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Abstract

Fourteen Syrah red wines with different initial composition and antioxidant properties (polyphenols, antioxidant capacity, voltammetric behaviour, colour parameters and SO_2) were selected. Three different accelerated ageing tests (AATs) were then performed on these wine: thermal test at 60°C (60°C-ATT), enzymatic test with laccase (Laccase-ATT) and chemical test with H_2O_2 (H_2O_2-ATT). The results showed high correlations between the initial phenolic composition and antioxidant properties of the samples. Partial least squares (PLS) regressions were used in order to establish some models that can predict the AATs test results based on their different initial composition and antioxidant properties. The PLS regression models had overall very good accuracy and involved different explaining variables for each test. The models taking into account all the measured parameters and the phenolic composition alone showed good predictive capacities with correlation coefficients (r^2) > 0.89.

Keywords:

Wine

Syrah

Oxidation

Polyphenols

Ageing

Antioxidant

1. Introduction

Wine ageing is an important step in the elaboration of high-quality wines, especially for red wines. The process is divided into two key steps. The first one is the maturation phase (bulk storage in barrel or tank) and the second one is the ageing phase after bottling (A. Waterhouse et al., 2016). During wine ageing, many reactions take place, which will modify and improve the organoleptic parameters of the wine over time (Tao et al., 2014).

Oxygen is one of the most important parameters in all these reactions throughout the wine making process, especially during the maturation phase. Indeed, it is known that the good quality of a red wine is associated with a moderate and controlled exposure to oxygen throughout the winemaking and ageing process (Ugliano, 2013). The addition of oxygen is generally necessary during the maturation of red wines. This occurs during different stages of the winemaking process or micro-oxygenation during barrel ageing (exchange through the barrel) or during bottle ageing through the cork. It is particularly important for the improvement of red wine quality and will stabilise the colour and improve the mouthfeel (Kilmartin, 2009).

From an organoleptic point of view the bouquet of the wine changes radically during wine ageing. This change is the result of a complex set of chemical reactions and in most cases, oxygen contributes to these reactions. There are two different possible general chemical

pathways: the oxidation of aroma compounds and also the formation of new aroma compounds by oxidation. In particular the formation of oxidative aromas such as acetaldehyde or sotolon, and the formation/degradation of volatile sulphur compounds such as hydrogen sulfide or methanethiol (Ferreira et al., 2014; Ugliano, 2013).

In addition to the evolution of aromas, the colour of red wine also changes during red wine ageing. These changes are due to the evolution of the native anthocyanins extracted during the maceration of grapes. Their content decreases over time (Alcalde-Eon et al., 2006), as they react with other constituents of the wine such as flavanols (Salas et al., 2004) or with yeast metabolites such as acetaldehyde or pyruvic acid or glyoxal to form new pigments (Fulcrand et al., 1998). The new anthocyanins formed possess more stable structures and lead to stabilisation of wine colour (Echave et al., 2021), as well as bathochromic and hypsochromic changes (Brouillard et al., 1997). In addition, condensation between flavan-3-ols and anthocyanins by yeast derivatives can yield to the formation of yellow-orange xanthylium derivatives (A. Waterhouse et al., 2016). The formation of all these new pigments during ageing is responsible of the observed changes in wine colour, i.e. from red-purple hue to a brick-red hue (Ribéreau-Gayon & Peynaud, 1966).

Concerning mouthfeel properties, a decrease in astringency and bitterness is also observed during red wine ageing. This is due to oxidative and non-oxidative polymerisations and precipitation of phenolic compounds such as tannins especially during storage in the bottle (Echave et al., 2021).

All these evolutions can take place quickly depending on the wine studied and influence the wine ageing capacity. This capacity is also linked to the different terroirs and grape variety used, the winemaking process and the stopper used during bottle ageing. In general, high quality red wines require a period of bottle ageing to reach an optimum before consumption (Gambuti et al., 2013). The ageing capacity is then related to the capacity of a wine to

71 undergo oxidation over time (A. L. Waterhouse & Miao, 2021). It is therefore necessary to
72 understand how the wine evolves during an exposure to oxygen.

73 When oxygen is dissolved in wine, it can rapidly react with many wine components,
74 especially with phenolic compounds which are one of the main substrates for oxidation
75 (Oliveira et al., 2011). Although individual phenols may react very differently to oxygen, the
76 total phenol composition of a wine can give an estimate of its ability to react with oxygen
77 (Singleton, 1987) and then to resist to oxidation. As shown by Ferreira et al (Ferreira et al.,
78 2015) the average oxygen consumption rate is related to the total polyphenols measured by
79 the Folin-Ciocalteu method. Phenolic compounds with ortho-catechol groups (anthocyanins,
80 proanthocyanidins and flavan-3-ols) can form highly reactive species such as quinones,
81 radicals and hydrogen peroxide due to the catalytic action of metals such as copper and iron
82 (Kilmartin, 2009; A. L. Waterhouse & Laurie, 2006). These species may induce the oxidation
83 of various other wine components in particular, SO_2, phenolics, carbonyls and thiols (Ferreira
84 et al., 2015; Ugliano, 2013).

85 Previous studies have shown that higher pH (Singleton, 1987) and addition of ellagitannins
86 can increase oxygen consumption rate (Vivas & Glories, 1996). Furthermore, oxygen
87 consumption rate is positively correlated with concentrations of metals such as copper,
88 tannins rich in epigallocatechin and epicatechin-3-O-gallate (Carrascón et al., 2018; Ferreira
89 et al., 2015).

90 Accelerated ageing tests (AATs) were recently developed in our laboratory (Deshaies 2020)
91 in order to accelerate the oxidation process for red wines. These tests consist of monitoring
92 oxygen consumption of red wines in the presence of hydrogen peroxide (H_2O_2-ATT) or
93 laccase (laccase-ATT) oxidising agent or a temperature increase at 60°C (60°-ATT).
94 Moreover, as these ageing tests use different physico-chemical conditions, they will target

different chemical pathways and may also depend on the type of wine and on its initial composition.

The objectives of this article are:

- To obtain chemical characterization of Syrah red wine samples.
- To obtain the antioxidant capacity (radical scavenging capacity and ferric reducing capacity) of these wines.
- To determine the cyclic voltammetry behaviour of these wines.
- To build PLS regression models based on wine composition and antioxidant capacity to predict AATs results for these wines.

