Effects of oenological tannins on aroma release and perception of oxidized and non-oxidized red wine: A dynamic real-time in-vivo study coupling sensory evaluation and analytical chemistry

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1 # Effects of oenological tannins on aroma release and perception of oxidized and non-oxidized
2 red wine: a dynamic real-time *in-vivo* study coupling sensory evaluation and analytical
3 chemistry.

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18

19

20 **Highlights**

21 • A dynamic sensorial evaluation of 6 wines is coupled to dynamic aroma release recording
22 • Addition of ellagitannin extract impacts the dynamic of sensations of oxidized wine
23 • Addition of ellagitannin extract impacts the length of aroma release in mouth
24 • Addition of ellagitannin extract preserves fruitiness under oxidative conditions

25

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26 **Abstract**

27 Addition of oenological tannins claims to have a positive impact on wine stability, protection from oxidation
28 and likely sensory persistence. However, their role on red wine aroma during oxidation is controversial. The
29 present study aims at investigating the effect of addition of oenological tannins on wine flavour (mainly
30 aroma) before and after air exposure. Temporal Dominance of Sensations, a dynamic sensory evaluation,
31 was coupled with a dynamic chemical measurement (nosespace analysis) using a Proton-Transfer-Reaction
32 Mass-Spectrometer connected to the nasal cavity of 17 assessors. Results showed that the oxidation of a non-
33 oaked Pinot Noir red wine decreases the fruity aroma dominance and increases the maderised and prune one.
34 A contextual decrease of the fruity ethyl decanoate and increase of oxidative Strecker aldehydes are
35 observed. Ellagitannins but not proanthocyanidins preserved perception of fruitiness and prevented increase
36 of maderised notes. Moreover, ellagitannins increase the aroma persistence mainly in the non-oxidized wine.

37

38 **Keywords**

39 PTR-ToF-MS – Temporal Dominance of Sensations

40 Proanthocyanidins

41 Ellagitannins

42 Red wine oxidation

43 Oenological tannins

44

45 **1. Introduction**

46 Oxidation is one of the main chemical phenomena affecting the organoleptic properties of wine during its
47 evolution/ageing. Since the pioneering work of Pasteur, numerous studies have been dedicated to
48 characterizing the impact of oxidation on wine quality. It is currently accepted that a slow and constant
49 aeration through the different steps of wine making and ageing has a positive effect on red wine sensory
50 quality, while a fast and excessive oxidation can significantly alter this quality, negatively impacting colour,
51 flavour, and mouthfeel (Ugliano, 2013). The early oxidative ageing is one of the main widespread worldwide
52 defects in oenology (Franco-Luesma et al., 2019; Ugliano, 2013) and it corresponds to a short wine shelf-
53 life.
54 Oxidative transformation of wine compounds modifies the structure and the properties of molecules
55 belonging to different chemical families, and affects compounds involved in wine colour and flavour (i.e.
56 olfaction, taste, and oral somatosensory inputs). Thus, oxidation tends to decrease wine astringency, but also
57 to modify its fruity and floral notes. Oxidized wines are characterized by the increase or the appearance of
58 the following olfactory descriptors: raisin, overripe character, rancid, dried fruit, caramel, farm-feed, cooked
59 vegetables, boiled potato, hay, sweet and Madeira/Porto (Cullere et al., 2007; Escudero et al., 2000; Silva
60 Ferreira et al., 2003; Ugliano, 2013). During the last two decades, several studies aimed at gaining a deeper
61 understanding of the molecular origin of aroma evolution through wine ageing and oxidation. They
62 characterized the evolution of wine volatile organic compounds (VOCs) in terms of quantity and quality
63 during the oxidation processes, in some cases trying to observe their perception pattern during wine tasting.
64 The results show that aroma changes related to excessive oxygen exposure are due to the oxidation of VOCs
65 leading to the formation of new aroma active compounds and to the decrease/disappearance of several VOCs
66 (Escudero et al., 2000). For example, under oxidative conditions, aldehydes increase, while polyfunctional
67 thiols decrease significantly, especially in white wines (Ugliano, 2013). Recently, Carrascon and co-workers
68 (Carrascon et al., 2015) observed that at low levels of SO_2, β-damascenone, E-whiskylactone, and methyl
69 vanillate are the preferred targets of free radical species. It has been recently observed that ethyl esters and
70 acetates decrease during oxidation of red wines produced from Corvina grapes (Picariello et al., 2020).
71 However, the nature of the reactions involved is not clear, as esters can also be easily hydrolysed (Carrascon

