Evaluation of oenological tannins for preventing the light-struck taste

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Abstract

The light-struck taste (LST) is a fault occurring in white and rosé wines associated to the formation of volatile sulfur compounds (VSCs) due to the reactions between riboflavin (RF) and methionine (Met). We investigated the possible preventing effect of 15 commercial tannins of different origin in model wine added with RF and Met, under oxic and anoxic conditions, and submitted to standardized light-exposure. All the tannins limited the degradation of Met in comparison to the tannin-free samples. Lower concentrations of VSCs were found in presence of tannins even under anoxia, condition favouring their formation. The sniffing trials evidenced the minor perception of cooked cabbage note with added tannins. The multivariate analysis showed the presence of flavan-3-ols was related to the formation of DMDS and DMTS, while tannic acid was related to MeSH, both the behaviors occurring under oxic condition. The study highlighted the ability of tannins to prevent the LST.

Keywords: methionine; riboflavin; volatile sulfur compounds; phenols; multivariate analysis.

1. Introduction

The light can induce a number of detrimental reactions in wine, such as formation of off-flavours, loss of vitamins and discoloration of the pigments. A well-known light-dependent fault that can occur in white wine is the light-struck taste (LST). This defect causes the appearance of certain off-flavours associated to cooked cabbage, onion and garlic odours-like due to the formation of volatile sulfur compounds (VSCs), namely methanethiol (MeSH) and dimethyl disulfide (DMDS) (Maujean et al., 1983a). They originate from light-induced reactions involving riboflavin (RF) and methionine (Met) (Maujean et al., 1978). RF, or vitamin B_2, acts as photosensitizer prompting photo-oxidative reactions that can occur in two possible pathways. When RF is exposed to light, it reaches the excited triplet state that may react directly with triplet oxygen. The latter is converted to singlet oxygen and ground state RF is generated (Type II pathway) (Cardoso et al., 2012). Singlet oxygen promotes non-radical reactions with electron rich compounds (e.g., with double bonds) that result oxidised (Min & Boff, 2002). Triplet RF can also directly reacts with compounds able to donate electrons, such as phenols and amino acids, including Met (Type I mechanism) (Cardoso et al., 2012). In this pathway, Met transfers two electrons to triplet RF resulting fully reduced. Met is then decarboxylated to imine which is easily hydrolysed generating methional. The latter is also chemically unstable, photosensitive and, through a retro Michael reaction, decomposes to MeSH and acrolein. Two molecules of MeSH can yield dimethyl disulfide (DMDS) (Maujean & Seguin, 1983b). Furet and co-authors (2022) recently suggested DMDS is originated from a dimer cation radical species, reaction occurring in a short time and without oxidizing species. MeSH and DMDS have low perception threshold up to 2-10 μg/L and 20-45 μg/L, respectively (Mestres et al., 2000; Solomon et al., 2010). The occurrence of the two pathways depends on the concentration of oxygen. In anoxic conditions only Type I occurs, whereas in the presence of oxygen, both mechanisms may

take place to a different extent depending to the susceptibility to oxidation of the compounds present in the reaction environment (Min & Boff, 2002; Grant-Preece et al., 2017).

The content of RF in must is up to few tens of micrograms per litre (Riberau-Gayon et al., 2006), but it increases during alcoholic fermentation due to the activity of *Saccharomyces cerevisiae* (Mattivi et al., 2000; Fracassetti et al., 2017). RF amount can further increase due to prolonged contact with yeast lees (Andrés-Lacueva et al., 1998). The addition of yeast-based formulations (e.g. inactivated and autolyzed yeasts) can further increase the final concentration of RF in wine (Fracassetti et al., 2017). The average content of Met in wine is about 3-5 mg/L (Riberau-Gayon et al., 2006; Grant-Preece et al., 2017; Sartor et al., 2021), depending to grape cultivar, vineyard treatments, winemaking conditions, fermentation yeast performing and its autolysis, thus rising up to 15 mg/L (Soufleros et al., 2003; Fiechter & Mayer, 2011).

Certain technological strategies have been proposed to prevent the formation of LST (Fracassetti et al, 2021a). The removal of RF to concentrations lower than 80-100 μg/L can represent a suitable approach to decrease the risk of LST appearance (Pichler, 1996; Fracassetti et al., 2019). The treatment with some adjuvants, such as active charcoal or bentonite, was effective to achieve this purpose (Pichler et al., 1996; Fracassetti et al., 2017). However, these adjuvants are not specific for binding RF and they can also deplete some desirable wine aromas (Riberau-Gayon et al., 2006). The addition of condensed tannins showed a positive effect in prevent LST (Maujean & Seguin, 1983b). Hydrolysable tannins were also assayed in model wine resulting effective as lower concentrations of VSCs were found following the light exposure (Fracassetti et al., 2019). Moreover, the chestnut tannin in mixture with sulfur dioxide (SO_2) and glutathione (GSH) was recently investigated in both model wine and white wine. The effectiveness of hydrolysable tannins in preventing LST was highlighted also when hydrolysable tannins were combined use of other antioxidants (i.e., SO_2+GSH) (Fracassetti et al., 2021b).

Oenological tannins are a group of phenolics including polymers of flavan-3-ol units, the condensed tannins, and glucosides of gallic acid (gallo tannins) or ellagic acid (ellagitannins), the hydrolysable tannins. Commercial tannins with diverse chemical structure, botanical origin, and extraction process (e.g. with water or solvent) are available for winemaking (Versari et al., 2013). The oenological tannins show great heterogeneity in terms of phenolic composition, antioxidant capacity and oxygen consumption rate (Pascual et al., 2017; Vignault et al., 2018; Ugliano et al., 2020). Similar differences were evidenced even for oenological tannins obtained from the same source (Vignault et al., 2018; Watrelot et al., 2020).

Based on the promising effectiveness of tannins against LST, the aim of this study was to investigate the protective effect of fifteen commercial oenological tannins, either condensed or hydrolysable, in model wine. Both oxic and anoxic conditions were considered; the degradation of RF and Met, the formation of VSCs and the sensory impact were determined. Due to the high variability of oenological tannins, a multivariate approach was carried out in order to clarify the influence of the chemical composition of tannins (Fracassetti et al., 2021d) on LST formation. To the best of our knowledge, this is the first study considering a wide range of tannins in preventing LST and looking for a possible relation between their composition and their capability to counteract the formation of LST.