2. Material and methods

2.1. Reagents, solvents, and standards

Hydrogen peroxide solution 30% (ACS reagent) and Folin-Ciocalteu reagent were obtained from Carlo Erba Reagents (Peypin, France). Laccase from Trametes versicolor (0.78 U/mg), gallic acid, phloroglucinol, catechin, Trolox, 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), potassium persulfate, sodium hydrogen sulfite (NaHSO3) and hydrochloric acid (37%) were obtained from Sigma-Aldrich (Saint-Quentin Fallavier, France). Ferric chloride, 2,4,6-tri(2-pyridyl)-s-triazine (TPTZ) and ferrous sulfate heptahydrate were purchased from Alfa Aesar (Kandel, Germany). Oenin Chloride was obtained from Extrasynthese (Genay, France).

2.2.Wines samples

Fourteen red wines samples (100% Syrah) from the same vintage (2019) were obtained in different wineries from Côtes du Rhône and Languedoc-Roussillon area (Table 1).

Code	Grape variety	Denomination of origin	Vintage	Ageing

LR12-B19	Syrah	Languedoc-Roussillon	2019	Oak barrel	
LR7-C19	Syrah	Languedoc-Roussillon	2019	Tank	
LR10-B19	Syrah	Languedoc-Roussillon	2019	Oak barrel	
LR1-C19	Syrah	Languedoc-Roussillon	2019	Tank	
LR8-B19-1	Syrah	Languedoc-Roussillon	2019	Oak barrel	
LR8-B19-2	Syrah	Languedoc-Roussillon	2019	Oak barrel	
LR9-B19-1	Syrah	Languedoc-Roussillon	2019	Oak barrel	
LR9-B19-2	Syrah	Languedoc-Roussillon	2019	Oak barrel	
LR6-B19	Syrah	Languedoc-Roussillon	2019	Oak barrel	
LR4-B19	Syrah	Languedoc-Roussillon	2019	Oak barrel	
LR2-B19	Syrah	Languedoc-Roussillon	2019	Oak barrel	
CR1-C19	Syrah	Côtes du Rhône	2019	Tank	
CR12-B19-1	Syrah	Côtes du Rhône	2019	Oak barrel	
CR12-B19-2	Syrah	Côtes du Rhône	2019	Oak barrel	

Table 1. Characteristics of the red wines samples

Three 750 mL bottles of each wine were opened and slowly homogenized under nitrogen and aliquots of 50 mL tubes were then immediately frozen at -80 °C.

2.3. Accelerated ageing tests (ATTs)

Three accelerated ageing tests were applied to wine samples as described by Deshaies et al. (Deshaies et al., 2020). Each wine sample was saturated with air by vigorously shaking a 500 mL flask containing 50 mL of wine for 10 s, after which the cap was opened (5 s) to let fresh air enter the flask. This saturation operation was repeated three times. Then, 11 mL of saturated red wine was added into a Hermetic Pyrex 11 mL cultures tubes (VWR 734-4224, Radnor, PA, USA) containing Pst3 oxygen sensors (Presens-Precision Sensing GmbH,

144 Regensburg, Germany). For the Laccase-ATT 50 µL of a 10 $g \cdot L^{-1}$ laccase from *Trametes*
145 *versicolor* solution was added. For H_2O_2-ATT, 20 µL of hydrogen peroxide solution (30%)
146 was added. Finally, the thermal treatment was performed by heating the 11 mL of wine at 60
147 °C. The dissolved oxygen rate concentration was followed during the tests by
148 oxoluminescence. Assuming first order kinetic, oxygen consumption rate constants (k) for
149 each AATs were calculated.

150 **2.4.Total polyphenols and anthocyanins analysis**

151 **2.4.1. Total Polyphenols Content (TPC) determination**

152 The determination of the total phenolics compounds content in red wine was
153 determined by the Folin–Ciocalteu method and performed on a microplate reader (BMG
154 Labtech, Germany) using spectrophotometric detection and microliter 96-well plates,
155 according to the method described in Deshaies et al. (Deshaies et al., 2021). Briefly, fifty
156 microliters of diluted wine (1:100, v/v) or standard were mixed with 50 µL of Folin–Ciocalteu
157 reagent (1:5, v/v) and 100 µL of sodium hydroxide solution (0.35 $mol.L^{-1}$) in each well.
158 After a period of 3 min of incubation, the absorbance of each sample was monitored at 750
159 nm. A standard curve of gallic acid (0 - 200$mg.L^{-1}$) was prepared and the results were
160 expressed as $g.L^{-1}$ of gallic acid equivalents (GAE).

161 **2.4.2. Total Polyphenols Index (TPI) determination**

162 Total Polyphenols Index (TPI) were determined by UV–visible spectrophotometry
163 (Agilent Carry 60 spectrometer, Agilent) at 280 nm with 1 cm cells after dilution in water
164 (1:100 v/v) (Ribéreau-Gayon, 1970).

165 **2.4.3. Total Anthocyanins (TA) determination**

166 Total Anthocyanins contents were determined as described by Ribéreau-Gayon and
167 Stonestreet (Ribéreau-Gayon & Stonestreet, 1965). One mL of wine samples, 1 mL of ethanol
168 0,1% HCl and 20mL of H_2O 2% HCl were mixed. Then 10 mL of this solution were placed in

two test tubes. In one of them was added 4 mL of water (1) and in the other one 4 mL of a 15% solution of $NaHSO_3$ (2). After 15 min, absorbance at 520 nm of each tube was measured (Agilent Carry 60 spectrometer, Agilent, Les Ulis, France). The concentration of total anthocyanins (TA) in $mg.L^{-1}$ was calculated by the following equation :

$$TA\ (mg.L^{-1}) = 875 \times (A1 - A2)$$

2.5.Antioxidant capacity measurements

2.5.1. Determination of radical scavenging activity using ABTS (TEAC assay)

ABTS antioxidant capacity was performed on a microplate reader (BMG Labtech, Germany) using spectrophotometric detection and microliter 96-well plates, according to Muller et al. (Muller et al., 2010) with slight modifications. To generate ABTS radicals, 5mL of ABTS solution (7 mM) was added to 5 mL of a potassium persulfate solution (2.45 mM) prepared in sodium acetate buffer (50 mM, pH = 4,6). The mixture was incubated at room temperature in the dark all night. The stock solution was diluted with acetate buffer to an absorbance of 0.7 ± 0.02 at 734 nm. Twenty microliters of diluted red wine (1:100, v/v) or standard were mixed with 200 µL of ABTS solution. After 7 min, the absorbance was measured at 734 nm. A standard curve of Trolox (0 - 250 $\mu mol.L^{-1}$) was used. Results (Trolox Equivalent Antioxidant Capacity) were expressed as $mmol.L^{-1}$ Trolox Equivalent (TE).