72 et al., 2015). Carrascon et al. (2015) reported that the concentration of isoeugenol, vanillin and ethyl vanillate
73 increases after exposing wine to oxygen, while their increase was not correlated to O_2 consumption.
74 It has been observed that wine oxidative notes could be more perceivable during tasting (retronasal) than
75 during sniffing (orthonasal) from the glass (Piombino et al., 2019) and that some VOCs involved in oxidative
76 notes perception were better released under condition simulating wine tasting in small sips (Genovese et al.,
77 2015). This suggests that the perception of oxidative molecular markers can be impacted by factors affecting
78 their portioning and release, such as the non-volatile matrix composition and saliva.
79 Wine contains different classes of polyphenols (e.g. tannins), which exhibit antioxidant properties. Two
80 mechanisms could promote the antioxidant capacity of polyphenols: scavenging of reactive oxygen species
81 and reactive nitrogen species and ion chelation. The chelation of Fe^{2+} ions by polyphenols increases their
82 oxidation to Fe^{3+} ions in the presence of oxygen, decreasing the quantity of Fe^{2+} that could participate in the
83 Fenton reaction and produce hydroxyl radicals (Waterhouse et al., 2016). In red wines, the antioxidant
84 capacity has been mainly attributed to tannins (Waterhouse et al., 2016). Tannins are usually divided into
85 two groups: i) oligomers and polymers of flavan-3-ols, named condensed tannins or proanthocyanidins, and
86 ii) non-flavonoids polymers, named hydrolysable tannins (Waterhouse et al., 2016).
87 Condensed tannins are naturally present in red wines, since they are extracted from grapes seeds and skins
88 during the maceration process. They differ in their constitutive units [(+)-catechin, (-)-epicatechin, (+)-
89 gallocatechin, (-)-epicatechin gallate, (-)-epigallocatechin], their sequences, the positions of interflavanic
90 linkages, (C4-C6 or C4-C8 in the B-type series, with additional C2-O-C7 or C2-O-C5 bonds in A-type
91 structures), their lengths and the presence of substituents (e.g., galloyl or glucosyl groups) (Versari et al.,
92 2013).
93 Hydrolysable tannins are composed of two subgroups: gallotannins and ellagitannins, that are polyol
94 (generally D-glucopyranose) acylated respectively with gallic or ellagic acid. Ellagitannins originate from
95 wood during oak-barrels ageing (Versari et al., 2013).
96 Besides their extraction during winemaking and oak-barrel ageing, both proanthocyanidins and hydrolysable
97 tannins can be added to wine as oenological tannins. Their use in winemaking is a long-used and common
98 technological practice. Up to date, they are authorized by the International Organization of Vine and Wine
99 (OIV) to facilitate the clarification/stabilization of wines and musts, to promote the expression, stabilisation

