect wine from autoxidation, which naturally occurs because of the intrinsic permeability to oxygen of stoppers (Karbowiak, Mansfield, Barrera-García, & Chassagne, 2010; Ugliano, 2013; Waterhouse et al., 2016).

The antioxidant properties of SO_2 in wine have already been the subject of several studies, and if various mechanisms have been proposed, an integrated description of its impact on the chemistry of wine ageing remains to be elucidated. Results from experiments run on model wine have shown that oxygen is supposed to react only weakly with molecular SO_2, but its reaction with hydrogen peroxide prevents ethanol oxidation via the Fenton reaction (Danilewicz, 2007; Waterhouse & Laurie, 2006). Due to its inherent nucleophilic character, the bisulfite ion (HSO_3^-) easily reacts with carbonyl-containing compounds, in particular with quinones, either to form sulfonic adducts or to reduce them back to dihydroxybenzenes (Danilewicz, Seccombe, & Whelan, 2008; Maujean, 2001). However, it was also shown that these reactions depend on various parameters, including the structure of the involved phenolics, the concentration of transition element catalysts or the composition of organic acids (Danilewicz, 2011, 2014; Makhotkina & Kilmartin, 2013). Through untargeted LC–MS analyses, Arapitsas et al. showed that SO_2 added to wine takes part in various reactions including the formation of sulfonated adducts of indole-3-lactic hexoside, tryptophol, glutathione, cysteine and pantetheine (Arapitsas et al., 2016). They further demonstrated that the sulfonation of metabolites with an indole scaffold dominated in white wines (Arapitsas, Guella, & Mattivi, 2018). However, and except for indole-3-lactic acid, for which the adducts could reach concentrations up to 8 mg/L, most of these identified adducts appeared to exist at low concentrations in white wine, thus suggesting that the actual chemistry related to SO_2 combinations certainly involves a significantly higher diversity of wine metabolites.

SO_2 can be added at different times of the winemaking of whites, starting with the protection of the must before, during, or immediately after pressing. Then if malolactic fermentation is desired, SO_2 is added at the end to protect the wine during ageing, which can proceed in barrels or in tanks. Finally, SO_2 is added before bottling. Although the first must addition assumes mostly antioxidasic and antiseptic purposes, several studies have shown that it has an impact on the long-term oxidative

stability of white wines (Cheynier, Masson, Rigaud, & Moutounet, 1993; Ugliano, 2013)(Cejudo-Bastante, Hermosín-Gutiérrez, Castro-Vázquez, & Pérez-Coello, 2011; Schneider, 1998) (Cheynier, Souquet, Samson, & Moutounet, 1991). Recently, and through untargeted Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR-MS) metabolomics, we have further shown that hundreds of masses and likely thousands of must and wine compounds are actually involved in chemical mechanisms related to SO_2 addition to the must, including amino acids, organic acids, carbohydrates or polyphenols, and that these compounds, which are still present after several years of bottle ageing, allow discrimination of bottle aged white wines according to the SO_2 concentration added at pressing (Chloé Roullier-Gall et al., 2017).

However, in a context where there is a trend over recent years to limit the use of SO_2 (Ancín-Azpilicueta, Jiménez-Moreno, Moler, Nieto-Rojo, & Urmeneta, 2016), and consequently an increasing demand for alternatives, understanding molecular mechanisms by which SO_2 operates as antioxidant during the long-term bottle ageing of wine is a prerequisite. Measuring an antioxidant capacity of wine and relating it to the many potentially involved antioxidant compounds remains a challenge. In order to get antioxidant capacity of wine, electrochemical methods have proved to be interesting alternative strategies to classical spectrophotometric methods, which monitor the formation or the disappearance of radical-related chromogenic compounds (Hoyos-Arbeláez, Vázquez, & Contreras-Calderón, 2017). Different authors have indeed attempted to provide estimated antioxidant capacity index or electrochemical index of wines, based in particular on voltammetric analyses (Gao et al., 2015; Kilmartin, Zou, & Waterhouse, 2001; Lino et al., 2014). It has been shown in particular, that anodic currents related to the oxidation of selected polyphenols could be selectively monitored (Makhotkina & Kilmartin, 2009). Considering representative wine polyphenols (catechin, caffeic acid and quercetin) in model wine conditions, these authors further showed that in the presence of SO_2, electrochemical oxidation could lead to semiquinone radicals, which can be either reduced back to the original polyphenol, or further oxidized to the quinone form. However, in combination with HPLC-MS analyses, they demonstrated that depending on the polyphenol structure, quinones could either react with SO_2 and produce new derivatives, or undergo chemical reactions to produce several new