2.5.2. Ferric-Reducing Antioxidant Power (FRAP assay)

FRAP antioxidant capacity determination was performed on a microplate reader (BMG Labtech, Germany) using spectrophotometric detection and microliter 96-well plates, according to Sulleria et al. (Suleria et al., 2020) with slight modifications. To prepare the FRAP reagent, 300 mM sodium acetate buffer (pH 3.6), 10 mM TPTZ solution in 40 mM HCl and 20 mM ferric chloride were mixed in a ratio of 10:1:1 (v/v/v). Then, 20 µL of diluted

wine samples (1/ 100; v/v) and 280 µL FRAP reagent were mixed in a 96 well plate followed by incubation at 37 °C for 5 min, and absorbance was measured at 593 nm. A standard curve of ferrous sulfate heptahydrate (0 - 100 µmol.L^{-1}) was used. Results were expressed as mmol.L^{-1} Fe^{2+} equivalent.

2.5.3. Electrochemical measurements

Cyclic voltammetry measurements were performed as described in Deshaies et al. (Deshaies et al., 2021). Wine samples were diluted (1:75 v/v) in model wine (ethanol 12% (v/v)., 33 mmol.L^{-1} tartaric acid and pH 3.6 adjusted with 1 mol.L^{-1} NaOH) and then 200 µL of diluted sample were dropped on the electrode surface. The measurements were performed in the range of -0.2 V to 0.8 V (vs. $Ag_{(s)}$) with a scan rate of 100 mV.s^{-1} using 4 mm diameter single-walled carbon nanotubes-modified screen-printed carbon electrode SWCNTs-SPCE (Dropsens, Oviedo, Spain) in a three-electrode configuration (carbon counter electrode and silver reference electrode). The experiments were performed on a potentiostat/galvanostat (Autolab PGSTAT 302N) using software Nova 2.1.5 (Metrohm, Herisau, Switzerland).

Different charges corresponding to antioxidant capacities can be extracted from voltammograms (area under the anodic curve until the chosen potential) (Deshaies et al., 2021). The total charge Q_{800mV} corresponds to all oxidizable phenolic compounds of the wine. Q_{240mV} can be assigned to the most oxidizable polyphenols with the highest reducing capacity. Q_{520mV} corresponds to all the compounds which oxidize until this potential and the difference Q_{520mV}-Q_{240mV} can be afterwards calculated. Finally, Q_{240mV}/Q_{800mV} ratio indicates the contribution of the most antioxidant compounds to the total antioxidant capacity of wines.

2.6. Free sulfur dioxide determination

Free sulfur dioxide content was determined by aeration/oxidation according to reference method O.I.V–MA–AS323–04A described in the “Compendium of International

Methods of Analysis" published in 2009 by the International Organisation of Vine and Wine (OIV).

2.7.Spectrophotometric colorimetric measurements

Total color intensity (sum of absorbance values at 420, 520 and 620 nm) and hue (ratio between A_{420} and A_{520}) were determined by UV-visible spectrophotometry (Agilent Carry 60 spectrometer, Agilent, Les Ulis, France) according to reference method OIV-MA-AS2-07B described in the "Compendium of International Methods of Analysis" published in 2009 by the International Organisation of Vine and Wine (OIV).

2.8.Determination of phenolic composition

2.8.1. UHPLC-DAD-MS analysis of monomeric anthocyanins

The quantification of monomeric anthocyanins was performed as described by Benbouguerra et al. (Benbouguerra et al., 2020) with slights modifications. After a filtration on a 0.45 µm PTFE filter (Macherey-Nagel, Düren, Germany), the wines were directly injected (7.5 µL) on a UPLC system Waters Acquity (Saint-Quentin-en-Yvelines, France) equipped with a reversed phase UPLC Acquity UPLC BEH C18 column (50 mm length, 2.1 mm internal diameter, 1.7 µm particle size) from Waters. The method used was a binary gradient with mobile phase A (0,1% v/v aqueous trifluoroacetic acid) and mobile phase B (acetonitrile). The column temperature was set at 50 °C. The 40 min elution method at flow 0.25 $mL.min^{-1}$ was 1% B (0 min), 1 - 8.8% (0-5 min) B, 8,8 - 20.6% (5-30 min) B, 20.6 - 96% (30-30.5 min) B, isocratic with 96% B (30.5 - 34 min), 96-1% (34 -34.1 min) B, and isocratic with 1% B (34.1 - 40 min). The detection was monitored at 520 nm with a photodiode array detector (PDA). External malvidin-3-O-glucoside calibration curve was used. Results were expressed as mg malvidin-3-O-glucoside equivalent (M3G eq).L^{-1}.

2.8.2. UHPLC-DAD-MS analysis of flavanols

The composition of flavanols was studied after acid-catalyzed depolymerization in the presence of a phloroglucinol as described by Carrascón et al. (Carrascón et al., 2018) with slights modifications. 400 µL of red wine sample were brought to dryness in a centrifugal solvent evaporator (Genevac, Ipswich, UK). The obtained residue was dissolved in 600 µL of a 50 $g.L^{-1}$ of phloroglucinol and 10 $g.L^{-1}$ of ascorbic acid in methanol-HCl 0.2 M solution The reaction was carried out at 50 °C for 20 min, then 600 µL of ammonium acetate 200 mM solution were added to stop the reaction. Then, the sample was centrifuged at 12,000 rpm for 10 min and the supernatant was injected in the same chromatographic apparatus as mentioned in section 2.8.1. The method used was a binary gradient with mobile phase A (0,1% v/v aqueous trifluoroacetic acid) and mobile phase B (acetonitrile). The elution method at flow 0.45 $mL.min^{-1}$ was 2% B (0 min), 2-6% B (0-10 min), 6-20% B (10-16 min), 20-99% B (16-16.1 min), isocratic with 99% B (16.1-18 min), 99-2% B (18-18.1 min) and isocratic with 2% B (18.1-22 min). The column temperature was 40 °C. Eluting peaks were monitored at 280 nm. This analyse allowed us to obtain the type and the relative proportions of the terminal and extension units of tannins. The molar ratio between total and terminal units gives access to the mean degree of polymerization (mDP). External catechin calibration curve was used and quantification was effected in equivalents of catechin. Concentration of epicatechin, epigallocatechin and epicatechin-3-O-gallate were estimated using their response factors relative to catechin.

2.9. Statistical Analysis

The ANOVA, correlation, PCA (Principal Component Analysis) and PLS (Partial least-squares) regression tests were performed using XLSTAT software (Addinsoft version 19.02, Paris, France). Tukey test was carried out and when p-values were < 0.05 the data were considered as significant. Pearson's correlation coefficient was carried out for the determination of correlations. PLS regression models were initially built with all the variables

studied and were further simplified by iterative elimination of variables with smaller contribution to the model. The optimum model was considered to be the one providing the highest coefficient of determination with the smallest number of variables. All analyses, experiments and tests were performed in triplicate.

