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Insight into the characterization of commercial oenological tannins

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ABSTRACT

The characterization in terms of phenolics, antioxidant capacity and oxygen consumption rate (OCR) was carried out for 15 commercial oenological tannins of different origin. A NMR approach was used to evaluate their average molecular weight, and their glycosidic and aromatic moieties. The investigated oenological tannins showed wide differences in their chemical properties. Total phenol index (TPI) ranged from 461±28 and 1018±57 mg gallic acid/g for cherry tannin and nut gall tannin, respectively. The antioxidant capacity, positively correlated with TPI, was higher for hydrolysable tannins ranging 3.05±0.06 and 12.06±0.71 mM Trolox/g for ABTS assay, and from 3.70±0.23 and 10.94±1.28 mM Trolox/g for DPPH assay. Relevant differences in OCRs were found and chestnut tannin showed the highest OCR. Wide range of molecular weights were found with nut gall 1 tannin showing the highest one, ranging from 790 to 1900 Da. This study improves and expands the actual knowledge of tannins supporting the suitability of NMR for the characterization of oenological tannins.

1. Introduction

Oenological tannins are a group of phenolics capable of both astringent and protein-binding behavior. Their use in winemaking is permitted by the International Organization of Vine and Wine (OIV) with the purpose of promoting the clarification of musts and wines (OIV, 2019a, 2019b). They are employed in winemaking to protect the wine against oxidation, improve wine body and mouthfeel, as well as to stabilize the color of red wines (Neves et al., 2010). Nonetheless, the addition of tannins must not be responsible for bringing further aroma or color to wine (OIV 2019b). The oenological tannins are also capable to react with thiols when they are in an oxidized state. Previous study on model wine solution have demonstrated that both ellagitannins and proanthocyanidins have significant effect in decreasing ethanethiol concentration (Vivas et al., 2003). Recently, the tannins showed the ability to prevent the appearance of light-struck taste in both model wine (Fracassetti et al., 2019) and white wine (Fracassetti et al., 2021).

Diverse commercial tannins are available for the oenological purposes above mentioned. They differ to each other for chemical structure, botanical origin, and extraction process (e.g. with water or organic solvent). From the chemical point of view, tannins include polymers of flavan-3-ol units, the condensed tannins derived from grape (both seeds and skin), quebracho, acacia and tea. The hydrolysable tannins are glucosides of gallic acid (gallo tannins) or ellagic acid (ellagitannins); tannins extracted from nut gall, tara, chestnut, cherry, and oak belong to this group. Other tannins include also those extracted from lemon being a mixture of both condensed and hydrolysable tannins (Ezeabara et al., 2014). These tannins are available either as dry material or liquid solution; information about the origins and recommended additions are usually indicated by the producers. However, concentration of phenols is not generally indicated and the applied extraction method (i.e. solvent, water) is unknown in most of the cases. Oenological tannins derived from different sources and have been classified from high to low prices, in the following order: skins > seeds > stems ≥ quebracho tannins ≥ other vegetal sources (Vivas et al., 2004). Commercial oenological tannins showed relevant differences in terms of concentration of total phenols, total tannins, and gelatin index (Obreque-Slíer et al., 2009; Vignault et al., 2018). Based on the origin, as expected, differences are also due to their composition (e.g. proanthocyanidins, ellagitannins). Such aspects should be considered when they are added during the winemaking, because they may lead to different effects on evolution of the wine (Chen et al., 2016; García-Estevez, Perez, Soares, & Mateus, 2017; Panero et al., 2015). The hydrolysable tannins are naturally extracted from the wood barrel during the wine aging as well as added

Abbreviations: TPI, Total phenol index; OCR, Oxygen consumption rate; mDP, Medium polymerization degree; Antiox, Antioxidant capacity.

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during winemaking. Among the source of wood tannins, those originating from oak show higher antioxidant activity (Bautista-Ortin et al., 2005). Despite that, many winemakers prefer to use condensed tannins; this might be due to their ability to improve some sensory properties of the wine, such as aroma complexity, as well as to stabilize the color of red wine. The ellagitannins have the properties above mentioned, but they are more expansive in comparison to condensed tannins. The sensory and antioxidant properties of oenological tannins were assayed by Li et al. (2020) and results showed that the mixture at ratio 1:1 hydrolysable: condensed tannins performed the best in young red wines. Moreover, the limited impact on intensity of astringency was found in red wine added with tannins after one year of aging (Rinaldi et al., 2017). The use of tannin can limit oxidation phenomena in white winemaking. This is because certain condensed and gallo tannins can denature polyphenol-oxidase enzymes, like tyrosinase and laccase, protecting must against the enzymatic oxidation (Vignault et al., 2019). Ellagic and gallo tannins have also the ability to chelate metal ions, such as iron and copper, counteracting the chemical oxidation in must and wine (Canuti et al., 2020; Vignault et al., 2018). Tannins have a major role in the stabilization of the color of rosé wine taking part into the formation of copolymers. The latter are more stable, and the precipitation of pigments is constrained (Versari et al., 2013). The addition of tannins in must from red grape can protect anthocyanins towards oxidation from the begin of the winemaking process when the content of ethanol is low and the extraction of tannins from grape skins is still limited (Versari et al., 2013). Last but not least, tannins can react with the dissolved oxygen producing acetaldehyde which can favor the formation of anthocyanin-tannin or tannin-tannin adducts *via* ethyl bond. This polymerization reaction increases the phenol stability prior the bottling (Versari et al., 2013). Due to the importance of oenological tannins because of their several abilities, the further knowledge of oenological tannins represents a pivotal aspect for the major comprehension of the composition of the commercial products available. This is because of the wide variability of the oenological tannins based of their origin as well as on the extraction procedures.