compounds without SO_2 combination (Makhotkina & Kilmartin, 2013). This is one of very few examples combining electrochemical and mass spectrometric analyses of wine related samples; it also points to the limits of targeted molecular analyses, which are far from characterizing the more complete diversity of wine compounds potentially involved in the antioxidant process.

In this paper, combination of electrochemical oxidation strategies and ultra-high resolution FT-ICR-MS were used to characterize the antioxidant property of white wines from an untargeted molecular point of view. Seven year old bottled Chardonnay white wines, only differing by the concentration of SO_2 added to the must at pressing, were considered here in order to account for the intrinsic chemical diversity related to the various chemical pathways, through which SO_2 is involved during the first years of bottle ageing.

2. Experimental

2.1 Wine Samples.

Chardonnay wines, from Montagny appellation in Burgundy, France and from the 2007 vintages, were analyzed. For each of the conditions, 3 biological replicates (bottles) were analyzed. These wines were originated from one single must, which was treated with two different concentrations of sulfur dioxide (0 and 8 $g.hL^{-1}$) immediately after pressing, and before alcoholic fermentation. All wines were bottled after supplementation of a constant SO_2 addition to ensure a free SO_2 concentration of the order of 35 mg/L. Free and total SO2 concentrations were measured after 6 months of bottle ageing. Wines with 0 $g.hL^{-1}$ SO_2 addition at pressing contained in average 17.5 mg/L free SO_2 and 91 mg/L total SO_2 and wine with 8 $.hL^{-1}$ SO_2 addition at pressing contained 17.5 mg/L free SO_2 and 120.5 mg/L total SO_2. Electro oxidation experiments were done between January and June 2015.

2.2 Oxidation by Electrochemistry.

2.2.1 On-line electrochemistry: An electrochemical μ-PrepCell™ Roxy (Antec, Netherlands) equipped with a conductive diamond (MD) working electrode was used (Supplemental Figure 1). A Pd/H_2 electrode was used as a reference electrode (RE) and titanium electrode as a counter electrode. Samples were analyzed at three different electrical DC potentials (400 mV, 800 mV and 1200 mV). Additionally the samples were mass spectrometrically acquired without applying a potential (0 mV). The liquid outlet of the electrochemical cell was connected directly to the electrospray source of a FT-ICR-MS (Bruker Daltonics, Germany), which is used to record mass spectra. Diluted Wine (1/20) in a solvent mixture of (50:50 water : methanol) was pumped through the electrochemical cell at a flow rate of 10 $\mu L.min^{-1}$. The cell volume is 11 μL and the working electrode area is 1.9 cm^2. The residence time of the wine in the μ-PrepCell™ Roxy cell was 1 min and 6 s.

2.2.2 Off-line electrochemistry: EC experiments were acquired using a DLK-70 Web-Pstat™ Potentiostat (Analytical Instrument Systems, Flemington, USA) (Supplemental Figure 1). A self-made copper electrode was used as working electrode (WE), an Ag/AgCl electrode was used as a reference electrode (RE) and Pt electrode as a counter electrode. The working electrode area is 1.3 cm^2. The surface area is calculated by assuming cylindrical geometry of wires measured to be 75 um in diameter each. For each sample, a new copper WE electrode was used in order to ensure same conditions and unmodified surface. 2 ml of each sample were treated with constant potentials (500 mV, 1000 mV and 1500 mV) applied between the copper WE and Ag/AgCl RE. Wine samples were collected after 10 min of EC treatment. After electrochemical treatment the samples were analyzed by direct infusion FT-ICR-MS.