3. Results

3.1. Composition and antioxidant properties of Syrah red wines

For this study, fourteen French Syrah red wines of 2019 vintage were collected directly from tanks and oak barrels in different winery of the Languedoc-Roussillon and Cotes du Rhône area. Before accelerated ageing tests, several parameters were measured on samples, including spectrophotometric measurements such as Folin-Ciocalteu total polyphenol content (TPC), total polyphenol index (TPI), total anthocyanins (TA) and absorbances at 420, 520 and 620 nm for chromatic characteristics. Trolox equivalent antioxidant capacity (TEAC) by the ABTS assay, ferric reducing antioxidant power (FRAP) and cyclic voltammetry parameters (charges at various potentials) were measured in order to characterize the antioxidant capacity. Free sulfur dioxide determination was carried out by aeration/oxidation method. Analysis of monomeric anthocyanins and polymeric flavanols (after acid-catalyzed depolymerization) by UPLC-DAD-MS were also performed. Results are shown in Supplementary material (Table S1 - S5).

3.1.1. Syrah red wines composition

In order to study the phenolic diversity and to identify possible groupings of the red wine samples, a principal component analysis of the composition data was performed. Figure 1 shows the loadings and scores plots in the space described by the 1st and 2nd factors (total explained variance of 48,42%). The first factor is mainly related to the richness in anthocyanins (total and monomeric) and the second factor is positively correlated with the composition in tannins.

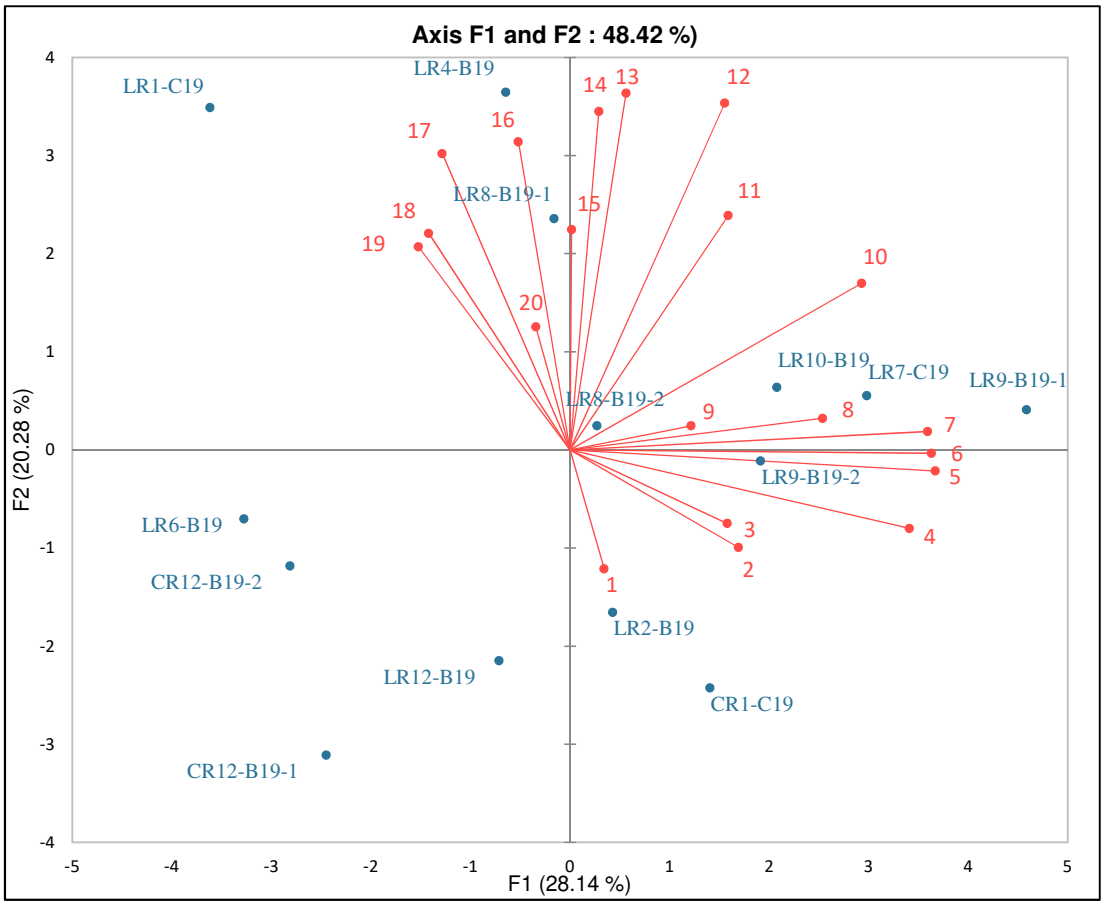

Figure 1. PCA analysis of composition data (wine samples and variables); 1: mDP; 2: Epicatechin extension unit; 3: Catechin extension unit; 4: Acetylated anthocyanins; 5: Coumaroylated anthocyanins; 6: Total monomeric anthocyanins; 7: Glycosylated anthocyanins; 8: Free SO_2; 9: Catechin terminal unit; 10: Total anthocyanins; 11: Total flavanols; 12: TPC; 13: Epicatechin-3-O-gallate extension unit; 14: TPI; 15: Epicatechin terminal unit; 16: Epigallocatechin extension unit; 17: % of galloylation; 18: Epicatechin-3-

O-gallate terminal unit; 19: % of prodelphidin; 20: Epigallocatechin terminal unit (explained variance of 48,42%)

The PCA showed that the samples were well distributed according to the composition variables. One sample seems to stand out from the others (LR1-C19), looking at the compositional data, this sample was characterised by a low concentration of anthocyanins (molecular and total) and a higher-than-average concentration of condensed tannins. Overall, the samples represented a good diversity, with no clustering by winery, origin, and type of ageing. This dataset and samples were then ideally adapted for applying PLS regression in order to construct models for predicting AATs.