On these bases, the aim of this study was to chemically characterize fifteen different commercial oenological tannins in terms of phenolic composition and antioxidant capacity. Their ability of oxygen consumption was also investigated with and without sulfur dioxide (SO_2). A NMR approach was used to obtain further information regarding their structure. This study is expected to expand the understanding of the composition of commercial tannins, considering also those from the same botanical origin. Opportunities regarding their use in specific winemaking processes will be also considered.

2. Materials and methods

2.1. Chemicals and reagents

Methanol (99.9%), ethanol (96%), acetonitrile (99.9%), Folin-Ciocalteu reagent (99.9%), gallic acid (97.5–102.5%), sodium carbonate (99.5%), tartaric acid (99.5%), hydrochloric acid (37%), methyl cellulose (1500 cP), phloroglucinol (> 99%), catechin (98%), epicatechin (> 98%), ascorbic acid (99%), sodium acetate (> 99%), pentagalloyl glucose (> 96%), tannic acid, dextran, dimethylaminocinnamaldehyde,2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS), Trolox (97%), potassium metabisulfite (> 98%), copper sulfate pentahydrate (98%), iron sulfate heptahydrate (98%), sodium hydroxide (98%), trifluoroacetic acid (99%) and hydrochloric acid (37%) were purchased from Merck (Darmstadt, Germany). Procyanidin A (> 97%) was purchased from Extrasynthese, (Genay Cedex, France) All the chemicals were of analytical reagent grade, at a minimum. HPLC grade water was obtained from a Milli-Q system (Millipore Filter Corp., Bedford, MA, USA).

2.2. Description of commercial tannins

Fifteen commercial oenological tannins listed in Table S1 were analyzed. Tannins, in a powder form, were provided by Dal Cin Gildo Spa (Concorezzo, MB, Italy).

2.3. Chemical characterization of phenolics

For the phenolic characterization of the commercial tannins, total phenol index, tannin content, determination of proanthocyanidins, reactivity to SO_2 were determined.

2.3.1. Total phenol index

2.3.1.1. Folin-Ciocalteu assay. The phenol content in tannin samples was assessed as reducing ability by the Folin-Ciocalteau method (Scalbert et al., 1989).

Sample solutions at 1 g/L of each tannin were prepared in methanol/water 50/50 (v/v) and then serially diluted up to 20 times in the same solvent.

The reaction solutions were prepared as follows: 2.5 mL of diluted Folin-Ciocalteu reagent diluted (10 times with water), were added to 0.5 mL of sample solution properly diluted and 2 mL of sodium carbonate (75 g/L). The absorbance at 765 nm was measured after incubation of the reaction solution for 1 hour at 20±2 °C in the dark.

The calibration curves were obtained with gallic acid. A five-points calibration curve was obtained in methanol/water 50/50 (v/v) at concentrations in the range 10–100 mg/L. Results were expressed as mg gallic acid/g of tannin (Scalbert et al., 1989; Fracassetti et al., 2020). Triplicate determination was carried out.

2.2.1.2. Absorbance at 280 nm. The total phenol index (TPI) was determined by the spectrophotometric method reported by Fracassetti et al. (2016).

Tannin samples were dissolved in model wine solution (tartaric acid 5 g/L, ethanol 12% (v/v), pH 3.2 adjusted with sodium hydroxide) at 1 g/L and then properly diluted in water up to 10 times in order to read an absorbance lower than 1 AU.

A five-points calibration curve was obtained with tannic acid and (+)-catechin stocks prepared in model wine solution (1 g/L) and diluted with water (5–100 mg/L). The calibration curve obtained with tannic acid was used for the quantification of hydrolysable tannins, while calibration curve obtained with (+)-catechin was used for the quantification of the condensed tannins. Results were expressed as mg of gallic acid/g of tannin or as mg of (+)-catechin/g of tannin for hydrolysable tannins and condensed tannins, respectively (Vignault et al., 2018). Triplicate determination was carried out.

2.2.2. Determination of free gallic acid

For the determination of gallic acid, the oenological tannins (5 g/L) were dissolved in water/methanol 50/50 acidified with formic acid 1% (v/v) (Watrelot et al., 2020). An Acquity HClass UPLC (Waters, Milford, MA, USA) system equipped with a photo diode array detector 2996 (Waters) was used. The separation column was a Kinetex RP18 (150 × 2.1 mm, 2.6 μm, 100 Å) (Phenomenex, Torrance, CA, USA) kept at 30 °C. The chromatographic separation was carried out using trifluoroacetic acid 0.2% (v/v) in MilliQ-treated water (solvent A) and acetonitrile (solvent B) as eluting solvents. The UPLC separation was achieved by an elution gradient (1% to 10% of solvent B in 10 min) at a flow rate of 0.8 mL/min. The sample preparation was carried out in triplicate and the solutions were filtered prior the injection. Six-points calibration curve was obtained with gallic acid (1–100 mg/L) dissolved in the same solvent (water/methanol 50/50 acidified with formic acid 1%). Chromatographic data acquisition and processing were performed by Empower 3 software (Waters).

2.2.3. Tannin content

Tannin content (richness) was determined by exploiting the ability of methyl cellulose (MC) to precipitate tannins. The absorbance reading at 280 nm before and after tannin precipitation enables the selective measurement of tannin only (subtractive approach) (Sarneckis et al., 2006; Vignault et al., 2018).