2.3 Ion Cyclotron Resonance Fourier Transform Mass Spectrometer.

Ultrahigh resolution mass spectra were acquired using a solariX FT-ICR-MS instrument (Bruker Daltonik, Bremen, Germany) equipped with a 12 Tesla superconducting magnet and an Apollo II electrospray ionization source operated in the negative ionization mode. 50 μL of the samples were diluted in 1 ml of methanol prior to injection and introduced into the micro electrospray source at a

flow rate of 120µL.h^{-1} using a syringe pump. The MS was externally calibrated based on cluster ions of arginine (10 ppm). The mass range is *m/z* 100 to 1000 Da. 300 scans were accumulated in an acquisition for each sample and spectra were acquired with a time domain of 4 mega-words. Spectra were internally recalibrated using a composed list of fatty acids and recurrent wine compounds, applying linear calibration until *m/z* 1000, with mass errors below 50 ppb. Peaks with a signal to noise ratio (S/N) of 4 and higher were used for further data processing. In addition to an automated theoretical isotope pattern comparison, the generated formulas were further validated by setting sensible chemical constraints (N rule; O/C ratio ≤ 1; H/C ratio ≤ 2n+2; element counts: C ≤ 100, H ≤ 200, O ≤ 80, N ≤ 3, S ≤ 3 and P ≤ 1). FT-ICR-MS peak alignment and filtering of masses were performed in MS Excel 2010 (Microsoft, Redmond, USA) with maximum error thresholds of 1 ppm and filtered for masses occurring at least in 10% of all samples (Roullier-Gall, Witting, Gougeon, & Schmitt-Kopplin, 2014).

3. Results and discussion

3.1 On-line EC-MS simulation of oxidative ageing

Initially, on-line EC-MS experiments were performed to obtain information on the electrochemical oxidation behavior of white wine depending on the SO_2 dose. Two wines, from identical musts, treated immediately after pressing with two different concentrations of sulfur dioxide (0 and 8 g.hL^{-1}) were subjected to DC potentials (from 0 to 1200 mV). A commercial electrochemical flow-through cell (µ-PrepCell^{TM}) including a conductive diamond working electrode, a palladium counter electrode and a Pd/H_2 reference electrode was used. The hierarchical cluster analysis based on the matrix of all masses for wines with 0 and 8 g.hL^{-1} SO_2 initially added to the must is presented in Figure 1. The SO_2 dose (0 or 8 g.hL^{-1} SO_2) was used as initial condition prior to apply any electrical potential. Samples were clustered into two groups (Figure 1A). The first group corresponds to sulfur treated samples (8 g.hL^{-1} SO_2 in blue), independently of the applied voltage (0, 400, 800 and 1200 mV) in addition to the zero SO_2 added sample (0 g.hL^{-1} in grey) prior electrochemical oxidation (0 mV). The second group

corresponds to wines without SO_2 addition at pressing (0 g.hL^{-1} in grey) after electrochemical oxidation (400, 800 and 1200 mV) (Figure 1A).

Wines without SO_2 addition at pressing showed significant compositional changes (chemical reactivity) when a DC potential of 400 mV is applied. This was the minimum required DC voltage, below which no significant modifications could be observed with our FT-ICR-MS detection, although some phenolics can oxidize already below that value (Makhotkina & Kilmartin, 2013; Ugliano, 2016). With increasing DC voltages from 400mV to 1200mV, more compositional changes and sharper signal intensity changes could be experimentally observed with the on-line EC system (Figure and Supplemental Figure 2). Wines without SO_2 addition at pressing (0 g.hL^{-1} of SO_2 in grey) showed the highest internal variation of elemental chemical compositions due to electrical potential (Figure 1A). The composition of sulfur treated samples (8 g.hL^{-1} of SO_2 in blue) appeared to be less impacted by electrochemical oxidation (Figure 1A). Additional conclusions can be elucidated considering the masses that show a significant change in peak intensity after electrochemical treatment (van Krevelen diagrams shown in Figure 1B and C).

