RELATION BETWEEN PHENOLIC CONTENT, ANTIOXIDANT CAPACITY, OXYGEN CONSUMPTION RATE OF DIVERSE TANNINS

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Introduction

Enological tannins can be divided into two main groups, according to their chemical structure: condensed and hydrolysable tannins.
Condensed tannins are oligomers or polymers of flavan-3-ols, that differ from one another for the proportion of monomeric units and for the degree of polymerization. They are also called proanthocyanidins due to the red pigments formed in concentrated acids upon oxidative heating (Bate-Smith reaction) (Celzard et al., 2015).
Hydrolysable tannins include gallotannins (glucose esters of gallic acid) and ellagitannins (glucose esters of ellagic and/or hexaydroxydiphenic acids) (Versari, du Toit, & Parpinello, 2013).
The galloyl groups may further yield more complex hydrolyzable tannins through esterification or oxidative crosslinking (Hagerman, 2002).
Enological tannins are extracted from different botanical species, including oak, chestnut, quebracho, tara, galla and grape.
The chemical composition of commercial tannins and consequently their chemical and technological properties are strongly influenced by the botanical origin, the part of the plant used (Vivas, Vivas De Gaulejac, & Nonier, 2002) and the extraction protocol (Bosso, Guaita, & Petrozziello, 2016).
Several properties are attributed to the wide range of commercial tannins, and recently a great interest was addressed to the antioxidant activity, a very important quality, especially in the perspective to produce wines with lower sulphites content. Anyway, despite the necessity for winemakers to know the impact of tannin additions to the wines, and the many studies available, a considerable uncertainty remains regarding the beneficial effects of enological tannins as antioxidant. The lack of univocal information derives from both the compositional variability of commercial tannins available on the market and the difficulty of testing the antioxidant activity, due to the absence of a standard method, to the complexity of the oxidation process and to the variability of wine composition.
Indeed, the antioxidant activity is related to different properties: capacity to scavenge superoxide radicals (Farhadi, Esmaeilzadeh, Hatami, Forough, & Molaie, 2016) and to consume dissolved oxygen (Pascual et al., 2017; Vignault et al., 2018), reduction capacity towards Fe(III), and chelating effect on Fe(II) with the consequent prevention of oxidative evolution mediated by Fenton-based reactions (Perez, Wei, & Guo, 2009);
Therefore, since antioxidant assays account for different mechanisms underlying the total antioxidant capacity, they often produce different and sometimes contradictory results (Magalhaes et al., 2014). For this reason, in order to have a complete and reliable characterization, it is important to use diverse chemical tests based on different mechanisms of antioxidant action (Magalhaes et al., 2014), and to support these tests with the measure of the oxygen consumption (Pascual et al., 2017).

Aim of the work

In this work, seven tannins with different botanical origin were characterized for the polyphenolic content and antioxidant capacity with a multitechnique approach, aimed at investigating the correlations between the different parameters studied and to find out the parameters most related the oxygen consumption kinetic.

Materials and methods

The study was carried out with the following seven tannins:

- E1A: ellagitannin from not toasted American oak
- E2F: ellagitannin from not toasted French oak
- GT: gallic tannin from tara
- Lb: tannin from lemon balm wood (non-enological);
- C1Sd: condensed tannin extracted from grape seeds
- C2Sk: condensed tannin extracted from grape skins
- C3Sd: condensed tannin obtained in our laboratory at CREA from Grignolino grape seeds

The polyphenols and tannins content were determined with spectrophotometric methods: total polyphenols by Folin Ciocalteu (GAE%), total polyphenols index (TPI%), total proanthocyanidin index (PC%) (Di Stefano et al., 1989); HPLC (phloroglucinolysis) for the condensed tannins content (CT%) and their mDP, and the percentage of monomeric units (Guaita et al., 2017), and gravimetric analysis (OIV method%) according to OIV Resolution 574-217.

The antioxidant capacity was investigated in terms of free radical scavenging activity (DPPH%) (Carmona Jimenez et al., 2014), ferric ion reducing antioxidant power (FRAP) (Benzie and Strain, 1999) and redox properties with linear sweep voltammetry (LSV_{1200mV}, LSV_{600mV} and R600%) (Kilmartin et al., 2001; Sanchez-Arribaz et al., 2012). According to literature, the area up to 1200 mV serves for total polyphenols determination, while the area up to 600 mV measures the easily oxidizable polyphenols, which contain galloyl and/or catechol moieties (Sanchez Arribas et al., 2012).

For the oxygen consumption test a model solution (12% v/v EtOH; pH 3,5) containing transition metals (5 mg/L Fe^{2+}, 0,15 mg/L Cu^{2+}) was used in order to better simulate the oxidation process occurring in wine.