Tannin sample solutions were prepared in model wine solution at 1 g/L. For the reaction mix, two reaction tubes (Tube A and Tube B) were prepared as following reported. For Tube A, 125 μL of tannin solution for condensed tannins or 75 μL for the hydrolysable tannins was added with 1 mL of MC solution, 1 mL of saturated ammonium sulfate solution and up to 5 mL water. For Tube B, MC solution was substituted with water. The reaction solutions were kept at 20±2 °C for 10 min and centrifuged (10 min at 2863 *x g)* (Hettich Mikro 220R, Tuttlingen, Germany). The supernatants were recovered and their absorbance was read at 280 nm.

The difference among the absorbance values read for tube A and tube B was calculated and adjusted for the dilution factor.

The calibration curves (five-points) were obtained with catechin and tannic acid (5–100 mg/L). The tannin richness for condensed tannins was expressed as catechin equivalents and for hydrolysable tannins as tannic equivalents per g of tannins. Triplicate determination was carried out.

2.2.4. Determination of proanthocyanidins

The proanthocyanidin content and profile were determined for the condensed tannins (Grape skin, Grape seeds, Tea 1, Tea 2, Quebracho, Acacia, Lemon).

2.2.4.1. Determination of condensed tannins with dimethylaminocinnamaldehyde. Condensed tannins were determined by reaction with dimethylaminocinnamaldehyde (DMCA) following the procedure reported by Prior et al. (2010).

The samples (1 g/L) were dissolved in ethanol:water 80:20 (v/v) and serially diluted up to 16 times in ethanol:water 80:20 (v/v), then 130 μL were added to 1.2 mL of DMCA to and incubated for 20 min at 20±2 °C in the dark. Finally, the absorbance at 640 nm was read.

A five-points calibration curve (2.5–80 mg/L) was obtained with procyanidin A stock solution (80 mg/L) prepared in ethanol:water 80:20 (v/v). Results are expressed as mg of procyanidins A/g of tannin. Triplicate determination was carried out for each commercial tannin.

2.2.4.2. Determination of proanthocyanidins by phloroglucinolysis. This determination is based on the analysis of proanthocyanidin cleavage products after acid-catalysis in the presence of excess of phloroglucinol (Kennedy & Jones, 2001).

Oenological tannin solutions 1 g/L in model wine solution were submitted to Solid Phase Extraction (SPE) with C_{18} cartridge (Phenomenex, Torrance, CA, USA) as it follows. After SPE activation with methanol and water, 5 mL of the sample solution was loaded followed by 5 mL of sulfuric acid 0.01 N and 20 mL of diethyl-ether. Proanthocyanidins were eluted with 15 mL of methanol and recovered by solvent evaporation under vacuum. The phloroglucinolysis reaction was carried out as follows: 2 mL of phloroglucinol solution (50 g/L prepared in acidified methanol containing hydrochloric acid 0.2 N) containing ascorbic acid (10 g/L). The reaction tubes were incubated at 50 °C for 25 min immediately soaked in ice bath and then added with 4 mL of sodium acetate solution 0.1 M. The samples were filtered with 0.22 μm PVDF membrane filter and analyzed by UPLC-UV. The chromatographic separation was carried out by a Acquity HClass UPLC (Waters, Milford, MA, USA) coupled with diode array detector 2996 (Waters). The column was a Kinetex RP18 (150×2.1 mm, 2.6 μm, 100 Å) (Phenomenex) thermostatted at 30 °C. The solvents were acetic acid 2.5% (v/v) in Milli-Q water (Solvent A) and acetonitrile (Solvent B). UPLC separation has been completed with an elution gradient (from 3% to 9% of Solvent B in 1.5 min, from 9% to 16% in 4.5 min, from 16% to 50% in 13.5 min) with a flow of 0.85 mL/min (Fracassetti et al., 2017). Six-points calibration curves were obtained with catechin and epicatechin (5–50 mg/L). Chromatographic data acquisition and processing were performed by Empower 3 software (Waters). The results are expressed as mg catechin/g of tannin. Triplicate determination was carried out. The average degree of polymerization (mDP) was calculated as molar ratio between the moles of the terminal units and the total moles of proanthocyanidins. Percentage of galloylation was calculated as molar ratio between the moles of gallic acid flavan-3-ol esters and the total moles of proanthocyanidins.

2.1.5. Reactivity to sulfur dioxide

Oxidized tannins were indirectly estimated after reaction with sulfur dioxide as, reported in Fracassetti et al. (2016). Briefly, the samples (1 g/L) were dissolved in model wine solution and added with sulfur dioxide (0.3% w/v). The absorbance at 280 nm was read before and after the addition of sulfur dioxide. The results are expressed as the % of oxidized tannins considering the sample without and with sulfur dioxide. Triplicate determination was carried out.

2.1.6. Nuclear Magnetic resonance

17 mg of tannin extracts were dissolved in 550 microliters of DMSO-d_6. ^{1}H NMR spectra were acquired at 25 °C with a Bruker AV600 spectrometer, operating at 600.13 MHz for the ^{1}H nucleus and equipped with a TXI probe with z-gradient. The spectra were processed with TOPSPIN 1.3 software. Pseudo two-dimensional DOSY experiments (Morris, 1992) were acquired using the pulse-program 'stebpgp1s'. The strength of the linear pulse gradient was incremented in 64 steps (8 scans for each linear gradient step) from 5% up to 95% of the maximum gradient amplitude. Diffusion delay of 400 ms.

Raw data were processed using the standard DOSY software present in the Bruker library.

The calibration curves were generated by measuring four model compounds in independent DOSY NMR experiments (Morris, 1992). Diffusion-order NMR spectroscopy (DOSY) experiments for calibration curve have been carried out on DMSO-d_6 solution of gallic acid (M_w 170), pentagalloyl glucose (M_w 940), tannic acid (M_w 1701) and dextran (M_w 10,000). Plotting logD versus log Mw of all model molecules in one diagram gives a linear correlation. A least-squared fitting of data leads to the relationship Log M_w = 2.1834 D(m^2/s) + 23.152.