3.1.2. Correlation between physico-chemical parameters, antioxidants capacity and the wine composition

Table S6 (Supplementary Data) represented Pearson correlation coefficients ($p(t) < 0.05$ and $p(t) < 0.01$) between wine composition (polyphenols and free SO_2), antioxidant capacity (TEAC and FRAP), cyclic voltammetry charges and chromatic characteristics (absorbances). Concerning the phenolic composition, the TPC measured by Folin-Ciocalteu method, was not highly correlated as expected with TPI ($R = 0.647$). This may be due to the fact that TPI is based on UV absorption while Folin-Cioccalteu is based on the reducing capacity of polyphenols. Besides, antioxidant capacity measured by colorimetric assays were well correlated with each other ($R_{TEAC/FRAP} = 0.823$). In addition to that, total polyphenols (TPC and TPI) and colorimetric antioxidant assays (TEAC and FRAP) were correlated with all electrochemical parameters especially with Q_{240mV} and Q_{800mV} ($R_{TEAC/Q240mV} = 0.828$, $R_{TPC/Q240mV} = 0.757$, $R_{FRAP/Q240mV} = 0.667$, $R_{TPI/Q240mV} = 0.626$ $R_{TEAC/Q800mV} = 0.764$, $R_{TPC/Q800mV} = 0.728$, $R_{FRAP/Q800mV} = 0.637$, $R_{TPI/Q800mV} = 0.728$) in accordance with previous studies (Benbouguerra et al., 2020; Deshaies et al., 2021; Newair et al., 2018). This could be explained by the fact that they are methods based on electron transfer from antioxidant to

oxidized compounds. Moreover, the different charge parameters measured by cyclic voltammetry were logically well correlated with each other ($R_{Q800mV/Q240mV}$ = 0.822, $R_{Q520mV/Q240mV}$ = 0.724, $R_{Q520mV/Q520mV-Q240mV}$ = 0.949). TPC was also strongly correlated with TEAC and FRAP assays respectively R = 0.83 and R = 0.891. These results are in accordance with the literature as the different phenolic compounds present in the wines will be the predominant source of the antioxidant activity of red wines (Šeruga et al., 2011). TPC and FRAP assays were also correlated with total flavanols obtained after acidic depolymerization (R = 0.682 and R = 0.698) as shown previously (Newair et al., 2018). Correlations were found between spectrophotometric assays (TPC, FRAP and TEAC) and epigallocatechin extension unit. In addition, the electrochemical charges Q_{800mV} and Q_{240mV} are also correlated with these polymeric units, R = 0.783 and R = 0.665 respectively. This could be explained by the fact that the pyrogallol group (triphenol on the B-ring of the flavonoid) has a higher reducing power (Kilmartin & Hsu, 2003). The colour intensity was well correlated with the epicatechin-3-O-gallate extension units (R = 0.728), which can be explained by the influence of the flavanol on the co-pigmentation reaction with anthocyanins leading to a hyperchromic effect (Boulton, 2001). At last, the composition in anthocyanins showed a positive correlation with free SO_2 levels, in agreement with literature (Christofi et al., 2021).

3.2. Comparison and PLS modelling of Accelerated Ageing Tests (AATs)

3.2.1. Comparison of Accelerated Ageing Tests

Syrah red wine samples were subjected to three different accelerated ageing tests: chemical (H_2O_2-ATT), enzymatic (laccase-ATT) and thermal (60 ◦C-ATT) (Deshaies et al., 2020) in order to study the influence of phenolic composition on the kinetic parameters of these AATs.

After air saturation, initial dissolved oxygen levels were between 7 and 9 ppm. The evolution of oxygen consumption was monitored using fluorescence oxygen sensors. Oxygen consumption rate constant (k) for each AATs were calculated by exponential regression of

dissolved O_2 concentration as a function of time. As showed in Table S7 (Supplementary Data) wine samples can differ in term of oxygen consumption kinetics parameters, these results were in agreement with the literature (Deshaies et al., 2021; Ferreira et al., 2015). Moreover, values differed between the tests and values ranged from 0.07 s^{-1} to 1.81 s^{-1} for H_2O_2-AAT, from 0.05 s^{-1} to 0.89 s^{-1} for Laccase-AAT and at last from 0.15 s^{-1} to 0.45 s^{-1} for 60°C-AAT. Globally, chemical test using H_2O_2 showed the highest oxygen consumption rate constants for all considered samples except for, LR8-B19-2, CR12-B19-1 and CR12-B19-2, for whom enzymatic test showed the highest consumption rate constants and thermal test for LR8-B19-1. Concerning Pearson correlation (Table 2), no significant correlations (p = 0.05) were found between the three different AATs.

Table 2. Pearson's correlation coefficients between the three different AATs

	H_2O_2-AAT	**Laccase-AAT**	**60°C-AAT**
H_2O_2-AAT	**1**	0.010	0.523
Laccase-AAT	0.010	**1**	0.310
60°C-AAT	0.523	0.310	**1**

These results suggest that different chemical mechanisms and targets are involved for each AATs as the oxidising agents or temperature conditions that will accelerate or slow down oxygen consumption. The concentration of these targets may therefore be a limiting factor in the protection of the wine against oxidation and consequently in its ageing potential.

3.2.2. Chemical Accelerated Ageing Tests: H_2O_2-AAT

As the AATs showed different results depending on the test and the wine used, it appeared necessary to investigate which parameters of the initial wine characteristics could impact them. First, in order to investigate the individual influence of the wine different parameters

372 (phenolic, antioxidant and chromatic properties), a full linear correlation study was
373 performed. The list of parameters with significant Pearson correlation coefficients ($p(t) < 0.05$) with the oxygen consumption constants for the H_2O_2-ATT (k_{H2O2}) are shown in Table 3.A.

376 *Table 3: A. Pearson's correlation coefficients measured parameters and oxygen consumption*
377 *rate constant for the chemical AAT. * represents significance at $p \leq 0.01$ and # represents*
378 *significance at $p \leq 0.05$.*
379 *B. PLS regression coefficients and quality parameters for the different models explaining the*

A.				**H_2O_2-AAT - Pearson correlation coefficients (# p(t)<0.05; * p(t)<0.01)**					
TPC	FRAP	TEAC	Epigallocatechin extension unit		Catechin terminal unit	Total flavanols	Q_{800mV}	Q_{240mV}	Q_{520mV}
0.654#	0.741*	0.654#	0.751*		0.622*	0.534*	0.631*	0.660*	0.574*

B. Modelling parameters	**PCs**	**r^2**	**H_2O_2-AAT - PLS Regression Model**				
Total	4	0.928	TPC	Epigallocatechin extension unit	Catechin terminal unit	Epicatechin-3-O-gallate terminal unit	A_{620}
			0.394	0.400	0.566	0.344	-0.382
Chemical composition	3	0.920	Epigallocatechin extension unit	Catechin terminal unit	Epicatechin terminal unit	Epicatechin-3-O-gallate terminal unit	Total flavanols
			0.689	0.641	-0.337	0.233	0.172
Antioxidant parameters	3	0.621	FRAP	$Q_{240\ mV}$	$Q_{520\ mV}$		
			0.547	0.139	0.217		