100 and preservation of colour in red wines, and to contribute to the antioxidant and antioxidasic protection of
101 compounds of the wine (*OIV. International Oenological Codex. COEI-1-TANINS: 2015*, 2015). Indeed, due
102 to their hydroxyl groups on aromatic rings, tannins also have the properties to interact with different
103 compounds and especially with proteins present in the wine, which are responsible for instability, or saliva of
104 the consumer (Canon et al., 2013). These interactions can lead to aggregation and precipitation of the
105 interactants (Canon et al., 2013). Moreover, during wine tasting, the aggregation of the mucosal pellicle by
106 tannins is thought to be at the origin of astringency perception(Ployon et al., 2018) while involving the
107 tethered MUC1(Canon et al., 2021), and it can also modify the ability of the mucosal pellicle to interact with
108 aroma compounds and change aroma persistence (Ployon et al., 2020). Some results testing the application
109 of tannins in winemaking either as antioxidants or as modulators of aroma persistence have been reported
110 (Versari et al., 2013). A wide range of oenological tannins are present in the market. Their antioxidant
111 capacity is one of the main targeted properties to protect wines against oxidation (Magalhaes et al., 2014;
112 Versari et al., 2013). Oenological tannins can be very useful in protecting musts and white wines against
113 browning and oxidation (Versari et al., 2013). However, their antioxidant capabilities are controversial, since
114 tannins with different compositions can show very different antioxidant properties (Magalhaes et al., 2014;
115 Vignault et al., 2018), and because tannin oxidation leads to the formation of reactive species such as *ortho*-
116 quinones (Petit et al., 2019; Singleton, 1987) that can modify wine VOCs patterns, via on one hand the
117 formation of new VOC (i.e. Strecker aldehydes) and on the other hand the consumption of other ones (i.e.
118 thiols). *Ortho*-quinones are highly reactive species, which can be involved in different chemical reactions
119 with other wine components, including in nucleophilic conjugate addition reactions with thiols (Petit et al.,
120 2019). These reactions can be at the origin of a decrease of volatile polyfunctional thiols concentration,
121 responsible for varietal fruity notes of many young wines produced from different varieties (Darriet et al.,
122 1995). *Ortho*-quinones are also involved in the formation of odour active Strecker aldehydes (Singleton,
123 1987)**.** Thus, it can be hypothesized that the addition of oenological tannins in wine influences the perception
124 of wine aromas, especially of oxidized wines, through different mechanisms, which impact the nature, the
125 concentration, and the release kinetics of aroma compounds.
126 The aim of the present work was to shed light on the impact of the addition of oenological tannins on wine
127 perception and on *in vivo* aroma release before and after oxidation. The impact of two different commercial

tannins (i.e., proanthocyanidins and ellagitannins) on the dynamic of sensory perception and aroma release of a red wine before and after air ~~exposition~~ exposure was investigated for the first time. In order to link analytical chemical measurements with sensory evaluation, this *in-vivo* study pioneered the coupling of Temporal Dominance of Sensations (TDS) (Pineau et al., 2009), a dynamic sensory method, with a dynamic approach of analytical chemistry consisting in the analysis of subject's nosespace by Proton Transfer Reaction-Time of Flight-Mass Spectrometer (PTR-ToF-MS) to study the impact of wine oxidation and oenological tannin use on wine flavour perception and release.

2. Materials and methods

2.1. Samples

2.1.1. Wine

A commercial Pinot Noir wine, labelled "Bourgogne Pinot Noir" and obtained with a standard ~~industrial~~ process from a winery located in Burgundy wine region (Domaine Jean-François Bouthenet, 71150 Cheilly-les-Maranges, France), vintage 2016, with no barrel ageing, was selected as base wine for both *in-vivo* and *in-vitro* experiments. This wine was considered the base wine of the study (BW).

2.1.2. Wine phenolic compound characterization

UPLC-DAD-MS analysis was performed on a Vanquish UPLC-DAD system (Thermo Fisher Scientific, Waltham, MA, USA) hyphenated with a Thermo Scientific Exploris 480 Orbitrap (Waltham, MA, USA) mass spectrometer equipped with an electrospray source, using a (10 × 1 mm i.d.) Acquity HSST3 column (Waters, Milford, MA; 1.7µm), thermostated at 35°C. The mobile phase consisted of water/formic acid (99/1, v/v) (eluent A) and acetonitrile/water/formic acid (79.5/19.5/1, v/v/v) (eluent B). Flow rate was 0.22 mL/min. The elution program was as follows: isocratic for 1.5 min with 2% B, 2-12% B (1.5-4.5 min), isocratic with 12% B (4.5-7 min), 12-24% B (7-12 min), 24-48% B (12-15 min), 48-60% B (15-16 min). The DAD signal was acquired from 200 to 650 nm. The mass spectrometer was operated in the negative ion mode (spray voltage, 2.5 kV ; sheat gas, 40 arbitrary unit ; auxiliary gas, 10 arbitrary unit ; sweep gas 2 arbitrary unit ; ion transfer tube temperature, 280°C ; vaporizer temperature, 300°C).