2. Material & Methods

2.1. Experimental plan

The experimental plan consisted of assessing the impact of different oenological tannins for counteracting the formation of LST. Model wine solution (Milli-Q water added with 5 g/L of tartaric acid, 12% of ethanol (v/v), at pH 3.2 adjusted with sodium hydroxide) was added with 200 μg/L of RF and 4 mg/L of Met. Oenological tannins of different origin and extraction method (Table S1), provided by Dal Cin Gildo Spa (Concorezzo, MB, Italy) in a powder form,

were tested at 50 mg/L. Both oxic and anoxic conditions were carried out, the latter was obtained by sparging nitrogen into the bottles for 180 minutes. All the solutions were contained in 100 mL clear glass bottles tightly closed without head space and exposed for 120 min to standardized conditions of fluorescent light (Fracassetti et al., 2019). Briefly, the used laboratory-made apparatus consisted of three fluorescence light bulbs placed 40 cm from each other. Three 100 mL bottles containing the test sample (triplicate trials) were each positioned between two light bulbs, i.e. at 20-cm distance. The compact fluorescent light bulbs (Philips) emitted cold light (6500 K) with a luminous flux of 3172 Lumen with high emission in the absorption wavelengths of riboflavin (370 and 440 nm). The apparatus was kept in a dark room with air conditioning set at 22 °C. The temperature was monitored in the proximity by a thermometer dipped in water in a 100 mL bottle placed in the centre of the apparatus and no temperature change was revealed (Fracassetti et al., 2019). The model wine solutions were kept at 20±2 °C and protected from light before and after the controlled light exposure by covering the bottles with an aluminium foil. Triplicate determinations were carried out for each experiment.

Tannins used for this study were provided by Dal Cin Gildo S.p.A. (Concorezzo, MB, Italy) (Table S1).

2.2. Analysis of riboflavin

Riboflavin (RF) was quantified by UPLC as reported by Fracassetti (2017, 2019) with slight modifications. Flavons were determined by UPLC (Acquity H-Class system) equipped with a fluorescence detector Acquity UPCL (Waters, Milford, MA, USA). The column was a Hypersil ODS C18, 3 μm, 100 x 3 mm (CPS Analitica). The flow rate was 0.5 mL/min, and the injection volume was 10 μL. The solvents were (solvent A) citrate buffer 50 mmol at pH 2.5 and (solvent B) methanol in gradient mode in which B was from 5% to 100% in 13.50 minutes followed by column washing and equilibration. The quantification was carried out

with a nine-points calibration curve at concentration ranging from 1 μg/L to 500 μg/L. The detection was performed at 420 nm and 530 nm for excitation and emission, respectively (Fracassetti et al., 2018). Data acquisition and processing were performed by Empower 2 software (Waters).

2.3. Analysis of methionine, methionine sulfoxide and methionine sulfone

Methionine (Met), Met sulfoxide and Met sulfone were determined by derivatization with *o*-pthaldehyde (OPA) as described by Fracassetti et al. (2017) with some modifications. The derivatization solution was prepared in a 10 mL volumetric flask by dissolving 250 mg of OPA in 1.5 mL of ethanol, adding 200 μL of 2-mercaptoethanol, and making up to the volume with borate buffer 0.4 M at pH 10.5. The derivatization reaction was performed as follows: 500 μL of borate buffer (0.4 M, pH 10.5), 200 μL of sample, 10 μL internal standard (norvaline 20 mg/L) and 100 μL of OPA. The reaction mixture was vortexed for 2 minutes, filtered with a PVDF 0.22 μm filter and injected. The chromatographic system was an Acquity UPLC (Waters) equipped with a fluorescence detector (Waters). The column was a Kinetex, 5 μm EVO C18, 100 A, 150 x 2.1 mm (Phenomenex, Torrance, CA, USA) maintained at 40°C. The flow rate was 1 mL/min and the injection volume was 10 μL. The solvents were (solvent A) citrate buffer 10 mM at pH 7.5 and (solvent B) methanol in gradient mode in which B was from 5% to 47% in 22 min. The quantification was carried out with the external method by using a six-points calibration curve obtained at concentration in the range 0.5-20 mg/L. The detection was performed at 335 nm and 440 nm for excitation and emission, respectively. Data acquisition and processing were performed by Empower 2 software (Waters).

2.4. Analysis of volatile sulfur compounds

153 The analysis of volatile sulfur compounds (VSCs) was performed following the method
154 proposed by Fracassetti et al. (2019, 2021b) with slight modification. VSCs were assessed
155 by Solid Phase Micro Extraction SPME-GC/MS. The sample was prepared as follows: 2.5 g
156 of magnesium sulphate heptahydrate were added to 10 mL of sample in a glass-vial that
157 was immediately hermetically closed. The fibre was carboxen-polydimethylsiloxane-
158 divinylbenzene (CAR-PDMS-DVB; 50/30 μm X 1 cm) (Supelco, Bellefonte, PA, USA). The
159 SPME was carried out with an autosampler (HTA autosampler, Brescia, Italy) set at the
160 following conditions: incubation for 5 minutes at 40°C; agitation 10 seconds and then 3
161 seconds off; extraction for 30 minutes; desorption for 20 minutes. The GC/MS equipment
162 was a Perkin Elmer Autosystem XL Gas Chromatograph coupled with a Turbomass Mass
163 Spectrometer (Perkin Elmer, Italy). The separation was achieved by a MEGA-5 MS column
164 (30+5 m x 0.250 mm x 0.250 μm) (MEGA S.r.l., Legnano, MI, Italy) and using helium as
165 carrier gas at 1 mL/min flow rate. The oven temperature was firstly set at 40 °C and held for
166 5 minutes, then ramped at 1.5°C/min up to 60°C; ramped at 4°C/min up to 150°C and held
167 for 5 minutes and finally ramped at 40°C/min up to 230°C and held for 10 minutes. The
168 transfer line temperature was set at 230°C and the source temperature at 250°C. The MS
169 detector registered the *m/z* in the range from 33 up to 350 Da. For the identification of the
170 target molecules, the ions were chosen according to the NIST library and Nguyen et al.
171 (2009). Duplicate injections of each sample were carried out. Results are expressed as the
172 relative concentration (μg/L) for MeSH referred to as d_6-DMS. For DMDS and DMTS, the
173 amounts were determined by the external standard method (0.1-100 μg/L). The Odour
174 Activity Values (OAVs) were determined as the ration between the amount of the VSC found
175 in the sample and the respective perception threshold. The perception threshold
176 concentrations were 2-10 μg/L for MeSH, 20-45 μg/L for DMDS and 0.1 μg/L DMTS. VOCs
177 were identified according to the NIST library, for an R match higher than 95% and the
178 retention time of the pure standards (Fracassetti et al., 2019, 2021b).