The model solution was oxygenated at saturation, then the seven tannins at three different doses (250, 500, 1000 mg/L) and 40 mg/L of SO_2 were added at bottling. A thesis was also prepared without tannins (Control). The bottles were then crown capped and stored at 20°C. The oxygen was measured over time using a luminescence based technology (NomaSenseTM O2 Trace - PreSens GmbH, Regensburg, Germany).

The addition of SO_2 to the model wine solution was aimed at accelerating the oxygen consumption rate and at shortening the duration of the test. Indeed, SO_2 reacts with quinones (the oxidized form of polyphenols) reducing them back to the corresponding phenolic forms or generating addition compounds (Michael-type 1,4 addition) that yield sulfonic acid, causing an acceleration in the oxygen consumption rate (Danilewicz et al., 2008).

Results

Fig. 1 shows the oxygen consumption kinetics of the Control and the seven tannins at three doses during 3 weeks, that was the time employed by the fastest oxygen consumer tannin to consume all the dissolved oxygen. E2F was distinguished from the other tannins for the highest oxygen consumption rate, while the lowest rate, among the enological tannins, was observed for the gallotannin GT, in agreement with literature. Three weeks after bottling, E2F was the only trial with an oxygen content lower than 1 mg/L for both the medium (0.50 g/L) and the highest (1 g/L) doses; in the latter case the dissolved oxygen content was close to zero, while

for the lowest dose (0.25 g/L) the oxygen content was 1.2 mg/L. As regards GT, three weeks after bottling the dissolved oxygen content was respectively 1.8 mg/L for the highest dose, 2.5 mg/L for the medium dose, and higher than 3.0 mg/L for the lowest dose. Regarding the tannins with an intermediate behavior, C1Sd and C2Sk were faster oxygen consumers than the other two, with similar consumption rates, superimposable to E2F for the 1 g/L dose.
The oxygen consumption trend was described for all trials (all tested tannins at three doses and Control) over 21 days by a quadratic equation and, only for the first five days, by a linear equation. Three indexes were calculated to determine the oxygen consumption rate for each dose: OCR21 for 21 days, OCR5 for the first 5 days and OCRd as the mean daily oxygen consumption rate.
In general, the oxygen consumption rate increased with the increase of the tannin dose, according to literature (Danilewicz et al., 2008). In particular, the influence of the tannin dose on the oxygen consumption rate varied with the tannin botanical origin: significant interactions were observed between kind and dose of tannins. A similar increase in the oxygen consumption rate was observed for all tannins when increasing the dose from 0.25 to 0.50 g/L; conversely, a lower increase was observed from 0.50 g/L to 1 g/L, only for some tannins, while no differences were noticed for the two ellagitannins E2F and E1A (Fig. 2).