2.2. Antioxidant capacity

The antioxidant capacity was determined by the 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) assays the procedure reported by Fracassetti et al. (2016). Briefly, tannin samples (25 g/L) were dissolved in methanol 70% (v/v) and serially diluted up to final concentrations of 0.125 g/L. For the ABTS assay, the reaction occurred by adding 1 mL of ABTS and 10 μL of diluted sample; for the blank add 10 μL of methanol 70% (v/v). The reaction mix was vortexed for 30 s, the absorbance at 734 nm was read and a second reading was carried out after 20 s. For the DPPH assay, the reaction was carried out with 2.45 mL of DPPH and 50 μL of diluted sample; for the blank add 50 μL of methanol 70% (v/v). The reaction solutions were incubated for 50 min at 20 °C in the dark and the absorbance at 515 nm was read.

The calibration curve was obtained with Trolox (0.16–5 mM). Results are expressed as mM Trolox/g of tannin. For each commercial tannin, the determination was carried out in triplicate.

2.3. Oxygen consumption rate

The oxygen consumption rate (OCR) was measured by chemiluminescence using an oxygen sensor spot (a ruthenium-based luminophore glued inside each bottle) and an optical system (OxySenseTM 101, DecisionLink, Inc., Las Vegas, NV).

Tannin samples were dissolved in model wine solution 500 mg/L of TPI determined by Folin-Ciocalteu method. The model solution was

added with iron (10 mg/L as $FeSO_4 \cdot 7H_2O$) and copper (0.5 mg/L using $CuSO_4 \cdot 5H_2O$) in accordance with Danilewicz (2013). Triplicate samples dissolved in model wine solution were transferred in 100 mL bottles without headspace and hermetically closed. The samples were stored at 20±2 °C in the dark. Oxygen was monitored twice a day for the first three days and then once a day for about two weeks and after shaking the bottle (Vidal et al., 2006; Fracassetti et al., 2013). The monitoring of oxygen consumption was carried out in the presence of sulfur dioxide (40 mg/L). As control, catechin (500 mg/L) and gallic acid (500 mg/L) were used, with and without sulfur dioxide.

The oxygen consumption rate (OCR, mg oxygen/L/day) was calculated as a ratio between the oxygen consumed and the days of monitoring.

2.4. Statistical analysis

Statistical analysis of the results was carried out with R software (R Core Team (2021). R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/) implemented with R-Studio (RStudio: Integrated Development for R. RStudio, PBC, Boston, MA URL http://www.rstudio.com/).

Shapiro test was performed to evaluate the normality of the distribution of the population; Levene test were performed to evaluate the homoschedasticity of the variances. When these two tests were satisfied, ANOVA was performed, in the other cases the Kruskal-Willlis non-parametric test were carried out. Significant differences were considered for $p < 0.05$.

Correlation indexes were determined among the TPI (Folin-Ciocalteau assay), antioxidant capacity, ratio Antiox/TPI, richness (ABS 280 nm and methyl cellulose assay), through the Pearson Correlation considering the critical value of 0.351 (df = 13, $\alpha = 0.2$); 0.441 (df = 13, $\alpha = 0.1$) and 0.514 (df = 13, $\alpha = 0.05$).

The equations of the calibration curves were calculated by linear regression analysis.

3. Results & discussion

The chemical characterization of the investigated tannins allows to clarify the composition of the commercial products implementing the actual knowledge related to the composition of the oenological tannins.

The highest values of total phenol index (TPI) were obtained for the two nut gall tannins (respectively 1019 ± 57 and 983 ± 36 mg gallic acid/g of tannin), being significantly higher than those found for tara (1 and 2), tea (1 and 2), quebracho and grape seeds tannins (Table 1). The two nut gall tannins showed also the highest content of gallic acid (235±12 and 348±17 mg/g of tannin; Table S2) being around 30% of TPI. As expected, gallic acid was negligible or lower than 10% in condensed tannins, while it was around 20% as average in hydrolysable tannins (Table S2). The lowest TPI was observed in cherry tannin (461 ± 28 mg gallic acid/g of tannin). The proper oxidative state of the investigated oenological tannins can be asserted as no detectable phenols reactive to SO_2 was found, except for tara 2 tannin which showed only negligible reactivity to SO_2 (4 ± 3%). This value is comparable with those previously reported by Fracassetti et al. (2016) related to polyphenol-based formula containing a mixture of plant gallic and ellagic acids extracted from grape, and plant ellagic acid and Arabic gum. Our TPI results differed to those obtained by Vignault et al. (2018), since these authors did not find significant differences between the average values calculated for each class of oenological tannins under study. Nonetheless, the individual characterization of diverse oenological tannins showed a high variability among them (20–78%), even when tannins have the same origin (Vignault et al., 2018). Our results agree with those reported by Canuti et al. (2020) with concern to TPI for oak tannins (611 mg gallic acid/g of tannin as an average for the five tannins tested vs. 558 mg/g in our study as average of two tannins tested) and slightly differed for grape seeds (601 mg gallic acid/g of tannin vs.

Table 1
Chemical characterization of the commercial tannins investigated in this study. [1]: Folin-Ciocalteu method; [2]: Ratio calculated between the antioxidant capacity determed by the DPPH assay and the total phenol index determined with the Folin-Ciocalteau assay; [3]: Methyl cellulose assay; [4]: absorbance reading at 280 nm. Different letters mean significant difference ($p < 0.05$). Legend: Antiox, antioxidant capacity; TPI, total phenol index.