HPLC-grade acetonitrile and formic acid were purchased from Merck. Gallic acid (>99%), phloroglucinol (>99%), ascorbic acid (>99%), caffeic acid (>98%), *trans*-caftaric acid (>98%), epicatechin gallate (>98%)

156 were purchased from Sigma-Aldrich. Procyanidin B2 (>90%), procyanidin C1 (>90%), (+)-catechin (>99%)
157 and (-)-epicatechin (>99%) were purchased from Extrasynthese.
158 Proanthocyanidin constitutive units were determined by HPLC-DAD after phloroglucinolysis carred out in
159 triplicate following a protocol adapted from Kennedy and Jones, 2001 (Kennedy & Jones, 2001). After
160 evaporation of 300 µL of wine with Genevac centrifugal evaporator, 500 µL of phloroglucinol/ascorbic acid
161 solution (respectively 50 and 10 g/L in MeOH/HCl 0.2 M) were added. After solubilisation with an
162 ultrasonic bath (10 min), the solution was heated (50 °C, 20 min). The phloroglucinolysis reaction was
163 stopped by placing the sample in ice and by adding 500 µL of ammonium formiate solution (12.6 g/L). The
164 solution obtained was centrifuged (HettichLab Technology, Tuttlingen, Germany) (15,000 rpm, 15 min)
165 before injection (0.5 µL).
166 The concentrations of proanthocyanidin units released after phloroglucinolysis were determined from peak
167 areas at 280 nm using calibration curves established using external standards, either commercial ((+)-
168 catechin, (-)-epicatechin, (-)-epigallocatechin, (-)-epicatechin 3-gallate) or purified in our laboratory
169 (phloroglucinol derivatives). The total concentration of proanthocyanidins was calculated as the sum of
170 concentrations of all constitutive units. The mean degree of polymerization (mDP) was calculated as the ratio
171 between the summed molar concentrations of all released constitutive units and the summed molar
172 concentrations of lower constitutive units.
173 For analysis of lower molecular weight phenolic compounds, wine was injected directly (0.5µL) after
174 centrifugation (15,000 rpm, 15 min) in triplicate. Identifications were performed by comparison of retention
175 times, UV-visible and MS data with those of standards. The concentrations of gallic acid and flavanol
176 monomers, dimers and trimers were calculated from peak areas at 280 nm and those of hydroxycinnamic
177 acids from peak areas at 320 nm, using calibration curves established with commercial standards.
178
179 *2.1.3. Wine oxidation procedure*
180 The oxidation procedure was conducted by saturating the wine samples with air, as previously described
181 (Ferreira et al., 2015) with few modifications. In the specific, air saturation was performed by gentle shaking
182 250 mL of wine in a closed 500 mL flask for 10 s, successively opening the cup for 10 s to allow fresh air to
183 enter and repeating the same operation two more times.

184 For the *in-vivo* experiments, the 250 mL of air-saturated wine were then transferred in dark amber glass
185 bottles of 500 mL with a screwed cap, resulting in headspace volume to liquid volume (V_{HS}/V_L) ratio of 1,
186 and directly stored in an incubator (XB112, France Etuves, Chelles, France) in the dark at +25 °C for seven
187 days, when the first saturation cycle was considered complete (Ferreira et al., 2015). At that time, the
188 samples were considered ready for *in-vivo* experiments.
189 For the *in-vitro* experiments, following the air-saturation, 5.5 mL volumes of each sample were aliquoted
190 and distributed in screw capped vials of 11 mL, resulting in the same V_{HS}/V_L ratio equal to 1 as for the *in-*
191 *vivo* part. Finally, the samples were stored in the incubator in the dark at +25°C for seven days. After seven
192 days (t=1week), the saturation cycle was considered complete, the vials representing oxidized samples were
193 taken out from the incubator and analysed.
194 *2.1.4. Wine sample preparation for in-vivo experiments*
195 Measurements were carried out during the consumption of the same red wine ~~in~~ under six different
196 conditions (3x2 factorial design. The base wine (BW) was treated with two different commercial tannin
197 extracts: i) a commercial extract of oak ellagitannins, named QUERTANIN® (Laffort, Bordeaux, France), at
198 50 mg/L that led to a wine coded as Base Wine Ellagitannins (BWE), and ii) a commercial grape seed extract
199 rich in proanthocyanidins named TANIN VR GRAPE® (Laffort, Bordeaux, France), at 200 mg/L, that led to
200 the Base Wine Proanthocyanidins (BWP). These three samples (BW, BWE, BWP) were then submitted to
201 the oxidation procedure described above to obtain the oxidized base wine (OW) and the oxidized base wine
202 spiked with ellagitannins at 50 mg/L (OWE) and proanthocyanidins at 200 mg/L (OWP), resulting in six
203 wine samples: BW, BWE, BWP, OW, OWE, OWP. These two concentrations correspond to the highest
204 concentration recommended by the supplier for each extract and it has been checked that the aroma
205 perception of BW, BWE and BWP was similar through preliminary intra-laboratory sensory tests. This
206 preliminary result was confirmed during the sensory analysis of the wine (cf result and discussion). The
207 chemical characterisation of the two commercial tannin extracts has been previously reported (Harbertson et
208 al., 2012). The concentration of proanthocyanidin and ellagitannin measured in their respective extract was
209 200 and 339 mg/g.
210 Tannins were added to 50 mL of BW 45 min before the experiment giving BWE and BWP. After 15 minutes
211 of incubation at room temperature, 10 mL of samples, which correspond to 1 sip, were put into the glasses