380 *the H_2O_2-AAT results.*

381 Results of linear correlation revealed, that H_2O_2-AAT was positively correlated with the total
382 polyphenols content (TPC) and the total flavanols content measured after acidic
383 depolymerization. These results were in agreement with previous studies for wine model
384 solution (Danilewicz, 2007) and for real red wine (Ferreira et al., 2015) where the average
385 rate of oxygen consumption was related to total polyphenols. This highlights the antioxidant
386 character of total polyphenols. Moreover, the use of hydrogen peroxide, which is notably
387 involved in the Fenton reaction (A. L. Waterhouse & Laurie, 2006), will release a hydroxyl
388 radical ·OH that will accelerate the formation of the quinone of polyphenols and so oxygen

consumption is consequently increased. Noteworthy, a good positive correlation between the composition of epigallocatechin units and high H_2O_2-AAT was observed (R = 0.751), which can be explained by the fact that prodelphinidins have an important antioxidant power (Ferreira et al., 2015; Kilmartin & Hsu, 2003). In addition to that, a positive correlation with the catechin terminal unit was also noted (R = 0.622) was obtained. This positive influence of catechin on wine oxidation was in agreement with the literature (Márquez et al., 2019). Indeed, when hydrogen peroxide is added, the Fenton reaction is accelerated and accentuates the formation of acetaldehyde by oxidation of ethanol (Oliveira et al., 2011) which accelerates the oxygen consumption. The acetaldehyde thus formed will react with acetaldehyde-reactive-polyphenol (ARP) such as catechin to form ethyl-bridged polymers. Concerning the antioxidant parameters (TEAC, FRAP, Q_{800mV} Q_{240mV} and Q_{520mV}), as shown in section 3.1.b, these parameters were correlated with the polyphenol composition. It is therefore logical that they were also correlated with the H_2O_2-AAT values.

As the linear correlation study showed, the H_2O_2-AAT values were only weakly correlated with the individual parameters of the chemical composition or the antioxidant properties of the wine. This confirmed that the H_2O_2-AAT values were influenced by multiple factors and PLS models provided better results with satisfactory predictive power. The results of the most relevant variables and their normalized coefficients in the regression equation were summarised in Table 3.B. Three different PLS regression models were established considering: all the measured parameters (1), only the composition variables (2) and at last only the antioxidant parameters (3). PLS models (1) and (2) showed a good correlation between the predicted and experimental values of the H_2O_2-AAT values with a linearity coefficient (r^2) of 0.928 and 0.920 respectively (Figure S1, Supplementary Data). However, for the model (3) which take only the antioxidant parameters into account, the model did not give a high linearity (r^2 = 0.621) which means that the oxidation mechanisms of the H_2O_2-

AAT were less related to these parameters. In addition to that, some variables were only relevant for the multivariate model (Table 2.B.) and it was not possible to establish a linear correlation for them with the H_2O_2-AAT results. These variables included epicatechin-3-O-gallate terminal unit and A_{620} for model (1) and epicatechin terminal unit and epicatechin-3-O-gallate terminal unit for model (2). This means that there was no direct relationship of the concentration of these parameters on oxygen consumption, but the interactions between these compounds were important in the oxidative process.

PLS models (1) and (2) suggested that red wine rich in the terminal epicatechin-3-O-gallate unit had faster chemical oxidation kinetics and thus higher H_2O_2-AAT values. This result is in agreement with previous study (Carrascón et al., 2018) which showed that epicatechin-3-O-gallate units were rapidly consumed in wines with high oxygen consumption rates. This result highlights the reducing properties of this flavan-3-ol through the presence of a trihydroxyl group in the galloyl moiety that may be important in radical scavenging. Additionally, model (2) indicated that the epicatechin end unit was negatively related to the H_2O_2-AAT values. This result could be surprising but previous studies (Carrascón et al., 2018; Ferreira et al., 2015; Márquez et al., 2019) showed the negative role of epicatechin polymeric units on oxygen consumption and wine oxidation. A possible explanation for this result could be the possible formation of chelation complexes between the hydroxyl functions of the polyphenols and some metal ions (iron and copper) which may disrupt the catalytic oxidation cycle (Perron & Brumaghim, 2009). At last, it was observed in the model (1) that the negative contribution of the absorbance at 620 nm corresponding to the absorbance of polymeric pigments whose maximum absorption wavelength induces a blue-violet tone through hyperchromic shift (Boulton, 2001). This result could be explained by the fact that polymeric pigments have a higher resistance to oxidation than native pigments, thus resulting in a lower oxygen consumption rate and lower H_2O_2-AAT values.

3.2.3. Enzymatic Accelerated Ageing Test: laccase-ATT

For the Laccase-AAT, the oxidation of the sample is catalysed by laccases which are polyphenoloxidaze (PPO) enzymes derived from fungi. The laccases use molecular oxygen to oxidise polyphenols through free radical reaction mechanism. Polyphenols are oxidation substrates for these enzymes which can have two types of activity, monophenol oxidase activity characterised by the hydroxylation of a monophenol to a diphenol and diphenol oxidase activity corresponding to the oxidation of dihydroxybenzenes to benzoquinones (Oliveira et al., 2011).

In order to investigate the parameters influencing the oxygen consumption kinetics of laccase-AAT, a chemometric analysis similar to the chemical test was performed. First, a full linear correlation study was performed and the list of parameters showing significant Pearson correlation coefficients ($p(t) < 0.05$) with the oxygen consumption constants for laccase-AAT ($k_{laccase}$) are compiled in Table 4.A.

*Table 4: A. Pearson's correlation coefficients measured parameters and oxygen consumption rate constant for the Laccase-AAT. * represents significance at $p \leq 0.01$ and # represents significance at $p \leq 0.05$.*

B. PLS regression coefficients and quality parameters for the different models explaining the oxygen consumption rate constant for the Laccase-AAT.

A.	**Laccase-AAT - Pearson correlation coefficients (# p(t)<0.05; * p(t)<0.01)**	
Catechin extension unit	mDP	
0.638#	0.750*	

B. Modelling parameters	**PCs**	**r^2**	**Laccase-AAT - PLS Regression Model**					
Total	4	0.956	TPI	Acetylated anthocyanins	Epicatechin extension unit	Epicatechin-3-O-gallate extension unit	Free SO_2	A_{620}
			0.319	0.877	0.382	-0.542	-0.901	0.412
Chemical composition	4	0.980	TPI	TA	Catechin extension unit	Epicatechin extension unit	Epicatechin-3-O-gallate extension unit	Free SO_2

				0.271	0.706	0.452	0.367	-0.579	-0.742
	Antioxidant parameters	4	0.186	FRAP	Q_{800mV}	$Q_{240\ mV}$	Q_{240mV} / Q_{800mV}		
				0.320	5.591	-6.797	3.744		

457

458 The results of the linear correlation revealed that Laccase-AAT was positively correlated with
459 only two variables, the catechin extension unit and the mean degree of polymerization (mDP).
460 Concerning the catechin extension units of tannins were correlated (R = 0.638), it has been
461 reported in the literature that polyphenols of the 1-2 dihydroxybenzene type such as catechol
462 or galloyl groups are preferential substrates for oxidation by laccases (Deshaies et al., 2020;
463 Oliveira et al., 2011). An unexpected and outstanding result is that Laccase-AAT is positively
464 correlated with the mDP (R = 0.750). Indeed, steric hindrance plays an important role during
465 oxidation reactions by laccases, the reactivity of laccases towards different substrates is all the
466 weaker as the substituents are voluminous (Nicolas et al., 2003). It seems that oxygen
467 consumption during enzymatic oxidation was impacted by the size of the condensed tannins.
468 However, the mDP values (table S8, Supplementary Data) were low and the range of the
469 values limited (min = 1.94 and max = 3.79), so there was not an important variation of this
470 parameter between the samples.