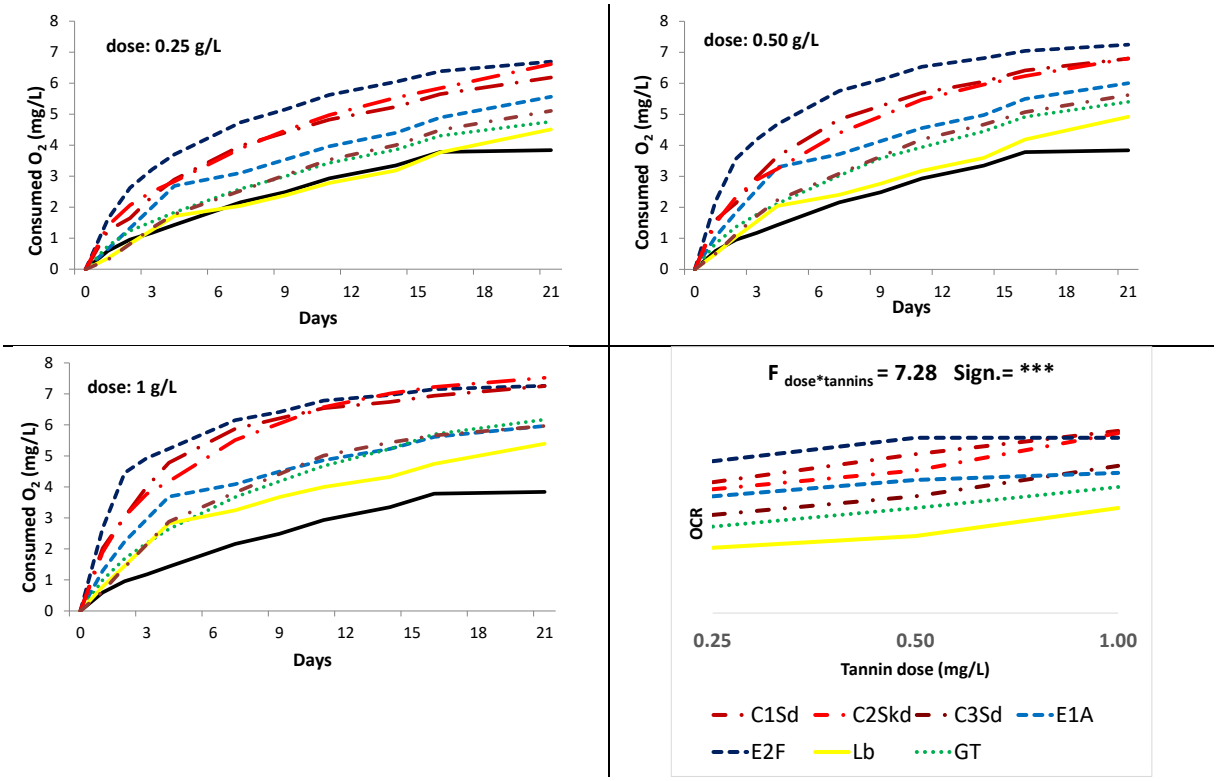

Figure 1: Oxygen consumption trend of control and tannins at three doses in an oxygen-saturated model wine solution and interactions between tannin type and dose on the oxygen consumption rate

Table 1 shows the correlation between all the indexes calculated to express the oxygen consumption rate during 21 and 5 days (Table 1a), and the correlation between all the studied parameters used to determine the polyphenols and tannins content, the antioxidant activity and the oxygen consumption rate (Table 1b).
All the oxygen indexes, calculated for each dose, were highly correlated to each other: the trend observed in the first five days reveals what happens in a longer period of 21 days.
This result suggests the possibility to shorten the oxygen consumption test at 5 days.
Regarding all the studied parameters, significant correlations were observed between the polyphenolic content (GAE%, TPI% and OIV method%) and the antiradical power (DPPH%), between the oxygen consumption rate (OCRd_0.25, OCRd_0. 5 OCRd_1) and the redox properties (LSV_{1200mV}), between the ferric reducing capacity (FRAP) and the redox properties.

Furthermore, good correlations were observed between the FRAP data and the oxygen consumption rate, possibly due to the fact that both tests are based on the polyphenolic oxidation process by Fe(III).

	OCRd_0.25	OCR21_0.25	OCR5_0.25	OCRd_0.5	OCR21_0.5	OCR5_0.5	OCRd_1	OCR21_1
OCRd_0.25	1							
OCR21_0.25	0.96 **	1						
OCR5_0.25	0.91 **	0.93 **	1					
OCRd_0.5	0.98 **	0.99 **	0.92 **	1				
OCR21_0.5	0.94 **	1.00 **	0.91 **	0.98 **	1			
OCR5_0.5	0.89 **	0.94 **	1.0 **	0.92 **	0.92 **	1		
OCRd_1	0.93 **	0.9 **	0.76 *	0.93 **	0.89 **	0.74	1	
OCR21_1	0.92 **	0.91 **	0.74	0.94 **	0.93 **	0.74	0.94 **	1
OCR5_1	0.93 **	0.95 **	0.95 **	0.95 **	0.94 **	0.96 **	0.84 *	0.85 *

	GAE %	TPI %	OIV method %	DPPH%	FRAP	LSV_{1200mV}	$LSV_{600\ mV}$	OCRd_0.25	OCRd_0.5
GAE %	1								
TPI %	0.88 **(1)	1							
OIV method%	0.95 **	0.8 *	1						
DPPH%	0.89 **	0.921 **	0.9 **	1					
FRAP	0.7	0.33	0.78 *	0.44	1				
LSV_{1200mV}	0.44	0.24	0.63	0.33	0.78 *	1			
$LSV6_{00\ mV}$	0.38	-0.01	0.44	0.26	0.55	0.2	1		
OCRd_0.25	0.30	0.21	0.49	0.29	0.6	0.84 *	0.01	1	
OCRd_0.5	0.41	0.28	0.55	0.33	0.67	0.81 *	0.04	0.98 **	1
OCRd_1	0.56	0.50	0.73	0,61	0.63	0.78 *	0.13	0.93 **	0.93 **

Table 1: Pearson correlation matrix of the variables that describe the oxygen consumption kinetics, the polyphenolic composition and the antioxidant activity of tannins.
(1), ** represent significance at p ≤ 0.05 and 0.01 respectively.*