Tannin		Total phenol 1ndex [1]	Antioxidant capacity		Ratio Antiox/ TPI (x 10^{-3}) [2]	SO_2 reactivity	Total phenol index [3]		Tannin content [4]	
		mg gallic acid/g	ABTS assay *mM Trolox/g*	DPPH assay		%	*mg tannic acid/g*	*mg catechin/g*	*mg tannic acid/ g*	*mg catechin/g*
Condensed tannins	Acacia	580±8 ^{cd}	4.05±0.31 ^{ef}	4.51±0.68 ^{ef}	9.7	n.d.		742±25 ^{f}		431±26 ^{f}
	Grape skin	477±13 ^{e}	3.05±0.06 ^{f}	3.70±0.23 ^{f}	7.8	n.d.		873±42 ^{e}		226±18 ^{g}
	Grape seeds	813±30 ^{b}	5.87±0.02 ^{cdef}	5.75±0.43 ^{cdef}	7.1	n.d.		1125±43 ^{d}		717±19 ^{de}
	Quebracho	802±24 ^{b}	5.86±0.23 ^{cdef}	5.32±0.73 ^{cdef}	10.0	n.d.		1453±33 ^{c}		1098±12 ^{b}
	Tea 1	860±25 ^{b}	7.43±0.23 ^{bc}	7.42±0.48 ^{bc}	7.8	n.d.		2162±29 ^{a}		1333±93 ^{a}
	Tea 2	856±34 ^{b}	7.79±0.08 ^{cde}	6.41±0.88 ^{cde}	6.6	n.d.		1584±21 ^{b}		1196±58 ^{b}
Hydrolysable tannins	Cherry	461±28 ^{e}	4.08±0.27 ^{def}	4.62±0.32 ^{def}	7.5	n.d.	396±4 ^{g}		110±6 ^{h}	
	Chestnut	577±20 ^{cd}	6.72±0.11 ^{cde}	6.21±0.33 ^{cde}	10.8	n.d.	469±18 ^{g}		202±5 ^{gh}	
	Nut gall 1	1019±57 ^{a}	11.33±0.15 ^{a}	10.94±1.28 ^{a}	9.9	n.d.	1070±86 ^{d}		862±33 ^{c}	
	Nut gall 2	983±36 ^{a}	12.06±0.71 ^{a}	10.64±1.46 ^{a}	10.6	n.d.	1123±56 ^{d}		770±9 ^{cd}	
	Oak 1	463±18 ^{e}	4.21±0.16 ^{ef}	4.56±0.31 ^{ef}	10.8	n.d.	386±4 ^{g}		158±16 ^{gh}	
	Oak 2	652±22 ^{c}	7.10±0.15 ^{bcd}	6.93±0.47 ^{bcd}	10.7	n.d.	485±3 ^{g}		148±0 ^{gh}	
	Tara 1	796±28 ^{b}	7.55±0.29 ^{ab}	9.21±0.93 ^{ab}	11.6	n.d.	1018±28 ^{d}		666±5 ^{e}	
	Tara 2	817±38 ^{b}	7.32±0.25 ^{a}	9.98±0.52 ^{a}	12.2	4.24±3.2	1051±24 ^{d}		699±24 ^{de}	
	Lemon	545±22 ^{de}	4.78±0.17 ^{cdef}	5.25±0.56 ^{cdef}	8.6	n.d.	466±13 ^{g}		162±5 ^{gh}	

Table 2
Pearson Correlation, critical value: 0.351 for df = 13, α = 0.2 (*); 0.441 for df = 13, α = 0.1 (**); 0.514 for df = 13, α = 0.05 (***). Legend: TPI, total phenol index; ABS, absorbance.

	TPI (Folin-Ciocalteau assay)	Antioxidant capacity (ABTS)	Antioxidant capacity (DPPH)	Ratio Antiox/TPI	TPI (ABS 280 nm)	Tannin content (methyl cellulose assay)
TPI (Folin-Ciocalteau assay)	1					
Antioxidant capacity (ABTS)	0,877***	1				
Antioxidant capacity (DPPH)	0,818***	0.912***	1			
Ratio Antiox/TPI	0,096	0,425*	0,643***	1		
TPI (ABS 280 nm)	0,702***	0,390	0,321	−0,369	1	
Tannin content (methyl cellulose assay)	0,814***	0,520***	0,436*	−0,307	0,957***	1

813 mg/g in our study) and nut gall tannins (820 mg gallic acid/g of tannin 1001 mg/g in our study as average of two tannins tested). Other authors recently showed TPI for grape seeds (715 mg gallic acid/g of tannin as average of two tannins) and nut gall tannins (1014 mg gallic acid/g of tannin) that are in accordance to our findings (Paissoni et al., 2022). The TPI values assessed by the absorbance reading at 280 nm were lower for condensed tannins than those obtained with the Folin-Ciocalteu assay, while the opposite was observed for hydrolysable tannins. This could be dependent to the maximum of absorbance (i.e. at 280 nm for ellagitannins and at 370 nm for ellagic acid) as well as to different calibration curves used according to tannin structure being more discriminating and accurate than the Folin-Ciocalteu method even though it is not specific for tannins but the Folin-Ciocalteau reagent detects all types of phenolic compounds (Vignault et al., 2018). Nonetheless, the phenol abundance of the oenological tannins tested here was confirmed. An overestimation was found in case of quebracho and tea tannins; this could be attributed to the presence of galloylated derivates (Wang and Helliwell, 2001; Obreque-Slíer et al., 2009) leading to higher absorbance values due to the reading of both flavanols and gallic acid. The tannins content ranged from 1333 ± 93 mg tannic acid/g of tannin for tea 1 to 110 ±65 mg tannic acid/g of tannin for cherry (Table 1). The highest values were obtained for condensed tannins; nonetheless, a heterogeneous richness was observed mainly depending on the origin of the tannin. This variation is in agreement with previous observations, indicating that ellagitannins from oak are generally characterized by lower content in phenolics (Pascual et al., 2017; Vignault et al., 2018: Ugliano et al., 2020).