2.5 Sensory analysis

Astringency and bitterness were evaluated for the oenological tannins investigated. The panel was composed by 13 expert judges (8 females, 5 males) with an average age of 30 years. The judges were trained with model wine and white wine spiked with caffeine (up to 540 mg/L) for bitterness and a grape seeds tannin (up to 600 mg/L). The white wine was added with 80 mg/L of each commercial tannin and stored at 15±2°C for three weeks before the sensory evaluation. Fifteen triangle tests were performed in total by comparing the white wine with and without tannins, one for each tannin, in three different sessions. The panellists took a break of 10 minutes among the triangle tests planned in the same session.

For the evaluation of LST, the sensory analysis was carried out by a panel of 9 expert judges (4 males and 5 females) with an average age of 30 years. The judges had to give a score for the cooked cabbage descriptor to every sample in a range between 1 (not perceived) and 9 (extremely perceived). The panellists were firstly trained with model wine samples spiked with Met (4 mg/L) and RF (200 µg/L) and exposed to light for two hours in standard conditions in order to make the judges confident with the perception of cooked cabbage note. The judges were calibrated by sniffing model wine solutions spiked with Met (4 mg/L) and RF (200 µg/L) exposed to light for increasing time up to two hours (Fracassetti et al., 2021b). A reference sample (model wine, spiked with Met 4 mg/L, RF 200 µg/L and exposed to the light up to 2 hours) was served during the sensory session. Each sample was evaluated just after the bottle opening and served at temperature 18±2°C covering the glass. The repeatability (R) of every judge was considered and it was calculated as follows:

$$R = \frac{|x1 - x2|}{y} \times 100$$

Where *x1* and *x2* were the scores assigned by the judge to the replicates and *y* was the deviation of scores assigned. The replicability value was set at 75%; lower value led to the

exclusion of the judge (Fracassetti et al., 2020). The average scores of the three replicates were considered.

2.6. Statistical analysis

Statistical analysis was performed with SPSS Win 12.0 program (SPSS Inc., Chicago, IL, USA). One-way ANOVA was carried out to identify the significant differences between the tannins. Significant differences were judged by a post-hoc Fischer LSD ($p < 0.05$). Pearson correlations were calculated among Met degradation, Met-sulfoxide, MeSH, DMDS, DMTS and sensory score, and certain parameters related to the characterization of tannins, including the Total Phenol Index (Folin-Ciocalteau assay), antioxidant capacity, richness (ABS 280 nm and methyl cellulose assay) (critical value for df = 14: 0.383, α = 0.2; 0.426, α = 0.1; 0.497, α = 0.05). The Principal Component Analysis (PCA) was performed with Statistica 12 software (Statsoft Inc., Tulsa, OK, USA) on auto-scaled data considering the chemical characterization of oenological tannins, concentrations of VSCs and the sensory scores. Two approaches were used: in the first one the active variables were the chemical characteristics of the tannins obtained in the previous part of this work (Fracassetti et al., 2021d). In the second PCA, the active variables were the VSCs investigate in this study.

3. Results & Discussion

Hydrolysable tannins have recently proved to limit the formation of LST in model wine (Fracassetti et al., 2019) and white wine (Fracassetti et al., 2021b). Specifically, the tannins from chestnut, nut gall, and oak were investigated. Besides the hydrolysable tannins, the condensed tannins were suggested as preventing strategy against the appearance of LST (Maujean et al., 1983a). With regards to the oenological tannins, several studies evidence their relevant heterogeneity in terms of phenolic composition, antioxidant capacity, oxygen consumption rate, average molecular weight, even when they are obtained from the same

source (Vignault et al., 2019; Ugliano et al., 2020; Fracassetti et al., 2021d ; Paissoni et al., 2022). For these reasons either hydrolysable or condensed tannins from different origin and obtained with diverse extraction procedures were screened as possible oenological tool to prevent the formation of LST. Tannins can affect bitterness and astringency when an increase of phenols higher than 40 mg/L is carried out (Robichaud and Noble, 1990). In order to exclude any influence on taste and mouthfeel properties, astringency and bitterness perception was evaluated in white wine added up to 80 mg/L of the investigated tannins. As no significant difference was found (data not showed), the adopted addition of tannins was 50 mg/L, also in accordance with a previous research (Fracassetti et al., 2021b).

The model wine was adopted in this study, thus designed to avoid interferences and accurately monitor the light-induced reactions of RF and Met occurring in the presence of the investigated tannins. As reported in Fracassetti et al. (2019), the wine composition can slow down the RF degradation and model wine can allow to isolate the light-induced reactions. Consequently, the data obtained in model wine can effectively predict the preventing effect of tannins against LST appearance. We are conscious some differences can arise between model wine and white wine in terms of LST intensity. Nonetheless, the effectiveness of tannins, specifically those from chestnut, was found in both model wine and white wine (Fracassetti et al., 2021b). This makes of significance the screening in model wine that allows to understand the possible differences between the tannins tested and to exclude the impact of other wine components into the light-induced mechanisms.

3.1 Degradation of riboflavin

The average concentration of RF prior to the light exposure was 194.25 ± 4.18 µg/L. The adopted light exposure did lead to the complete photodegradation of RF in two hours (Fracassetti et al., 2019). Maybe such light condition could increase the photodegradative rate of RF in comparison to that one of the scale retail trade or grocery stores. Nonetheless,

256 the photodegradation of RF can occur in a reasonably short time under the applied
257 standardized and controlled conditions allowing to highlight and differentiate the possible
258 effectiveness of the investigated oenological tannins. As previously observed (Fracassetti
259 et al., 2019), RF was not detected in any of the samples after the discreet illumination (2 h)
260 adopted, irrespective of the oxic/anoxic conditions and the tannin added.
261
262 3.2. Impact on methionine and its derivatives
263 The decrease of Met concentration was found after light exposure in all the conditions tested
264 (Table 1). In particular, grape seeds tannin showed the highest Met degradation (-39%)
265 followed by oak 2 (-34%) and tea 2 (-29%) tannins under oxic condition. For the other tannins
266 tested, the Met loss was 24±2% as average. These values are to a certain extent higher
267 than those reported by Fracassetti et al. (2019) maybe due to the slightly higher
268 concentration of Met used in the present study (4 mg/L *vs.* 3 mg/L). In anoxic condition, the
269 Met degradation was generally higher than under the oxic one, with the exception of grape
270 seeds, tea 2 and quebracho tannins that showed comparable behavior independently to the
271 oxygen dissolved (Table 1). The tannin-free model solution (control) showed the highest Met
272 degradation (-42%), followed by oak 2 (-40%) and grape skin (-39%) tannins. The lower
273 decrease of Met observed in the presence of tannins suggests their role as photoprotectors
274 (Fracassetti et al., 2020c). As previously mentioned, the higher concentration of degraded
275 Met in comparison to a previous study (Fracassetti et al., 2019) could be ascribed to the
276 higher concentration of Met considered in this study. The nature of tannins does not seem
277 to affect the degradation of Met as condensed and hydrolysable tannins did show a common
278 behavior independent to their group. For example, acacia tannin and chestnut tannin did not
279 show a significant different decrease of Met after the light exposure. Similarly, grape seeds
280 tannin and oak 2 tannin showed a comparable decrease of Met. As a consequence, the
281 degraded Met did not allow to cluster the investigated tannins within condensed and