212 for sensory evaluation. Bottles were closed with a vacuum wine stopper and stored at 10 °C up to the next
213 session. 10 mL of oxidized samples were taken from the bottles stored into the incubator and put into the
214 glasses.
215 The samples were served in tulip shape 100 mL (±10) volume black glasses covered with a lid to avoid
216 sample evaporation before sensory evaluation. Each sample was prepared in triplicate (3 glasses of 10 mL,
217 each corresponding to 1 sip). Products were presented in an anonymous manner with random three-digit
218 codes (using the same three-digit code for the replicate of each sample).
219
220 *2.1.5. Wine sample preparation for in-vitro experiments*
221 To avoid any bottle effect, three bottles of BW (750 mL) were mixed (final volume: 2250 mL). Successively,
222 6x350 mL of BW were transferred in 500 mL volume flasks. Four BW samples were mixed with: i)
223 ellagitannins at 50 mg/L and 200 mg/L that led to wines coded as BWE1 and BWE2, respectively, and ii)
224 proanthocyanidins at 200 mg/L and 400 mg/L, that led to the BWP1 and BWP2 wines, respectively. Tannins
225 were added directly to the 350 mL volume wines and left in contact with them for 15 minutes. The two other
226 BW samples were used as an oxidized reference without tannins (OW) and a reference conserved under
227 nitrogen atmosphere (OWN). BW, BWE1, BWE2, BWP1 and BWP2 represented the five starting points of
228 the oxidation period (t=0). A volume of 1 mL of each condition was sampled for the analyses and stored in
229 the fridge at +2 °C and took out at the analysis time.
230 The oxidized wine samples were prepared by submitting the remaining volume of the five wine samples to
231 one week oxidation, as reported above (Section 2 of Materials and Methods). The following samples
232 represented the first-week oxidation conditions (t= 1 week): i) oxidized base wine (OW), ii) oxidized base
233 wine spiked with ellagitannins at 50 mg/L (OWE) and at 200 mg/L (OWE2), iii) oxidized base wine spiked
234 with proanthocyanidins at 200 mg/L (OWP) and 400 mg/L (OWP2) and iv) based wine under nitrogen
235 atmosphere (OWN). OWN was stored in the fridge (+2 °C) and took out at the analysis time (t= 1 week).
236
237 *2.1.6. Aroma solution preparation*
238 An aroma solution was prepared for checking the instrumental repeatability throughout the analyses. Four
239 ketones were chosen: 2-pentanone, 2-hexanone, 2-heptanone and 2-nonanone. They were all purchased from