Besides the content of phenolics, the antioxidant capacity is an important parameter to take under consideration as it is a marker of scavenging ability (Wang and Helliwell, 2001) being also highly correlated to phenols (Table 2).

The antioxidant capacity was determined by means of ABTS and DPPH assays. With regards to the ABTS assay, the antioxidant capacity ranged from 3.05 ± 0.06 mM Trolox/g for grape seed tannin up to 12.06 ± 0.71 mM Trolox/g for nut gall tannin. These data were slightly higher in comparison to those reported by Paissoni et al. (2022), while they were comparable or slightly lower to the findings reported by Vignault et al. (2018). DPPH assay is considered a reliable method for the assessment of free radical scavenging activity of antioxidants compounds or extracts from plant and is also considered as one of the ideal and easy-to-apply colorimetric methods for evaluating the antioxidant properties (Mishra et al., 2011). Even considering the DPPH assay, the antioxidant capacity found for the tannins investigated in this study was higher in comparison to the data reported by Paissoni et al. (2022). As expected, with both ABTS and DPPH assays, hydrolysable tannins showed higher value of antioxidant capacity than the condensed ones (Table 1) in accordance to Vignault et al. (2018). Nut gall and tara tannins showed comparable and the highest antioxidant ability values (7.32–12.06 mM Trolox/g of tannin for ABTS assay; 9.21–10.94 mM Trolox/g of tannin for DPPH assay) (Table 1). For these oenological tannins, the highest values of antioxidant capacity/TPI ratio were also observed suggesting a great potential of these oenological tannins in preventing oxidative mechanisms. Grape skin tannin showed the lowest value of antioxidant capacity (3.70 ± 0.23 mM Trolox/g of tannin). Overall, the lower ratio determined for condensed tannins suggests protective abilities against oxidations lower than the hydrolysable ones.

Considering the proanthocyanidins, the grape seeds tannin was the most abundant by means of both DMCA assay and phloroglucinolysis (Table 3). The mDP values for grape skin and seeds tannins were in accordance with previous observations (Vignault et al., 2018). Grape seeds tannin showed also the highest polymerization degree, even than that of grape skin tannin. This unexpected finding could be attributable to the extraction procedure that might be more efficient for the grape seed tannin. Nonetheless, the highest galloylation levels were found in lemon, quebracho and tea tannins. In particular, for the latter two tannin samples, the high galloylation degree can explain the overestimation of tannin richness (Table 1) for these formulas, as later described (Harbertson et al., 2014). The low values of mDP suggest the limited impact of the tannins investigated here in terms of astringency as previously reported (Richaud & Noble, 1990).

Our results highlight the high heterogeneity of the oenological tannins even those having the same botanical origin confirming the findings showed in previous studies (Vignault et al., 2018; Watrelot et al., 2020).

Nuclear Magnetic Resonance (NMR) spectroscopy is a very efficient tool used for structural identification of organic compounds. All ^{1}H NMR spectra showed a complex profile with many overlapped resonances. Nevertheless, signals assigned to the aromatic moiety of polyphenolic compounds were observed in the region between 8.0 and 6.0 ppm for both type of tannins. Moreover, the region between 6.0 and 3.0 ppm included mainly signals attributable to polyhydroxylated systems (carbohydrates or polyols) (Table 4). In particular, the gallate moieties lie between 6.4 and 7.6 ppm and the anomeric protons of glycosides were observed between 4.30 and 5.00 ppm. (Figure S3 and Figure S4). In some samples a sharp signal at 6.92 ppm was evident that was assigned to the H2/H6 aromatic protons of gallic acid; this attribution was also confirmed by comparison with a sample of gallic acid standard. A NMR approach was used to evaluate the average molecular weight of the considered tannins. For the molecular weight dispersions of the components in different tannin samples, DOSY experiments were performed. The diffusion coefficient values (D) evidenced some differences in the molecular weights of the tannins in the considered extracts. In particular, the nut gall 1 tannin showed the higher molecular weights, ranging from 790 to 1900 Da consisting with the presence of tetra, esa, epta and undeca galloylglucose. Also nut gall 2 and tara tannins showed high molecular weights (esa and epta galloylglucose). On the contrary, all the other samples displayed a D values corresponding to molecular weights of small gallotannin molecules (gallic acid and digalloylglucose). Tea 1 tannin contained one, two and three units (Table 5). These results agree with those previous described (Watrelot et al., 2020).

Concerning the oxygen consumption rate, oxygen was consumed with according to a second-order kinetic (Pascual et al., 2017). A different behavior related to the composition of tannins was found as well as depending to the presence of sulfur dioxide. In the absence of sulfur dioxide, the tannins were clearly clustered in three groups (Fig-

Table 3
Profile of proanthocyanidins (mg/g tannin) determined with phloroglucinolysis and [1]dimethylaminocinnamaldehyde (DMCA) assay. Different letters mean significant difference ($p < 0.05$). Legend: mDP, medium polymerization degree.