hydrolysable. Nonetheless, phenols can play an impact on Met loss related to the oxygen dissolves as significant relative correlation was found among total phenol index (determined by both Folin-Ciocalteau method and absorbance reading at 280 nm) and the richness in the case of anoxic trial, being -0.529 ($p < 0.05$), -0.5282 ($p < 0.05$), -0.412 ($p < 0.1$), respectively (Table 2) (chemical characterization data is reported in Fracassetti et al., 2021d). On the contrary, no significant correlation was evidenced in oxic condition (Table 2). This can be due to the oxidation of Met arising to different compounds (Barata-Vallejo et al., 2010), including Met sulfoxide and methionine sulfone. The latter was not detected in any of the sample (data not showed) accordingly to previous studies (Fracassetti et al., 2020b, 2021b). Met sulfoxide was found in all the samples, either exposed (0.17-0.74 mg/L) or not (0.07-0.53 mg/L) to light. In most of the cases, an increase was observed for the samples exposed to light, with few exceptions in oxic (acacia, chestnut and lemon tannins) and anoxic (quebracho tannin) conditions (Table 1). Grape seeds tannin showed the highest increase, more evident under oxic condition as occurred for most of the tannins. As previously stated for the degradation Met, the formation of Met sulfoxide does not seem to be dependent to the specific tannin added. Nonetheless, a significant negative correlation was found between the formed Met sulfoxide and the degraded Met only when oxygen was present (Table 2).

3.3 Formation of volatile sulfur compounds

The concentration of VSCs detected after the light exposure under oxic condition were generally lower in comparison to the anoxic one (Table 3). Specifically, the levels of DMDS were up to 30-folds higher in anoxic condition and those of DMTS up to more than 100-folds. However, the latter was negligible (< 0.1 µg/L) when most of the tannins were added in the presence of oxygen (Table 3). Both DMDS and DMTS were significantly lower in the presence of tannins in oxic condition (Table 3) indicating their effectiveness in limiting the

appearance of LST (Fracassetti et al., 2019, 2021b). We can suppose the phenols can act as a stabilizer of the dimer cation radical being involved in the formation of DMDS (Furet et al., 2022). A further support of the radical stabilizing effect played by phenols was given by the negative significant correlations found between DMDS and DMTS towards total phenol index (determined by both Folin-Ciocalteau method and absorbance reading at 280 nm) and the richness under both oxic and anoxic conditions (Table 2). The increase of MeSH was not so evident between oxic and anoxic conditions and it resulted strongly dependent to the different tannins investigated. In particular, as previously found (Fracassetti et al., 2019), no significant difference in MeSH content was observed in oxic condition. On the contrary, the addition of tannins led to a significantly lower MeSH for most of the tannins added in comparison to the control in anoxic condition, with the exception of chestnut, nut gall 2, tara 1 and tara 2 tannins. Significant negative correlations were found between MeSH and total phenol index (determined by both Folin-Ciocalteau method and absorbance reading at 280 nm) and the richness in anoxic condition, while they were not in oxic condition (Table 2). When no oxygen is present, only the Type I mechanism can take place (Grant-Preece et al., 2017; Fracassetti et al., 2019). Tannins could compete with Met in accepting the electrons from RF in excited state (T_1), which turns into reduced RF. Tannins can also quench RF in excited state (T_1) (Vaish & Tollin, 1970). These competing mechanisms can limit the formation of MeSH. On the contrary, in oxic condition, the single oxygen generated in the Type II mechanism could oxidize the phenols (Fracassetti et al., 2019) limiting their protective effect for the formation of MeSH. Surprisingly, the addition of oak 2 tannin did not result significant different for DMDS and DMTS in comparison to the model wine without tannin in anoxic condition. To explain this behaviour, differences in phenol abundance and nature for this tannin can be excluded since the phenolic composition was consistent to the other tannins investigated (Fracassetti et al., 2021d). As declared by the supplier, oak 2 tannin was extracted from toasted wood (Table S1). The toasting changes both the quantity

and the quality of the extractable substances (Carpena et al., 2020), being possible to differentiate barrel toasting levels by considering its volatile and semi volatile compounds (Chatonnet et al., 1999). The toasting causes thermal degradation of several compounds, including carbohydrates, resulting in furanic compounds; lignin or hemicellulose with the consequent volatile phenols formations and acids, which by dehydration result in oak lactones (Chira & Teissedre, 2013). As recently suggested by Furet at al. (2022), the formation of DMDS can occur as a consequence of the formation of radical species, we can hypothesize the limited radical-binding capability of toasted wood as a consequence of the wood changes above mentioned. However, none of the other investigated hydrolysable tannins were produced from toasted wood. Further investigation is necessary to confirm such hypothesis.

Our results confirmed that the formation of VSCs is favored under anoxic conditions. Considering the total content of VSCs (expressed in nmoles), the lowest concentrations were found in the presence of tea 1 (20.6 nmol/L), tea 2 (19.8 nmol/L) and grape seed tannin (20.9 nmol/L) in oxic conditions. The latter was also the most effective in anoxic condition (50.6 nmol/L) followed by grape skins (52.6 nmol/L) and quebracho (59.4 nmol/L) tannins, while among the hydrolysable tannins, tara 2 tannin showed the lowest VSCs (63.0 nmol/L). Grape seeds tannin could be a promising condensed tannin for LST prevention with tea 2 tannin and, among the hydrolysable ones, tara 2 tannin.