Some metabolites were characterized by decreasing intensities as a result of the oxidation process and some others were newly formed (Figure 1B and C, supplemental Figure 3). Masses, whose signal intensity decreased as a function of the applied electrical potential, were considered reactive compounds toward electro-oxidation. These reactive compounds, which are located in the polyphenol and peptide regions, as seen in the Van Krevelen diagrams, represent mostly sulfur containing species (CHOS and CHNOS) (Figure 1 and Supplementary Table 1). Masses, whose signal intensities significantly increase as a function of applied electrochemical DC potential, are considered electro-oxidation products. These products are mainly nitrogen containing compounds (CHON, orange) and CHO compounds (CHO in blue) (Figure 1 and Supplementary Table 1). According to Supplementary Table 1, a larger number of reactive compounds were found in wines without SO_2 addition at pressing (385 decreasing masses) compared to wines for which the must received 8 g.hL^{-1} of SO_2 (222 decreasing masses). Interestingly, the number of oxidation products was of the same order for the two

types of wines (171 increasing masses for wine without SO_2 addition at pressing and 157 for SO_2 treated musts) (Supplementary Table 1). The comparison of van Krevelen diagrams of reactive compounds and products toward electro-oxidation highlighted a shift from low to higher O/C ratio as expected for oxidation processes (Roullier-Gall et al., 2016). Specific formulas of reactive compounds are distributed along O/C ratio from 0.2 to 1 whereas those of products are mainly located between 0.4 and 1 (Figure 1B and C). Altogether, bearing in mind that all wines received SO_2 at bottling, and that high additions of SO_2 to the must tend to produce wines with higher levels of combined SO_2 (as shown by SO_2 levels after bottling), our results suggest first that those compounds reacting to electrochemical oxidation are not necessarily those, which had reacted with SO_2 added to the must, and second that those compounds reacting to electrochemical oxidation are in particular sulfur- and nitrogen-containing compounds, which may either be native compounds from the grape or produced by fermentation. Therefore, these results provide unprecedented insights into the distinct chemical mechanisms involved in the reactivity toward electrochemical oxidation of bottle aged Chardonnay white wines, as a consequence of distinct levels of SO_2 initially added to the must.

Combining the on-line EC device with FT-ICR-MS is a fast and simple issue for getting a quick insight into the oxidative resistance of wines. On the other hand, the off-line system allows for a better control of the experimental setup. For example, the on-line system cannot tolerate high concentration of infused wine due to oversaturation of the working electrode and also because the electrospray ionization source, which is directly connected to the on-line system, cannot deal with high ion abundance which would consequently cause ICR cell ion oversaturation problems. For the off-line electrochemical approach, it is possible to use larger wine concentrations and flexibly extended oxidation periods for achieving better oxidation efficiency. The final oxidation products can be consequently further diluted in methanol to suit the electrospray source, and ICR-MS operation requirements. The extent of electrochemical oxidation is easily controllable by manipulating the time of electrochemical treatment and the ratio of electroactive surface area to fluid (i.e. higher surface area yields a faster reaction rate). For example, the time constraint in the on-line system is 1.1 min, whereas there is actually no time constraint for the off-line EC approach.