Tannin	Constitutive units				Terminal units			mDP	% Galloylation	DMCA [1]
	Epigallocatechin	Catechin	Epicatechin	Epicatechin gallate	Catechin	Epicatechin	Epicatechin gallate			
Acacia	6.27 ± 1.27 ^{c}	3.92 ± 0.18 ^{c}	2.15 ± 0.13 ^{c}	0.35 ± 0.01 ^{c}	11.69 ± 0.37 ^{c}	6.90 ± 0.68 ^{c}	0.47 ± 0.01 ^{a}	1.46 ± 0.05 ^{d}	17.04 ± 2.27 ^{d}	66.6 ± 4.9 ^{c}
Grape skin	2,61 ± 0.21 ^{d}	62.24 ± 4.73 ^{b}	9.38 ± 0.22 ^{b}	2.34 ± 0.26 ^{b}	19.65 ± 2.81 ^{b}	14.94 ± 1.59 ^{b}	0.31 ± 0.02 ^{b}	2.53 ± 0.10 ^{b}	3.61 ± 0.31 ^{e}	206.9 ± 8.5 ^{b}
Grape seeds	22.55 ± 0.11 ^{a}	427.62 ± 6.13 ^{a}	15.60 ± 0.83 ^{a}	34.36 ± 0.21 ^{a}	47.07 ± 1.67 ^{a}	26.49 ± 0.38 ^{a}	0.31 ± 0.01 ^{b}	5.65 ± 0.01 ^{a}	7.92 ± 0.08 ^{e}	583.6 ± 31.3 ^{a}
Quebracho	3.29 ± 0.28 ^{d}	0.12 ± 0.00 ^{c}	0.80 ± 0.01 ^{d}	0.24 ± 0.00 ^{c}	3.95 ± 0.44 ^{d}	0.87 ± 0.02 ^{d}	0.24 ± 0.00 ^{b}	1.60 ± 0.01 ^{d}	31.38 ± 0.02 ^{c}	54.2 ± 4.5 ^{c}
Tea 1	6.18 ± 0.14 ^{c}	1.43 ± 0.09 ^{c}	0.34 ± 0.00 ^{d}	0.24 ± 0.00 ^{c}	0.40 ± 0.02 ^{d}	4.90 ± 0.22 ^{c}	0.42 ± 0.03 ^{a}	1.99 ± 0.02 ^{c}	41.05 ± 0.51 ^{b}	n.d.
Tea 2	0.51 ± 0.01 ^{e}	1.51 ± 0.07 ^{c}	0.55 ± 0.06 ^{d}	0.24 ± 0.00 ^{c}	0.42 ± 0.05 ^{d}	6.23 ± 0.23 ^{c}	0.44 ± 0.05 ^{a}	1.28 ± 0.00 ^{d}	8.47 ± 0.00 ^{e}	n.d.
Lemon	13.33 ± 1.20 ^{b}	1.09 ± 0.00 ^{c}	0.94 ± 0.24 ^{d}	nd	2.93 ± 0.66 ^{d}	2.58 ± 0.03 ^{d}	0.41 ± 0.00 ^{a}	2.84 ± 0.31 ^{b}	57.46 ± 4.90 ^{a}	< 1 ^{d}

Table 4
Signals area in the ranges 6.0–4.0 ppm and 8.0–6.0 ppm.

Tannin		8.0–6.0 ppm	6.0–4.0 ppm
Condensed tannins	Acacia	49.02	50.99
	Grape skins	46.12	53.88
	Grape seeds	35.36	64.64
	Quebracho	62.95	37.05
	Tea 1	51.37	48.63
	Tea 2	48.46	51.54
Hydrolysable tannins	Cherry	26.04	73.96
	Chestnut	23.45	76.55
	Nut gall 1	79.44	20.56
	Nut gall 2	69.51	30.49
	Oak 1	26.32	73.69
	Oak 2	29.30	70.70
	Tara 1	60.19	39.81
	Tara 2	59.95	40.05
	Lemon	29.35	70.65

ure S5), while two clusters were identified with sulfur dioxide (Figure S6). In both trials, chestnut tannin showed a faster oxygen consumption, suggesting it could have a relevant role in preventing the wine oxidation. This result was in accordance with the finding reported by Jeremic et al. (2020) who found the oxygen consumption was the highest in the presence of ellagitannins. In general, hydrolysable tannins did show a faster oxygen consumption than condensed tannins (Pascual et al., 2017). Other authors found ellagitannin increased the OCR (Jeremic et al., 2020). For the tannins investigated here, we could not cluster condensed and hydrolysable tannins in terms of OCRs as it was higher without sulfur dioxide for chestnut, lemon, tea 2 and acacia tannins being hydrolysable, hydrolysable/condensed (Ezeabara et al., 2014) and condensed tannins, respectively (Figure S4). Similarly, the higher OCRs were found either for hydrolysable or condensed tannins in the presence of sulfur dioxide (Table 6).

The impact of sulfur dioxide was evident as OCRs increased for all the tannins investigated (Table 6) accordingly to previous study (Pascual et al., 2017; Ugliano et al., 2020). In the trials with sulfur dioxide, the residual dissolved oxygen was 20% in about two weeks (Figure S5). The only not significant difference between the two conditions was revealed for tara 1 tannins (0.28 ± 0.10 *vs.* 0.40 ± 0.04 mg oxygen/L/day without and with sulfur dioxide, respectively). The lowest difference, even if significant, was found for chestnut tannins (0.44 ± 0.00 *vs.* 0.48 ± 0.01 mg oxygen/L/day without and with sulfur dioxide, respectively). This could be attributed to the high oxygen consumption that this tannin showed even in the absence of sulfur dioxide making the contribution of sulfite negligible. For the other tannins, the OCRs significantly rose with sulfur dioxide. Both nut gall tannins showed low OCRs in the absence of sulfur dioxide, maybe due to their highest molecular weights, as following reported, leading to a strong impact of this antioxidant in consuming the dissolved oxygen. Moreover, oxidative loss of sulfur dioxide can be favored by gallic acid with a consequent increase of OCR. Our findings partly disagree with those reported by Ugliano et al. (2020) as we found a significant effect of oxygen consumption sulfur dioxide-mediated for oak tannins being comparable to that of tea 1 tannin, but twice in comparison to the increase found for tea 2 tannin. This further indicates the high variability of tannins of the same botanical origin that can be dependent to the raw materials as well as the extraction procedure and the overall preparation (Versari et al., 2013).