3.4 Perception of “cooked cabbage” note

In general, the cooked cabbage descriptor related to LST was highly perceived in anoxic condition (Table 4). All the tannins led to a significant lower perception of LST in comparison to the model wine without tannin under oxic condition. Nonetheless, no significant difference was found among the two conditions for grape seeds, grape skin, quebracho, cherry, tara 1and tara 2 tannins suggesting their comparable behaviour towards the perception of LST

360 with or without oxygen dissolved. As expected, under anoxic condition, no significant
361 difference was found between the addition of oak 2 tannin and the control showing a
362 comparable content of VSCs (Table 3) and, consequently, odor activity values (OAVs) that
363 were highly major than 1 for all the three VSCs investigated (0.5-4.5 and 1.6-15.5 for MeSH,
364 0.92-2.07 and 0.91-2.05 for DMDS, and 118.2 and 150.0 for DMTS, respectively for oak 2
365 tannin and control samples; Table 3). Even if the sensory scores were lower for grape skin,
366 nut gall 1, nut gall 2 and oak 1 tannins in comparison to the control, such difference was not
367 significant. This result was unexpected as low concentration of VSCs were found (Table 3).
368 Maybe these tannins could modify the perception of VSCs as the phenols can suppress,
369 accentuate or show negligible effect on the perception of the aroma compounds (Lund et
370 al., 2009).
371
372 3.5 Multivariate analysis
373 The multivariate approach was used in order to clarify the impact of the chemical
374 characterization of tannin on LST formation. Firstly, the chemical parameters of the
375 investigated tannins (Fracassetti et al., 2021d) was considered as active variables. The
376 Principal Component Analysis (PCA) explained 82 % of total variance with the two
377 components at 50 % and 32 %, respectively for Factor 1 and Factor 2 (Figure 1). Three
378 different clusters were clearly recognized. Chestnut, cherry, oak 1, oak 2 and lemon tannins
379 were included in the first cluster. These tannins belong to the hydrolysable group, with the
380 exception of lemon that contain a mixture of condensed and hydrolysable tannins (Ezeabara
381 et al., 2014). This cluster was correlated with DMDS in both oxic and anoxic conditions, and
382 DMTS and the sensory score in anoxic conditions. The second cluster comprised the
383 condensed tannins tested in this study (acacia, grape skin, grape seeds, quebracho, tea 1,
384 tea 2). This cluster was correlated with the highest oenotannins content. Nut gall (1 and 2)

385 and tara (1 and 2) tannins belonged to the third cluster. These gallotannins were correlated
386 with MeSH, in both oxic and anoxic conditions.
387 The concentrations of VSCs and the sensory scores as well as the decrease of Met were
388 considered the active variables in the PCA showed in Figure 2. The results indicated that
389 the first two components were significant in explaining 61 % of the total variance, 40 %of
390 which was explained by Factor 1 and 21 % by Factor 2. Oak 2 tannin resulted clearly
391 correlated with DMDS, DMTS and the sensory score in anoxic condition. After bottling, the
392 oxygen in the headspace of the bottle will be consumed quickly leading to nearly anoxic
393 condition in only one month (Dimkou et al., 2011). Due to this aspect, oak 2 tannin would
394 not be an effective tool in preventing LST as it could not be able to limit the formation of
395 VCSs once the anoxic condition is reached. The other tannins were far from oak 2 and a
396 clustering based on their chemical nature (condensed/hydrolysable) was not observed.
397
398 **4. Conclusions**
399 The results obtained in this study highlighted the ability of low concentration of oenological
400 tannins in preventing LST appearance as they can limit the formation of VSCs responsible
401 for this fault in both oxic and anoxic conditions.
402 The addition of oenological tannins also led to a lower degradation of Met for nearly all the
403 tannins in anoxic condition (except for oak 2 that was similar to the control).
404 To the best of our knowledge this is the first report that related a highly detailed chemical
405 characterization of the oenological tannins to their effectiveness against LST. The presence
406 of flavan-3-ols was related to the formation of DMDS and DMTS, while tannic acid was
407 related to MeSH, both under oxic condition. Nonetheless, as a restricted formation of LST
408 was confirmed in this study, the impact of certain ellagitannins and gallotannins can be an
409 advantage to avoid the appearance of LST. The oxygen consumption rate played by the
410 tannins should be considered as the formation of MeSH would be favored.

From the results obtained in this study and the multivariate approach including the chemical characterization of tannins (Fracassetti et al., 2021d), grape seeds, tea 2 and tara 1 can be considered the most promising tannins and they will be tested at bottling of white and rosé wines at winery level. Moreover, further investigations will be related to the addition of tannin mixture in both white and rosé wines.

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563

Figure captions

Figure 1: Projection of the (a) scores and (b) loading on the factor-plane obtained for the volatile sulfur compounds and the sensory scores of the investigated tannins, and the chemical characterization. The chemical characterization (in blue) (Saligari, 2022) as active variables, and the volatile sulfur compounds and the sensory scores (in red) were set as supplementary variables.

Legend: TPI (Folin), Total Phenol Index determined with Folin-Ciocalteau method; Antiox, antioxidant capacity determined with DPPH assay; Ratio TPI/Antiox, ration between Total Phenol Index and antioxidant capacity; Tannic acid (280nm), phenols determined by the absorbance reading at 280 expressed as tannic acid for hydrolysable tannins; Catechin (280nm), phenols determined by the absorbance reading at 280 expressed as catechin for condensed tannins; Oenotannins (tannic acid), tannins determined by the methyl cellulose assay expressed as tannic acid for hydrolysable tannins; Oenotannins (catechin), tannins determined by the methyl cellulose assay expressed as catechin for condensed tannins; ProACNs, proanthocyanidins determined by the dimethylaminocinnamaldehyde assay; Total 8.0-6.0 ppm, sum of NMR signals obtained between 8 and 6 ppm; Total 6.0-4.0 ppm, sum of NMR signals obtained between 6 and 4 ppm; O2 consum no SO2, oxygen consumption rate without sulfur dioxide; O2 consum SO2, oxygen consumption rate with sulfur dioxide; Met % diff ox, degraded methionine (%) under oxic condition (Table 1); DMDS ox, dimethyl disulfide (µg/L) formed under oxic condition (Table 3); DMTS ox, dimethyl trisulfide (µg/L) formed under oxic condition (Table 3); MeSH ox, methanethiol (µg/L) formed under oxic condition (Table 3); Sensory score ox, sensory score found in oxic condition (Table 4); Met % diff anox, degraded methionine (%) under anoxic condition (Table 1); DMDS anox, dimethyl disulfide (µg/L) formed under anoxic condition (Table 3);DMTS anox, dimethyl trisulfide (µg/L) formed under anoxic condition (Table 3); MeSH anox, methanethiol

589 (µg/L) formed under anoxic condition (Table 3); Sensory score anox, sensory score found
590 in oxic condition (Table 4).
591 **Figure 2.** Projection of the (a) scores and (b) loading on the factor-plane obtained for the
592 chemical characterization of the investigated tannins, and volatile sulfur compounds and the
593 sensory scores. The volatile sulfur compounds and the sensory scores (in blue) were set as
594 active variables; the chemical characterization (in red) (Saligari, 2022) as supplementary
595 variables.