The Factor Analysis calculated with GAE%, TPI%, OIV method, PC%, CT%, DPPH%, FRAP, LSV_{600mV}, LSV_{1200mV}, R600%, OCR21_0.25 and OCRd_0.25 identified three causes of variability between tannins (3 Factors) and the analytical parameters describing them.
Figure 2 shows the loadings and scores plots in the space described by the 1st and 2nd Factors and by the 1st and 3rd Factors. The First Factor explains for the 34.02% of the total data variability and it is related to the richness in polyphenols (OIV method, GAE%, TPI%) and to the antiradical capacity (DPPH%). The Second Factor explains the 27.4% of the total data variability, it is related to the tannin typology and distinguishes condensed from hydrolysable tannins. The second Factor is positively correlated with the condensed tannins content (PC% and CT%), while it is negatively correlated with the LSV results that express the content of easily oxidizable molecules (LSV_{600mV} and R600%), more abundant in hydrolyzable tannins. The Third Factor explains the 30% of the total data variability and it is related to the oxygen consumption kinetic. The third Factor is positively associated to the oxygen consumption kinetic indexes (OCRd_0.25 and b_0.25), and to LSV_{1200mV} and FRAP. The oxygen consumption rate was therefore associated to the capacity of polyphenols to reduce Fe(III) to Fe(II) (FRAP index) and to the total content of electroactive molecules (LSV_{1200mV}).

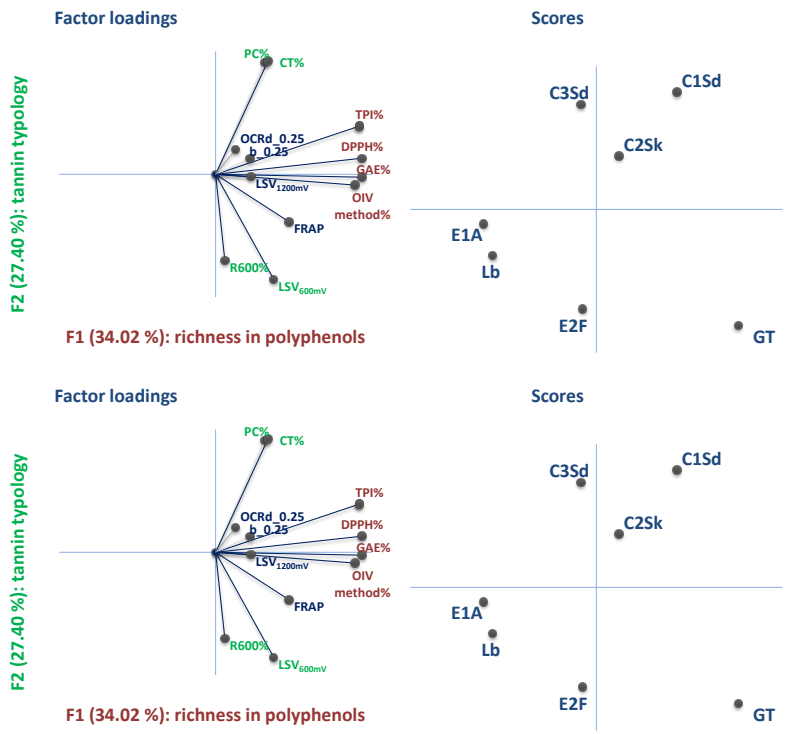

Figure 2: Representation of the loadings (variables) and the scores (tannins) in the planes respectively defined by the 1st (F1) and 2nd (F2) Factor and the 1st (F1) and 3rd (F3) Factor.

Conclusions and future perspectives

In general, these data confirm that different antioxidant tests produce different results (Magalhaes et al., 2014), not always correlated with the OCR (Pascual et al., 2017). According to literature (Magalhaes et al., 2014; Ricci et al., 2016), a significant correlation was observed between the polyphenols content (GAE%, TPI%, OIV method %) and the antioxidant capacity determined by DPPH assay, possibly due to the fact that these four assays measure the total number of phenol groups in the sample, rather than their reactivity. The parameter most correlated to the oxygen consumption kinetic was LSV_{1200mV}, proposed by Kilmartin et al. (2001) to determine the content of total oxidizable polyphenols in wine. This evidence suggests the possibility to use this index as a rapid test for measuring the oxygen consumption capacity. Conversely, no significant correlation was observed between OCR and Folin-Ciocalteu index (GAE%), probably because the oxygen consumption rate is influenced by the content of polyphenols susceptible to oxidation, while GAE% is not specific for this kind of compounds, since it oxidizes and quantifies also phenolic groups that are not oxidizable in air (Danilewicz, 2015).

Furthermore, some information can be obtained on the antioxidant property of the studied tannins: ellagitannins (oak) are excellent oxygen consumers with high ferric reducing capacity, gallotannins (tara) have slight capacity to consume oxygen but high antiradical power and good ferric reducing capacity, condensed tannins (grapes) are good oxygen consumer with high antiradical power.

The future perspective is to extend this study to a wider range of tannins, considering simultaneously the antioxidant capacity of the tested tannins in wine.

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