4. Conclusions

The oenological effects of tannins can allow the promotion of musts and wines clarification, the protection against oxidation, the improvement of certain sensory characteristics, such as body, mouthfeel and color (Neves et al., 2010). Moreover, they can prevent the appearance of light-struck taste in both model wine (Fracassetti et al., 2019) and white wine (Fracassetti et al., 2021). This study demonstrates the high hetero-

Table 5
Molecular weight (M) dispersions in tannin samples.

Tannin		*M* = 170–190	*M* = 300–490	*M* = 630–660	*M* = 790–850	*M* = 1000–1098	*M* = 1200–1400	*M* = 1800–1900
Condensed tannins	Acacia	X						
	Grape skins	X						
	Grape seeds	X						
	Quebracho	X	X					
	Tea 1	X	X	X				
	Tea 2	X	X					
Hydrolysable tannins	Cherry	X						
	Chestnut	X						
	Nut gall 1				X	X	X	X
	Nut gall 2	X	X				X	
	Oak 1	X	X					
	Oak 2	X						
	Tara 1		X			X		
	Tara 2	X						
	Lemon	X						

Table 6
Oxygen consumption rates (mg oxygen/L/day) with and without sulfur dioxide for the investigated tannins. Different letters mean significant differences ($p < 0.05$). Upper case letters refer to same tannin with/without sulfur dioxide; lower case letters refer to different tannins without sulfur dioxide and with SO_2.

	Tannin	*Without* sulfur dioxide	*With* sulfur dioxide
Condensed tannins	Acacia	$0.32 \pm 0.03^{\text{bc A}}$	$0.55 \pm 0.03^{\text{a B}}$
	Grape skin	$0.17 \pm 0.02^{\text{ef A}}$	$0.38 \pm 0.03^{\text{bcd B}}$
	Grape seeds	$0.14 \pm 0.02^{\text{f A}}$	$0.33 \pm 0.06^{\text{cd B}}$
	Quebracho	$0.14 \pm 0.00^{\text{f A}}$	$0.46 \pm 0.08^{\text{ab B}}$
	Tea 1	$0.20 \pm 0.01^{\text{ef A}}$	$0.40 \pm 0.01^{\text{bcd B}}$
	Tea 2	$0.30 \pm 0.02^{\text{bcde A}}$	$0.41 \pm 0.00^{\text{bcd B}}$
Hydrolysable tannins	Cherry	$0.22 \pm 0.04^{\text{cdef A}}$	$0.44 \pm 0.02^{\text{abc B}}$
	Chestnut	$0.44 \pm 0.00^{\text{a A}}$	$0.48 \pm 0.01^{\text{ab B}}$
	Nut gall 1	$0.13 \pm 0.03^{\text{f A}}$	$0.54 \pm 0.05^{\text{a B}}$
	Nut gall 2	$0.12 \pm 0.01^{\text{f A}}$	$0.46 \pm 0.06^{\text{ab B}}$
	Oak 1	$0.22 \pm 0.01^{\text{cdef A}}$	$0.44 \pm 0.03^{\text{abc B}}$
	Oak 2	$0.20 \pm 0.04^{\text{def A}}$	$0.41 \pm 0.01^{\text{bcd B}}$
	Tara 1	$0.28 \pm 0.10^{\text{bcde A}}$	$0.40 \pm 0.04^{\text{bcd A}}$
	Tara 2	$0.19 \pm 0.01^{\text{ef A}}$	$0.31 \pm 0.08^{\text{d B}}$
	Lemon	$0.34 \pm 0.01^{\text{ab A}}$	$0.41 \pm 0.03^{\text{bcd B}}$

geneity of the commercial oenological tannins. Our findings improve and expand the actual knowledge of tannins. Besides the methods commonly used for the characterization of tannins, the application of NMR can give useful information regarding the average molecular weight. The diverse composition and chemical behavior detected in commercial tannins highlights that their enological effectiveness can widely change regardless of the nominal biological source, as many other factors, maybe even the production batch, can affect tannin composition. This likely forces the user to test the tannin behavior of a new batch before its use.

Further studies will be carried to investigate the use of oenological tannins in preventing the light-struck taste with particular attention on limiting the formation of volatile sulfur compounds having a detrimental impact on sensory characteristics. The results obtained in this study will serve to clarify what are the main parameters as well as the possible synergic effect with relevant protection against the light-struck taste.

Declaration of Competing Interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper

CRediT authorship contribution statement

Daniela Fracassetti: Conceptualization, Methodology, Formal analysis, Resources, Data curation, Writing – original draft, Writing – review & editing. **Alberto Saligari:** Formal analysis, Investigation, Data curation, Writing – original draft, Writing – review & editing. **Natalia Messina:** Formal analysis, Investigation, Data curation, Writing – review & editing. **Rebecca Bodon:** Formal analysis, Investigation, Data curation, Writing – review & editing. **Stefania Mazzini:** Methodology, Formal analysis, Data curation, Writing – review & editing. **Gigliola Borgonovo:** Methodology, Formal analysis, Data curation, Writing – review & editing. **Antonio Tirelli:** Resources, Data curation, Writing – review & editing.

Data availability

Data will be made available on request.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.focha.2023.100218.

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