596 Legend: TPI (Folin), Total Phenol Index determined with Folin-Ciocalteau method; Antiox,
597 antioxidant capacity determined with DPPH assay; Ratio TPI/Antiox, ration between Total
598 Phenol Index and antioxidant capacity; Tannic acid (280nm), phenols determined by the
599 absorbance reading at 280 expressed as tannic acid for hydrolysable tannins; Catechin
600 (280nm), phenols determined by the absorbance reading at 280 expressed as catechin for
601 condensed tannins; Oenotannins (tannic acid), tannins determined by the methyl cellulose
602 assay expressed as tannic acid for hydrolysable tannins; Oenotannins (catechin), tannins
603 determined by the methyl cellulose assay expressed as catechin for condensed tannins;
604 ProACNs, proanthocyanidins determined by the dimethylaminocinnamaldehyde assay;
605 Total 8.0-6.0 ppm, sum of NMR signals obtained between 8 and 6 ppm; Total 6.0-4.0 ppm,
606 sum of NMR signals obtained between 6 and 4 ppm; O2 consum no SO2, oxygen
607 consumption rate without sulfur dioxide; O2 consum SO2, oxygen consumption rate with
608 sulfur dioxide; Met % diff ox, degraded methionine (%) under oxic condition (Table 1); DMDS
609 ox, dimethyl disulfide (µg/L) formed under oxic condition (Table 3); DMTS ox, dimethyl
610 trisulfide (µg/L) formed under oxic condition (Table 3); MeSH ox, methanethiol (µg/L) formed
611 under oxic condition (Table 3); Sensory score ox, sensory score found in oxic condition
612 (Table 4); Met % diff anox, degraded methionine (%) under anoxic condition (Table 1);
613 DMDS anox, dimethyl disulfide (µg/L) formed under anoxic condition (Table 3);DMTS anox,
614 dimethyl trisulfide (µg/L) formed under anoxic condition (Table 3); MeSH anox, methanethiol

615 (µg/L) formed under anoxic condition (Table 3); Sensory score anox, sensory score found
616 in oxic condition (Table 4).

Table1. Methionine (Met; mg/L) degradation and methionine sulfoxide (Met-sulfoxide; mg/L) formed under oxic and anoxic conditions after light exposure. The data are referred to the difference between the samples stored in the dark and those exposed to light. Different letters mean significant differences ($p < 0.05$). Upper case letters refer to same type of tannin in oxic/anoxic conditions; lower case letters refer to different tannins in the trials in oxic and anoxic condition separately.

	Met degraded		Met-sulfoxide formed	
Tannin	**Oxic**	**Anoxic**	**Oxic**	**Anoxic**
Acacia	0.78 ± 0.03 $^{ef\ B}$	1.25 ± 0.08 $^{cde\ A}$	-0.22 ± 0.07 $^{h\ A}$	0.16 ± 0.15 $^{bc\ A}$
Grape seeds	1.65 ± 0.04 $^{a\ A}$	1.57 ± 0.07 $^{abc\ A}$	0.60 ± 0.02 $^{a\ A}$	0.41 ± 0.01 $^{a\ B}$
Grape skin	1.11 ± 0.17 $^{bcde\ B}$	1.70 ± 0.07 $^{ab\ A}$	0.38 ± 0.05 $^{b\ A}$	0.15 ± 0.01 $^{bc\ B}$
Quebracho	0.87 ± 0.04 $^{cdef\ A}$	0.84 ± 0.04 $^{e\ A}$	0.12 ± 0.15 $^{ef\ A}$	-0.11 ± 0.07 $^{e\ A}$
Tea 1	0.93 ± 0.06 $^{cdef\ A}$	1.00 ± 0.18 $^{de\ A}$	0.27 ± 0.02 $^{bcde\ A}$	0.18 ± 0.00 $^{b\ B}$
Tea 2	1.35 ± 0.18 $^{ab\ A}$	1.10 ± 0.03 $^{de\ A}$	0.37 ± 0.00 $^{bc\ A}$	0.16 ± 0.03 $^{bc\ B}$
Cherry	0.86 ± 0.01 $^{cdef\ B}$	1.27 ± 0.04 $^{cde\ A}$	0.00 ± 0.00 $^{fg\ B}$	0.13 ± 0.04 $^{bcd\ A}$
Chestnut	0.71 ± 0.01 $^{f\ B}$	1.03 ± 0.04 $^{de\ A}$	-0.12 ± 0.00 $^{gh\ B}$	0.02 ± 0.03 $^{cde\ A}$
Nut gall 1	0.91 ± 0.08 $^{cdef\ B}$	1.23 ± 0.10 $^{cde\ A}$	0.21 ± 0.00 $^{de\ A}$	0.05 ± 0.02 $^{bcd\ B}$
Nut gall 2	1.19 ± 0.05 $^{bc\ B}$	1.54 ± 0.09 $^{abc\ A}$	0.21 ± 0.01 $^{de\ A}$	0.03 ± 0.00 $^{bcde\ B}$
Oak 1	0.95 ± 0.13 $^{cdef\ B}$	1.42 ± 0.12 $^{bcd\ A}$	0.26 ± 0.00 $^{bcde\ A}$	-0.01 ± 0.04 $^{de\ B}$
Oak 2	1.56 ± 0.07 $^{a\ B}$	1.93 ± 0.16 $^{a\ A}$	0.22 ± 0.04 $^{cde\ A}$	0.06 ± 0.00 $^{bcd\ B}$
Tara 1	0.81 ± 0.24 $^{def\ A}$	1.00 ± 0.13 $^{de\ A}$	0.22 ± 0.01 $^{cde\ A}$	0.14 ± 0.01 $^{bcd\ B}$
Tara 2	1.14 ± 0.13 $^{bcd\ A}$	1.36 ± 0.27 $^{bcd\ A}$	0.27 ± 0.07 $^{bcde\ A}$	0.18 ± 0.00 $^{b\ A}$
Lemon	0.83 ± 0.06 $^{def\ B}$	1.18 ± 0.13 $^{cde\ A}$	-0.19 ± 0.01 $^{h\ B}$	0.03 ± 0.06 $^{bcde\ A}$
Control	0.87 ± 0.09 $^{cdef\ B}$	1.85 ± 0.18 $^{a\ A}$	0.29 ± 0.00 $^{bcd\ A}$	0.14 ± 0.04 $^{bcd\ B}$

Table 2. Pearson Correlation of Met degradation, VSCs and chemical characterization of the oenological tannins (Fracassetti et al., 2021d). Critical values were: 0.383 for df = 14, α = 0.2 (*); 0.426 for df = 14, α = 0.1 (**); 0.497 for df = 14, α = 0.05 (***).