471 Overall, the linear correlation study showed that Laccase-AAT results are poorly correlated
472 with the measured wine initial parameters. This accelerated ageing test is then probably
473 dependent on multiple factors. This hypothesis was confirmed by the establishment of three
474 PLS regression models with good predictive values.

475 Models (1) and (2) showed high correlations between the predicted and experimental values
476 of Laccase-AAT with a linearity coefficient (r^2) of 0.956 and 0.980 respectively (Figure S2,
477 Supplementary Data). But model (3) showed no linearity (r^2 = 0.186) which means that the
478 antioxidant parameters alone are not able to predict this enzymatic test. The results of the

most relevant variables and their standardised coefficients in the regression equation are summarised in Table 4.B.

PLS Models (1) and (2) suggested that red wine with high total polyphenol index (TPI) had faster enzymatic oxidation kinetics ($k_{laccase}$) during the laccase-AAT test. This TPI index provided an estimation of the total phenolic compounds with a benzene ring. This result was in agreement with the literature (Deshaies et al., 2020; Oliveira et al., 2011; A. Waterhouse et al., 2016) where it was showed that polyphenols, in particular flavan-3-ols, were the main substrates for laccase oxidation. This result corroborated the positive influence of epicatechin extension units (model (1)) and catechin extension units (model (2)) on this accelerated enzymatic ageing test. In addition, catechin was the variable that contributes the most to the Laccase-AAT as it was both linearly correlated and relevant to the multivariate model (2). The epicatechin-3-O-gallate extension units were found to contribute negatively to both models (1) and (2). It would therefore appear that red wines with high concentrations of this polymeric unit would have lower Laccase-AAT values. Moreover, it was also reported (Cheynier & Ricardo da Silva, 1991) that galloylated procyanidins were not the main targets of oxidation reactions by polyphenol oxidase (PPO). Furthermore, the negative influence of the free SO2 content on model (1) and (2) can be noted. This result was consistent with the literature (Toit et al., 2006) where it has been shown that higher SO_2 concentration lower the laccase activity.

It was also observed that the concentration of acetylated anthocyanins had a positive impact on PLS model (1) and total anthocyanins (TA) on PLS model (2). At first sight, anthocyanins were not the main substrates for laccase oxidation and this limited activity was probably related to the presence of a glycosidic moiety that would cause steric hindrance. However, these compounds, although not direct substrates of the enzyme, can actively participate in browning by coupled oxidation reactions. The degradation of anthocyanins was strongly

accelerated in the presence of caffeic acid derivatives or catechins (Sarni-Manchado et al., 1997). As shown previously (Deshaies et al., 2020), during this accelerated test, a decrease in the anthocyanin ion intensity using LC-HMRS analysis occurred. Additionally, the PLS model (1) indicated that the Laccase-AAT was positively related to the absorbance at 620 nm.

3.2.4. Thermal Accelerated Ageing Test: 60°-ATT

For the thermal 60°-ATT, the increase in temperature accelerated and catalysed all endothermic reactions such as many oxidation reactions. Another chemometric analysis of the 60°-ATT was performed. A full linear correlation study was first obtained and the list of parameters showing significant Pearson correlation coefficients ($p(t) < 0.05$) with the oxygen consumption constants for 60°-ATT was compiled in Table 5.A.

*Table 5: A. Pearson's correlation coefficients measured parameters and oxygen consumption rate constant for the 60°-ATT. * represents significance at $p \leq 0.01$ and # represents significance at $p \leq 0.05$.*

B. PLS regression coefficients and quality parameters for the different models explaining the oxygen consumption rate constant for the 60°-ATT.

A

60°-ATT - Pearson correlation coefficients (# p(t)<0.05; * p(t)<0.01)								
FRAP	Glycosylated anthocyanins	Acetylated anthocyanins	Coumaroylated anthocyanins	Total monomeric anthocyanins	Catechin extension unit	Catechin terminal unit	Q_{520mV}	$Q_{520mV} - Q_{240mV}$
0.614#	0.536#	0.598#	0.688*	0.578#	0.637#	0.627#	0.585#	0.590#

B

Modelling parameters	PCs	r^2	60°-ATT - PLS Regression Model					
Total	4	0.967	FRAP	Acetylated anthocyanins	Catechin extension unit	Catechin terminal unit	$Q_{240\ mV}$	$Q_{520mV} - Q_{240mV}$
			0.267	0.426	0.207	0.386	-0.260	0.450
Chemical composition	4	0.892	Acetylated anthocyanins	Total monomeric anthocyanins	Epigallocatechin terminal unit	Catechin terminal unit	Epicatechin extension unit	Free SO_2
			0.495	0.341	-0.379	0.439	0.161	-0.370
Antioxidant parameters	4	0.748	FRAP	ABTS	Q_{240mV} / Q_{800mV}	$Q_{520mV} - Q_{240mV}$		
			1.083	-0.790	0.173	0.483		

Results of linear correlation revealed that 60°-ATT was only positively correlated with many parameters contrary to the two other tests. Outstandingly, it should be noted that all monomeric anthocyanins (glycolysed, acetylated, coumaroylated, total monomeric) measured

522 by UHPLC were positively correlated with the 60°-ATT. Due to the increase in temperature
523 (60°C), many reactions are catalysed and in particular the formation of highly reactive
524 quinones (Deshaies et al., 2020), which by metallic catalysis via the Fenton reaction will lead
525 to the formation of hydroxyl radicals, hydrogen peroxide and acetaldehyde (Oliveira et al.,
526 2011; A. L. Waterhouse & Laurie, 2006) and thus accelerate oxygen consumption. The
527 acetaldehyde formed can react with acetaldehyde-reactive polyphenols (ARP) such as
528 monomeric anthocyanins to form new polymerised pigments with flavanols (Echave et al.,
529 2021) and can lead to the formation of new anthocyanin-derived pigments such as
530 pyranoanthocyanins (Carrascón et al., 2018; Fulcrand et al., 1998). Moreover, anthocyanins
531 are also thermolabile compounds and chemical modifications such as deglycosylation when
532 the temperature is increased may occur. These different temperature-catalysed reactions
533 therefore increased the consumption of oxygen during the 60°-ATT.