240 Sigma-Aldrich (Steinheim, Germany). A gas chromatography–flame ionization detector (GC–FID) analysis
241 confirmed the purity of all aroma compounds (>99%). Four independent stock solutions were prepared in
242 absolute ethanol. From those solutions, 2 mL vials were prepared by adding each aroma compound to a 13%
243 ethanol solution to obtain a mixture of ketones at a final concentration of 0.1 µmol/L for each aroma
244 compound, strictly avoiding any headspace. They were stored ~~in the fridge~~ at -80 °C until the analysis
245 sampling.
246
247 *2.2. Subjects*
248 The jury was composed of 17 subjects aged between 22 and 59 years (10 females – mean age= 39±13; 7
249 males – mean age= 42±13) recruited from the Centre des Sciences du Goût et de l'Alimentation (INRAE,
250 Dijon, France) and selected based on their interest, motivation, and availability. They all have been informed
251 and have signed a consent form. They all were wine consumers and had previous experiences in performing
252 sensory tests on wine and TDS sensory measurements. They were asked not to drink any coffee or tea, not to
253 smoke and not to eat any food (chewing-gum included) 1 h before the sessions.
254
255 *2.3. Protocol of in-vivo analysis*
256 TDS and Nosespace analysis (NS) were performed simultaneously and required individual sessions that were
257 conducted in an air-conditioned room at 23 °C (±0.5). Each session lasted approximately 1 h. During each
258 evaluation session, subjects were connected to a Proton Transfer Reaction-Time of Flight-Mass Spectrometer
259 (PTR-ToF-MS). They were asked to evaluate a single-sip warm-up sample that preceded the six products (3
260 glasses of one sip per sample): BW, BWE, BWP, OW, OWE and OWP. The six products were analysed in
261 duplicate by each judge; therefore, for each panellist, two individual sessions were performed in two
262 different days. The complete design for the experiment was carried out in 9 days. The presentation orders
263 were set up following a Williams Latin square experimental design(Pineau et al., 2009) balancing order and
264 position effects.
265 The protocol of the sensory evaluation of one sample is represented in Figure 1. Briefly, the sensory
266 evaluation consisted in evaluating three consecutive repetitions of the same sample. Thus, for each sample,
267 three glasses containing one sip of 10 mL were presented to the subjects. The consumption of the three

glasses had to respect a strict protocol, which has been programmed using TimeSens 1.0. software (INRAE, Dijon, France). TimeSens controlled the sequence of events. For each event, instructions and timing were displayed on a screen in front of the subject.

The protocol of consumption consisted ~~of~~ in waiting 30 s before putting the first sample in the mouth, allowing to record the blank of the composition of the air from the nasal cavity by the PTR-ToF-MS. Dual-TDS evaluation started just after the panellists took the first sip in their mouth and click on "*Put in the mouth*" button displayed on the screen. Then, they had to keep the wine in mouth during 20 s, while selecting the perceived dominant attributes as a function of time. Inspiration of air by the mouth was allowed. After 20 s, a message indicated to the subjects that they had to spit off the wine. This step was validated once the subjects clicked on the appropriate button. The evaluation of the dominant sensations continued during 30 s. If the panellists did no longer perceive any aroma and/or taste, they were asked to click on "*No/No more aromas*" and/or "*No/No more tastes*" buttons. After these 30 s, the panellists had 10 s to evaluate astringency and oxidation intensities using two continuous intensity scales (from very low to very high). Then, they had to repeat this sequence two additional times: waiting 30 s, putting the sample in the mouth, and keeping it in mouth during 20 s while evaluating, spitting out the sample, continuing to evaluate the sample for additional 30 s and evaluating astringency and oxidation (10 s). At the end of the 3rd repetition, panellists were asked to wait 1 minute before the end of the PTR-ToF-MS acquisition. The whole TDS evaluation for one product lasted around 5 minutes in total. Between two successive samples, the judges had 3 minutes to clean their mouth as above exposed: ~~firstly~~ rinsing first with a solution of 0.1% apple pectin (Sigma-Aldrich, Saint-Quentin Fallavier, France), secondly with a solution of 1% sodium bicarbonate (Gilbert, France) and, thirdly with mineral water (Evian®, Donone, Evian-les-Bains, France).