Oxic condition

	Met Deg %	Met-sulfox	MeSH	DMDS	DMTS	Folin	Antiox	Abs 280 nm	Methyl cellulose
Met Deg %	1								
Met-sulfox	-0,590	1							
MeSH	0,246	-0,402*	1						
DMDS	0,338	-0,313	0,037	1					
DMTS	0,176	0,132	0,058	0,816***	1				
Folin	0,005	0,103	0,014	-0,744***	-0,701***	1			
Antiox	0,108	0,033	0,299	-0,724***	-0,594***	0,886***	1		
Abs 280 nm	-0,034	0,314	-0,406*	-0,526***	-0,442**	0,759***	0,494**	1	
Methyl cellulose	0,116	0,254	-0,342	-0,403*	-0,341	0,786***	0,533***	0,958***	1

Anoxic condition

	Met Deg %	Met-sulfox	MeSH	DMDS	DMTS	Folin	Antiox	Abs 280 nm	Methyl cellulose
Met Deg %	1								
Met-sulfox	-0,279	1							
MeSH	-0,333	0,015	1						
DMDS	-0,621***	-0,060	0,413*	1					
DMTS	-0,677***	-0,079	0,474**	0,962***	1				
Folin	0,563***	-0,171	-0,530***	-0,547***	-0,601***	1			
Antiox	0,464**	-0,167	-0,256	-0,425*	-0,450**	0,886***	1		
Abs 280 nm	0,626***	0,105	-0,528***	-0,473**	-0,582***	0,759***	0,494**	1	
Methyl cellulose	0,654***	-0,065	-0,413*	-0,443**	-0,511***	0,786***	0,533***	0,958***	1

Legend: Met Deg %, degraded methionine (%); Met-sulfox, methionine sulfoxide formed; MeSH, methanethiol; DMDS, dimethyl disulfide; DMTS, dimethyl trisulfide; Folin, Total Phenol Index determined with Folin-Ciocalteau method; Antiox, antioxidant capacity determined with DPPH assay; Abs 280 nm, phenols determined by the absorbance reading at 280 expressed as tannic acid for hydrolysable tannins and as catechin for condensed tannins; Methyl cellulose, tannins determined by the methyl cellulose assay expressed as tannic acid for hydrolysable tannins and as catechin for condensed tannins.

635 **Table 3.** Concentrations of volatile sulfur compounds (VSC) in oxic and anoxic conditions. Odor activity values are reported in brackets.
636 For the sum of the moles of VSCs, the moles of DMTS were not considered. Different letters mean significant differences ($p < 0.05$).
637 Upper case letters refer to same type of tannin in oxic/anoxic conditions; lower case letters refer to different tannins in the trials in oxic
638 and anoxic condition separately. Legend: MeSH, methanethiol; DMDS, dimethyl disulfide; DMTS, dimethyl trisulfide.