3.2 Off-line versus on-line coupling

We compared the on-line system and off-line system combined with FT-ICR-MS at 0 and 1000 mV for the same wine samples with and without SO_2 addition at pressing (Supplementary Table 2). With increasing DC voltages from 400 mV to 1200 mV, more compositional changes and sharper signal intensity changes had already been experimentally observed with the on-line system (Supplementary Figure 3). Based on these results, we used a standard potential of 1000 mV for off-line EC oxidation experiments. Using 10 min off-line electrochemical oxidation led to much more oxidation products with high m/z signal intensities (Figure 2). For samples without SO_2 addition (0 $g.hL^{-1}$), and using the off-line system, the total number of detected masses was higher, 7,637 versus 5,710 formulas for off-line versus on-line, independently of the DC voltage applied (Supplementary Table 2 and Figure 2). Histograms of unique formulae, colored according to chemical families (CHO, CHOS, CHNO and CHNOS), showed differences in the number of compounds for "cell off" (0 mV) and "cell on" (DC potential of 1000mV) as a function of the SO_2 dose (Figure 2). New compounds have been formed during the simulated oxidation (Figure 2 and Supplementary Table 2). Venn diagrams of the wine composition for "cell off" and "cell on" conditions confirm that numerous compounds appear and disappear during the simulated oxidation. According to venn diagrams and histograms of elemental formulas sorted by chemical families, similarities could be observed between both systems. In agreement with the literature (Arapitsas et al., 2018; Ugliano, 2016), masses corresponding to catechin/epicatechin and caffeic acid are examples for reactive compounds for both on-line and off-line systems, but with distinct behaviours according to the SO_2 initially added to the must (Supplementary Table 3). The concentration decrease for these two compounds appeared to be more significant for wines which had no SO_2 addition to the must (Supplementary Figure 4), consistently with the fact that these reactive compounds are less concentrated in these wines, and/or that some new compounds formed upon initial addition of SO_2 to the must exhibit higher antioxidant efficiency than these polyphenols, On the basis that a 1000 mV voltage would likely promote the oxidation of the A ring of catechin/epicatechin, such results shows that adding SO_2 to the must would limitate this reaction both for the monomer and the adduct. A close number of elemental formulas impacted by DC

voltage are detected using on-line and off-line systems and sulfur containing compounds (CHOS) were mostly consumed, whereas nitrogen containing compounds (CHON and CHONS) were mostly created regardless of the oxidation system used (Figure 2).

3.3 Resistance to electrochemical oxidation chemical fingerprints

Simulated electrochemical oxidation using the Off-line system coupled to FT-ICR-MS, did not only induce a complete appearance or disappearance of some compounds. It also changed relative concentrations of many others, represented by changes in peak intensity. More than 60% of the total masses were present in wine samples regardless of experimental conditions (cell off and on). As example, for wines without SO_2 added, 6662 masses, out of 7637, were present in both conditions (cell off and on) (Figure 2). However, changes in intensity induced by an electrochemical potential could be observed also for these omnipresent compounds. Specific masses with significantly higher intensities for "cell off" and "cell on" (1000 mV) samples were extracted for wines without SO_2 added (Figure 3). The "cell off" samples were discriminated based on 646 masses with significantly higher intensities than for "cell on" samples (ANOVA test, p-values ≤ 0.01). The corresponding elemental formulae were represented in van Krevelen diagrams according to H/C and O/C ratio and in histograms according to chemical family (CHO, CHOS, CHNO and CHNOS) (Figure 3). Discriminant masses for "cell off" showed a large variety of elemental formulas mainly with sulfur and nitrogen containing compounds (in red) consistently with previous results (Chloé Roullier-Gall et al., 2017). "Cell on" (1000 mV) samples were characterized by 1120 masses, which were significantly higher in signal intensities when compared to the wine samples prior to electro-oxidation (cell off) (ANOVA test, p-values ≤ 0.01). A large number of sulfur containing compounds (CHOS and CHONS) showed variation of intensity after the electrochemical oxidation and were found in areas corresponding to peptides, amino acids, and Maillard reaction products (Hemmler et al., 2017), suggesting the onset of a diversity of chemical reactions, which may include for instance Strecker degradation or Michael addition of amino acids/peptides to quinones (Bittner, 2006), when

polyphenols are consumed (Supplementary Table 3). In agreement with on-line experiments above (Figure 2), discriminant masses for oxidized samples (cell on in Figure 3B) were characterized by a higher number of sulfur containing compounds (46 CHOS compounds, in green) and nitrogen and sulfur containing compounds (133 CHNOS compounds, in red). Most of these masses were concentrated in the area of carbohydrates, and phenolic compounds, suggesting a possible sulfonation of these compounds. The comparison of these results to a recent study on the natural oxygenation of champagne wines (Roullier-Gall et al., 2016) revealed 38 common markers between low oxygenated champagne wines and markers of samples prior to electro-oxidation (cell off markers) and 13 common markers between high oxygenated champagne wines and markers of samples after electro-oxidation (Supplementary Table 4).