2.4. *Sensory analysis*

2.4.1. Panel Training

Considering that TDS sensory tests do not require lengthy training (Pineau et al., 2012), and that all participants had experience in TDS evaluation, only 2 training sessions were organized. During each session, subjects were asked to rinse their mouth firstly with a solution of apple pectin (0.1%) (Sigma-Aldrich, Steinheim, Germany), secondly with a solution of sodium bicarbonate (1%) provided by a pharmacy in Dijon

295 (Burgundy, France) and, thirdly with mineral water (Evian®, Danone, Evian-les-bains, France) (Esteban-
296 Fernãndez et al., 2016) and to wait 60 s between each sample.
297 *Session 1.* This session aimed at generating a list of aroma descriptors. Judges were asked to assess and
298 describe 7 wine samples in terms of aroma characteristics. The 7 wine samples were: 1) a Santenay 1er Cru
299 2016 (BW2), obtained from the same winery located in Burgundy (France) than BW; 2) BW2E; 3) BW2P;
300 4) OW2; 5) OW2E; 6) OW2P; 7) 11 days oxidized BW2.
301 *Session 2.* This session aimed at familiarizing the judges with the list of descriptors previously generated, in
302 order to reach a consensus on the definition of each attribute. Judges were asked to assess the aroma
303 characteristics of 9 wine samples, using the list of attributes previously generated, and to score their intensity
304 on the following numerical category scale: 1=very low, 2=low, 3=medium, 4=high, 5=very high. The sample
305 set was composed as following: 1) BW2; 2) BW2P; 3) OW2; 4) OW2E; 5) OW2P; 6) 10 days oxidized
306 BW2; 7) BW; 8) BWE (ellagitannins at 50 mg/L); 9) BW2 + ellagitannins at 100 mg/L.
307 During the two training sessions, the panellists were asked to score astringency and tastes (sweet, acid, and
308 bitter) intensities of the samples using the 5-point intensity scale described above. At the end of each training
309 session, the perceived sensations were discussed with the participants to prevent overlapping and
310 redundancies among terms and to help their memorization.
311
312 2.4.2. *Dual - Temporal Dominance of Sensations - Multi sips*
313 Dual-TDS consists of an arrangement on the computer screen of attributes belonging to two different sensory
314 modalities in two different columns (Figure SI 1). Using this type of sensory analysis method, the judges are
315 instructed that they can have only one dominant attribute at the same time in each column at any time. In
316 other words, the selection of a dominant attribute switches off only the dominant attribute from the same
317 column and not the other one (Schlich, 2017), defining as dominant a sensation that triggers the most
318 attention at any given moment. The subjects had the information that an attribute could be dominant several
319 times during the evaluation and that it was not necessary that all the attributes were selected as dominant
320 during the evaluation of each product.
321 The following seven aroma attributes were presented simultaneously with the taste attributes on the
322 computer screen, as represented in Figure SI 1: *Dried grass/Hay, Herbaceous/Green, Fruity,*

323 *Porto/Maderised, Animal, Ripe plums/Cooked fruits* and *Spicy*. For each judge, the attributes were displayed
324 in the same order during the whole sensory evaluation. However, their orders were randomised over the
325 subjects to avoid the risk that they choose preferentially the attributes from the top of the list (Pineau et al.,
326 2012).
327
328 *2.4.3. Software*
329 Data were recorded by TimeSens 1.0 (INRAE, Dijon, France). The Dual-TDS screens were designed in
330 French and translated to English for foreign judges.
331
332 *2.5. PTR-MS analysis of aroma release*
333 *2.5.1. In-vivo experiments*
334 The monitoring of the individual's nosespace was done through an home-made teflon nosepiece, that
335 connected both nostrils of the subjects via a light helmet to a Proton Transfer Reaction-Mass Spectrometer
336 (PTR-MS) instrument equipped with a Time-of-Flight (ToF) analyser (PTR-ToF 8000, Ionicon Analytik,
337 Innsbruck, Austria). Sampling was performed at a total flow rate of 400 mL/min with the transfer line
338 maintained at 110 °C. The helmet allowed subjects to move freely their head during the experiment.
339 Nosespace analysis (NS) was recorded at the same time than the evaluation of Temporal Dominance of
340 Sensations evaluation (TDS). $[H_2O+H]^+$ was used as reagent ion. Parameters of the PTR-ToF-MS instrument
341 were as following: drift pressure of 231 Pa, drift temperature of 80 °C, and drift voltage of 390 V, resulting
342 in electric field strength to number density ratio (E/N ratio) of 90 Townsend (Td, $1Td=10^{-17}$ $V.cm^2$). Data
343 were collected using the TofDAQ software provided by the manufacturer of the PTR-ToF-MS. Data
344 acquisition was performed at 1 mass spectrum ranging from *m/z* 0 to 226 per 0.100 s.
345
346 *2.5.2. In-vitro experiments*
347 Volatile compounds of the wine samples were analysed by direct injection – HS analysis. All the
348 measurements were performed using a commercial PTR-ToF-MS instrument (PTR-ToF 8000, Ionicon
349 Analytik GmbH, Innsbruck, Austria) with $[H_2O+H]^+$ as reagent ion (O_2^+ signal intensity was ca. 0.5% of the
350 $[H_2O+H]^+$ one). Succeeding several preliminary tests, parameters of the PTR-MS instrument were chosen