534 In addition to that, a positive correlation with the catechin polymeric unit (extension and
535 terminal unit) was obtained (R = 0.637 and R = 0.627 respectively) and was consistent with
536 the results of the H_2O_2-ATT. Indeed, when the temperature increased, the formation of
537 quinone by the Fenton reaction was accelerated and accentuated the formation of
538 acetaldehyde by oxidation of ethanol. The antioxidant capacity measured by the FRAP assay
539 was also positively correlated with 60°-ATT. Due to the fact that a wine with a high reducing
540 capacity have a better ability to resist to oxidation (Singleton, 1987) through its phenolic pool,
541 therefore a wine with a high antioxidant capacity will consume oxygen more quickly.

542 Two electrochemical parameters were correlated with 60°-ATT. The charge at 520 mV
543 (Q_{520mV}) which corresponds to all the compounds that oxidise up to this potential such as
544 catechin-type flavanols, malvidin-based anthocyanins and galloylated compounds. While the
545 difference of charges Q_{520mV}-Q_{240mV} which corresponds only to the charge resulting from
546 oxidation of malvidin-based anthocyanins and the second oxidation of ortho-diphenols such

547 as catechin (Benbouguerra et al., 2020; Deshaies et al., 2021; Newair et al., 2018). Positive
548 correlations between 60°-ATT and anthocyanins and catechin-based polymeric units were
549 also obtained. These compounds, which correspond to the electrochemical charge Q_{520mV} and
550 the difference of charges Q_{520mV}-Q_{240mV}, consequently explain these positive correlations.

551 As for the other two tests, the Pearson coefficients obtained did not allow us to establish clear
552 correlations when individual parameters were used. Three PLS regression models were
553 therefore built as for the H_2O_2-ATT and laccase-AAT. PLS modelling allowed to find highly
554 significant models linking the 60°-ATT values to the initial wine measured parameters. PLS-
555 Model (1) considering both compositional and antioxidant parameters showed the best
556 correlation between the predicted and experimental values of 60°-ATT with a linearity
557 coefficient (r^2) of 0.967. Model (2) taking into account only the compositional parameters
558 showed a lower correlation than model (1) with an r^2 of 0.892. Finally, contrary to the to the
559 H_2O_2-ATT and laccase-AAT, PLS-model (3) (antioxidant parameters) showed a linearity with
560 the antioxidant parameters with the lowest obtained correlation coefficient (r^2) of 0.748
561 (Figure S3, Supplementary Data). These results showed that it was necessary to consider all
562 the measured parameters to predict this thermal test. The results of the most relevant variables
563 for each PLS regression and their standardised coefficients in the regression equation have
564 been summarised in Table 5.B. There were variables that were only relevant for the
565 multivariate model, but for which it was not possible to establish a linear correlation with the
566 60°C-ATT.

567 Concerning the PLS-model (1), among the most important parameters, some were both
568 linearly positively correlated and relevant for the multivariate model, indicating a direct effect
569 of these compounds on this AAT result. The model suggested a positive influence of
570 antioxidant capacity measured by FRAP assay, acetylated anthocyanins, extension and
571 terminal catechin units, and the difference of electrochemical charge Q_{520mV}-Q_{240mV}. As

572 explained previously, these parameters were positively related to the oxygen consumption
573 during thermal AAT through the acceleration of oxidation reactions caused by the increase in
574 temperature (Deshaies et al., 2020). Unfortunately, PLS model (1) showed that the
575 electrochemical charge at 240 mV (Q_{240mV}) which represents the most easily oxidisable
576 polyphenols groups such as pyrogallol group (Kilmartin & Hsu, 2003) impacted negatively
577 this regression model. In addition to that, PLS-model (2) also showed negative impact of the
578 epigallocatechin terminal unit. However, some studies showed that certain polyphenols could
579 have a negative influence on oxygen consumption (Carrascón et al., 2018; Ferreira et al.,
580 2015). It has also been shown (Jovanovic et al., 1998) that epigallocatechin type flavanols
581 could form relatively stable complexes with metal ions that at pH of wine. These complexes
582 could therefore slow down the Fenton reaction by decreasing the concentration of free metal
583 ions. However, the opposite behaviour was obtained for the model (1) and (2) of the chemical
584 AAT, which could be explained by the addition of hydrogen peroxide in excess which
585 promoted the formation of an ethoxyl radical (Deshaies et al., 2020) which oxidised the wine
586 polyphenols more quickly.

587 Furthermore, according to model (2), the kinetic parameters of the thermal test were
588 positively influenced by the epicatechin extension units. Like catechin, this flavan-3-ol was
589 also substrate for non-enzymatic oxidation by the Fenton reaction (Oliveira et al., 2011).
590 Conversely, the free SO_2 content has a negative influence on the thermal ATT constant. This
591 may seem surprising as SO_2 being an antioxidant should accelerate oxygen consumption
592 (Danilewicz, 2007) but Carrascón et al. (Carrascón et al., 2018) showed that there was no
593 influence of SO_2 on the oxygen consumption of red wine. However, in our study, oxygen
594 consumption was monitored during accelerated ageing and when the temperature was
595 increased in acidic media like wine matrix, many chemical reactions were accelerated, in
596 particular reactions related to tannins, as depolymerisation reactions. These depolymerised

597 tannins may then react with SO_2 to give flavanol sulfonate (Bonaldo et al., 2020). These
598 reactions could therefore make SO_2 less available to react with oxygen.

599 ## 4. Conclusion

600 In this work, three accelerated ageing tests (60°C-ATT, Laccase-ATT and H_2O_2-ATT) were
601 applied to Syrah red wines with different phenolic compositions and antioxidant properties
602 showing that the results of the tests were not correlated with each other. These results
603 therefore revealed specific mechanisms for each accelerated ageing test. This was
604 corroborated by linear correlations and multivariate PLS models between the measured
605 variables and the accelerated tests that differed for each test.

606 Results also illustrated that some chemical and antioxidant parameters were useful variables
607 to predict the results of three different accelerated ageing tests. Although some individual
608 variables were linearly correlated with the AATs results, PLS models, using a multi-variable
609 approach, were much more precise and powerful to predict them.

610 These different accelerated ageing tests could be complementary for each other to predict red
611 wine ageing. In future work, other measurements involving wine samples undergoing
612 "natural" ageing were done to determine if one or more than one AAT(s) are needed to
613 predict red wine ageing kinetics.

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