During electro oxidation there is also a noticeable hydrogen loss. A decreasing number of hydrogen atoms in a molecule increases unsaturation and hence leads to higher double bond equivalent (DBE) values. However, since large molecules can potentially contain more double bonds and rings than small molecules, in this study the DBE was normalized to the total number of carbon atoms (DBE/C) Thus, the DBE/C ratio will increase with decreasing H/C ratio (Koch & Dittmar, 2006). Based on the DBE/C ratio comparison between “cell on” and “cell off” markers, structural information can be extracted. Indeed “cell off” markers appeared more confined in the small mass range between *m/z* 100-500 amu and are characterized by low DBE/C ratio, while “cell on” markers were more abundant in higher masses (*m/z* 250-550 amu) and characterized by higher DBE/C values (Figure 3C). “Cell off” markers would be described as non-oxidized compounds whereas “cell on” markers would therefore correspond to oxidized compounds, as confirmed by the wine oxidation mechanism leading to the presence of higher masses with a higher number of double bonds (Bittner, 2006; Nikolantonaki et al., 2014; Waterhouse & Laurie, 2006; Wildenradt & Singleton, 1974). A further oxidation signature was provided by the number of detected O-containing formulas as a function of m/z for “cell on” and “cell off” markers, which is clearly higher for the former (Figure 3D, Supplementary Figure 5 and 6 and supplementary table 5). Interestingly, a count of the number of possible transformations connecting the detected masses, and corresponding for instance to hydroxylation,

hydro-Peroxidation, dehydrogenation, hydrolysis/condensation, thiolation and direct sulfonation showed for instance, that (de)hydroxylation (±O) transformations would indeed be slightly more observed after electro oxidation (cell on) of wines without SO_2 added to the must, and even more observed after electro oxidation of wines with 8 $g.hL^{-1}$ SO_2 added to the must (Supplementary table 5). However, thiolation (±S) and sulfonation (±$S0_3$) transformations would exhibit a contrasted behavior, where they are similarly and significantly more observed after electro oxidation (cell on) of wines without SO_2 added to the must, but slightly less observed after electro oxidation of wines with 8 $g.hL^{-1}$ SO_2 added to the must. In contrast, sulfonation ((±H_2S0_3, corresponding to the addition of sulfites to carbonyls) would exhibit the opposite trend with much more transformations observed for cell-on (Supplementary Table 5). The reactivity of sulfonated compounds is indeed central to this study and these results illustrate how the resistance of wines to electro oxidation involves distinct reactional mechanisms depending on the fact that musts were initially protected with SO_2 or not. A closer look at masses corresponding to glutathione and glutamylcystein, and their sulfonated adducts further showed that such antioxidants exhibit distinct consumption behaviors upon electro oxidation (Supplementary Figures 5, 6 and 7). If all of these compounds appeared to be consumed upon electro oxidation (cell on), the consumption of glutathione and glutamylcystein was increased for wines with 8 $g.hL^{-1}$ SO_2 added to the must, whereas it is the opposite when their sulfonated adducts are considered (Supplementary Figure 7), *i.e.* sulfonated adducts formed upon SO_2 addition at bottling only (0 $g.\ hL^{-1}$ SO_2 added to the must) would be relatively more consumed after electro oxidation, than sulfonated adducts formed upon SO_2 addition both at pressing and bottling (8 $g.hL^{-1}$ SO_2 added to the must). Our results thus shows that such thiol S-sulfonate adducts formed upon ageing (Arapitsas et al., 2016; Bekker, Kreitman, Jeffery, & Danilewicz, 2018) may contribute to the overall antioxidant resistance of wines.

It must be noted though, that wines studied here were bottle aged for 6 years, and consequently may have already gone through a natural slow oxidation process, which means that cell off markers may already exhibit oxidized traits. The richness of information, which could be deduced for the decreasing and increasing number of annotated detected compounds, which are distributed along

several regions in the shown Van Krevelen diagram (Figure 3) confirmed that oxidation not only impacts polyphenolic compounds but also modify amino acids, carbohydrates and sulfur containing compounds.