Tannin	MeSH (μg/L)		DMDS (μg/L)		DMTS (μg/L)		Total VSCs (nmol/L)	
	Oxic	Anoxic	Oxic	Anoxic	Oxic	Anoxic	Oxic	Anoxic
Acacia	1.69 ± 0.83 $^{a\,A}$ (0.56-5.63)	1.40 ± 0.41 $^{b\,A}$ (0.47-4.67)	1.49 ± 0.63 $^{b\,B}$ (<0.1)	10.96 ± 4.99 $^{b\,A}$ (0.24-0.55)	< 0.1 $^{b\,B}$ (< 1)	4.26 ± 1.47 $^{bc\,A}$ (42.60)	51.0	145.5
Grape seeds	0.83 ± 0.58 $^{a\,A}$ (0.23-2.77)	1.12 ± 0.77 $^{b\,A}$ (0.37-3.73)	0.33 ± 0.21 $^{b\,B}$ (<0.1)	2.56 ± 0.91 $^{b\,A}$ (0.06-0.13)	< 0.1 $^{b\,A}$ (< 1)	0.23 ± 0.14 $^{c\,A}$ (2.30)	20.9	50.6
Grape skin	1.71 ± 0.82 $^{a\,A}$ (0.57-5.70)	1.33 ± 0.66 $^{b\,A}$ (0.44-4.43)	0.24 ± 0.33 $^{b\,B}$ (<0.1)	2.36 ± 0.92 $^{b\,A}$ (0.05-0.12)	< 0.1 $^{b\,B}$ (< 1)	0.59 ± 0.00 $^{c\,A}$ (5.90)	38.1	52.6
Quebracho	0.81 ± 0.42 $^{a\,A}$ (0.13-1.30)	1.09 ± 0.00 $^{b\,B}$ (0.36-3.63)	1.20 ± 0.07 $^{b\,B}$ (<0.1)	3.46 ± 0.51 $^{b\,A}$ (0.08-0.17)	< 0.1 $^{b\,A}$ (< 1)	0.43 ± 0.20 $^{c\,A}$ (4.30)	29.5	59.4
Tea 1	0.80 ± 0.67 $^{a\,A}$ (0.27-2.70)	0.60 ± 0.61 $^{b\,A}$ (0.20-2)	0.37 ± 0.03 $^{b\,B}$ (<0.1))	10.34 ± 0.23 $^{b\,A}$ (0.23-0.52)	< 0.1 $^{b\,B}$ (< 1)	0.89 ± 0.45 $^{c\,A}$ (8.90)	20.6	122.2
Tea 2	0.39 ± 0.04 $^{a\,A}$ (0.27-2.67)	1.20 ± 0.02 $^{b\,A}$ (0.40-4)	1.11 ± 0.34 $^{b\,B}$ (<0.1)	4.09 ± 0.53 $^{b\,A}$ (0.09-0.20)	0.13 ± 0.01 $^{b\,B}$ (1.3)	0.17 ± 0.04 $^{c\,A}$ (1.70)	19.8	68.4
Cherry	2.52 ± 1.17 $^{a\,A}$ (0.84-8.40)	1.52 ± 0.46 $^{b\,A}$ (0.51-5.01)	1.24 ± 0.51 $^{b\,B}$ (<0.1)	7.36 ± 0.82 $^{b\,A}$ (0.16-0.37)	< 0.1 $^{b\,B}$ (< 1)	2.34 ± 0.28 $^{c\,A}$ (23.40)	65.6	109.7
Chestnut	2.31 ± 0.96 $^{a\,A}$ (0.77-7.70)	2.78 ± 0.57 $^{ab\,A}$ (0.93-9.27)	1.37 ± 0.58 $^{b\,B}$ (<0.1)	7.98 ± 3.28 $^{b\,A}$ (0.18-0.40)	< 0.1 $^{b\,A}$ (< 1)	0.64 ± 0.43 $^{c\,A}$ (6.40)	62.5	142.4
Nut gall 1	2.05 ± 0.65 $^{a\,A}$ (0.68-6.83)	1.07 ± 0.65 $^{b\,A}$ (0.36-3.57)	0.81 ± 0.34 $^{b\,B}$ (<0.1)	7.22 ± 1.82 $^{b\,A}$ (0.16-0.36)	< 0.1 $^{b\,A}$ (< 1)	4.04 ± 4.11 $^{bc\,A}$ (40.40)	51.4	98.9
Nut gall 2	2.71 ± 1.68 $^{a\,A}$ (0.90-9.03)	2.08 ± 1.27 $^{ab\,A}$ (0.69-6.93)	0.19 ± 0.11 $^{b\,B}$ (<0.1)	3.89 ± 0.88 $^{b\,A}$ (0.09-0.20)	< 0.1 $^{b\,A}$ (< 1)	0.98 ± 1.30 $^{c\,A}$ (9.80)	58.3	84.5
Oak 1	0.88 ± 0.25 $^{a\,A}$ (0.29-2.93)	1.31 ± 0.12 $^{b\,A}$ (0.44-4.37)	0.78 ± 0.38 $^{b\,B}$ (<0.1)	5.48 ± 2.24 $^{b\,A}$ (0.12-0.27)	< 0.1 $^{b\,B}$ (< 1)	2.66 ± 0.24 $^{bc\,A}$ (26.60)	26.5	85.4
Oak 2	1.51 ± 0.16 $^{a\,A}$ (0.50-5.03)	1.34 ± 0.16 $^{b\,A}$ (0.45-4.47)	0.44 ± 0.49 $^{b\,B}$ (<0.1)	41.33 ± 19.62 $^{a\,A}$ (0.92-2.01)	< 0.1 $^{b\,A}$ (0.20)	11.82 ± 7.85 $^{ab\,A}$ (118.20)	36.0	466.5
Tara 1	1.19 ± 0.54 $^{a\,A}$ (0.40-3.97)	2.84 ± 1.39 $^{ab\,A}$ (0.95-9.47)	0.14 ± 0.03 $^{b\,A}$ (<0.1)	2.01 ± 1.43 $^{b\,A}$ (0.05-0.10)	< 0.1 $^{b\,A}$ (< 1)	0.46 ± 0.51 $^{c\,A}$ (4.60)	26.1	80.3
Tara 2	2.58 ± 1.12 $^{a\,A}$ (0.86-8,60)	2.50 ± 1.06 $^{ab\,A}$ (0.83-8.33)	0.12 ± 0.05 $^{b\,B}$ (<0.1)	1.04 ± 0.38 $^{b\,A}$ (0.02-0.05)	< 0.1 $^{b\,B}$ (< 1)	0.25 ± 0.08 $^{c\,A}$ (2.50)	54.9	63.0
Lemon	1.76 ± 0.16 $^{a\,A}$ (0.00-0.01)	1.24 ± 0.24 $^{b\,B}$ (0.41-4.13)	1.34 ± 0.62 $^{b\,B}$ (<0.1)	7.57 ± 2.29 $^{b\,A}$ (0.17-0.38)	< 0.1 $^{b\,B}$ (< 1)	2.85 ± 1.53 $^{bc\,A}$ (28.50)	50.9	106.2
Control	1.76 ± 0.40 $^{a\,A}$ (0.59-5.87)	4.66 ± 1.85 $^{a\,A}$ (1.56-15-56)	3.49 ± 1.41 $^{a\,B}$ (0.08-0.17)	41.03 ± 10.74 $^{a\,A}$ (0.91-2.05)	17.26 ± 4.73 $^{a\,A}$ (173.16)	15.00 ± 7.14 $^{a\,A}$ (150.66)	73.6	532.4

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640 **Table 4.** Sensory scores (average value ± standard deviation) for each tannin under oxic
641 and anoxic condition. Different letters mean significant differences ($p < 0.05$). Upper case
642 letters refer to same type of tannin in oxic/anoxic conditions; lower case letters refer to
643 different tannins in the trials in oxic and anoxic condition separately.

	Oxic	Anoxic
Acacia	2.0±0.3 ^{bA}	2.9±0.4 ^{bB}
Grape seeds	2.3±0.2 ^{bfA}	3.0±0.4 ^{bdA}
Grape skin	3.3±0.2 ^{cA}	4.4±0.4 ^{aceA}
Quebracho	2.1±0.3 ^{bdA}	2.9±0.4 ^{deA}
Tea 1	2.4±0.2 ^{bcA}	4.1±0.4 ^{abB}
Tea 2	2.2±0.3 ^{beA}	3.5±0.4 ^{bcB}
Cherry	2.3±0.3 ^{bfA}	3.0±0.4 ^{bcA}
Chestnut	2.1±0.3 ^{bdA}	3.2±0.4 ^{bcB}
Nut gall 1	2.2±0.2 ^{bdA}	4.5±0.4 ^{adB}
Nut gall 2	2.3±0.2 ^{bdA}	4.6±0.4 ^{aeB}
Oak 1	2.9±0.2 ^{ceA}	4.4±0.4 ^{aeB}
Oak 2	2.7±0.2 ^{cdfA}	5.5±0.4 ^{aeB}
Tara 1	2.4±0.2 ^{bfA}	2.7±0.4 ^{dA}
Tara 2	2.2±0.3 ^{bA}	3.1±0.4 ^{bA}
Lemon	2.0±0.3 ^{bA}	3.4±0.4 ^{bcdB}
No tannins	4.8±0.2 ^{aA}	5.1±0.4 ^{aA}
Model wine (dark)	1.1±0.2 ^{gA}	1.1±0.4 ^{fA}

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