4. Conclusions

In this work, the electrochemical simulation of oxidative reactions in wine coupled to FT-ICR-MS detection was investigated in order to unravel the diversity and nature of metabolites involved in the resistance of bottled wine towards electrochemical oxidation. Through the comparison of bottle aged chardonnay wines, which only differed by the amount of SO_2 added to the must at pressing, this study addressed for the first time the impact of SO_2 addition to the must, from a bottled wine ageing chemistry point of view. The on-line coupling of electrochemical oxidation and FT-ICR-MS detection gives a rapid access to wine electrochemical oxidation fingerprints but with low oxidation efficiency if coupled to high-resolution MS, whereas an off-line EC system allows for a more detailed investigation of oxidation products, due to time requirements which are disconnected for oxidation and MS acquisition. Besides know metabolites such as catechin/epicatechin and caffeic acid, sulfur-containing compounds (CHOS) appeared to be the most reactive (concentration decreasing with electrochemical oxidation), whereas nitrogen-containing compounds were mostly formed. From a methodological point of view, these results reveal an accessible chemodiversity associated with electrochemical oxidation. As such, they constitute an unprecedented basis for top down exploration of potential anti oxidation biomarkers and subsequent antioxidant alternatives to SO_2.

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Figure 1: Evolution of the composition of Chardonnay wines after electrochemical oxidation treatment. (A) Hierarchical cluster analysis of wine without SO_2 addition at pressing and wines treated with 8 $g.hL^{-1}$ SO_2 used as initial condition prior to applied electrical potential (0, 400, 800 and 1200 mV) using On-line system. Van Krevelen diagrams and histograms showing metabolites that have been strongly affected by electrical potential for (B) SO_2 treated samples and (C) samples without SO_2 addition at pressing. Van Krevelen diagrams from left show metabolites with decreasing signal intensities and from right show newly appeared metabolites as a result of oxidation. Elemental formulas in the van Krevelen diagram are colored according to their composition and are sized according to their intensity, CHO in blue, CHOS in green, CHON in orange and CHONS in red.

Figure 2: Composition of wines treated with on-line and off-line system, both with FT-ICR-MS detection. Venn diagrams of the total white wine composition for cell off (0 mV) and cell on (1000 mV) for two different sulfur dioxide doses (0 and 8 $g.hL^{-1}$ SO_2) and histograms illustrating the number of unique detected formulas divided into chemical families, in cell on and cell off, and for 0 and 8 $g.hL^{-1}$ SO_2 wine samples.

Figure 3: Standardized dataset from electro-oxidation experiments on wines with no SO_2 added, using the Off-line system coupled to FT-ICR-MS, and showing elemental formulas associated with significantly higher intensity according to the voltage applied (0 mV versus 1000 mV). High to low intensity profiles, van Krevelen diagrams (H/C versus O/C) and corresponding sum formula histograms for cell off markers (A) and cell on markers (B). These data were obtained from the hierarchical cluster analysis (ANOVA test, p-values < 0.01). (C) Diagram of the double bond equivalent normalized to the total number of carbon atoms (DBE/C) as a function of the mass range (*m/z* 100 to 800 Da) with median values highlighted for cell on and cell off markers (D) Diagram of the number of oxygen normalized to the number of hydrogen (O/H) as a function of the mass range (*m/z* 100 to 800 Da) with median values highlighted for cell on and cell off markers. Elemental formulas in van Krevelen diagrams are colored according to their composition and are sized according to their intensity, CHO in blue, CHOS in green, CHON in orange and CHONS in red.

Oxidation fingerprints obtained from electrochemical simulation coupled to FT-ICR-MS

S- and N-containing compounds were mostly impacted by electrochemical simulation.

Ultrahigh resolution mass spectrometry revealed the corresponding oxidation fingerprints.

This approach paves the way for exploration of potential antioxidation biomarkers.