351 and set up as following: drift pressure of 231 Pa, drift temperature of 80 °C, transfer line temperature 110 °C
352 and drift voltage of 390 V, resulting in electric field strength to number density ratio (E/N ratio) of 90
353 Townsend (Td, 1Td=10^{-17} V.cm^2). Data were collected using the TofDAQ software provided by the
354 manufacturer of the PTR-ToF-MS. Data acquisition was performed at 1 mass spectrum ranging from *m/z* 0 to
355 226 per 0.100 s.
356 For each wine samples, 300 µL were transferred into a 20 mL glass vial for the analyses. For aroma
357 solutions, 1 mL was sampled and transferred into a 20 mL glass vial for the analyses. A new vial was opened
358 for each analysis.
359 The vials were closed by a 3-way cap with silicon septum. A first way was connected to a Tedlar® bag
360 containing wet air. A second way was connected to the PTR-MS. Aroma injection was performed through
361 the third way. Two 3-way automatic valves were used to direct the airflow way through two parallel circuits.
362 The circuit connected to the glass vial with the sample is called "indirect", while the second circuit, directly
363 connected to the Tedlar® bag, is called "direct". The experiment started with the circuit in direct position.
364 Then, the circuit was turned to the indirect position and the air flow from the Tedlar® bag swept the glass
365 vial headspace to the PTR for 2 min. The composition of the gas was analysed by PTR-MS analysis.
366 The measurement order followed a Williams Latin square experimental design, and all the samples,
367 including the aromas solution, were analysed in triplicates.
368 *2.6. Data analysis*
369 *2.6.1. Dual TDS*
370 Dual-TDS is equivalent to two TDS run simultaneously. Thus, flavour and taste TDS data were each one
371 analysed separately by the usual TDS curves (Pineau et al., 2009). To compare two products, some TDS
372 curves of differences (Schlich & Pineau, 2017) were produced. TDS curves of differences are obtained as the
373 evolution along time of the difference between dominance rates of two products. Only points corresponding
374 to differences significantly (binomial test, p=0.10) higher or lower than 0 were produced.
375
376 *2.6.2. PTR-MS*
377 Mass spectra analysis was performed using IgorPro 6.36 (WaveMetrics, Inc. Portland, USA) with a
378 homemade procedure (Analyse_PTRMS_1.06.02.ipf). To guarantee high mass accuracy throughout the

379 analysis, the mass scale was calibrated following the peaks of known ions ($[H_2{}^{18}O+H]^{1+}$, *m/z*=21.022;
380 $[NO]^{1+}$, *m/z*=29.997; $[C_5H_8+H]^{1+}$, *m/z*=69.069). Area through the time of 194 ions have extracted giving the
381 corresponding curve of release. For all curves of release the average background signal during the 30 s
382 before introduction of the sample was subtracted for both *in-vivo* and *in-vitro* experiments. The curves have
383 been divided in three depending on the time of the respective repetitions. The area under the release curve
384 has been extracted for the 0-50 s period and every 5 s between 0 and 80 s for all repetitions of all
385 experiments. Background subtraction led to negative areas, suggesting that the signal was not coming from
386 the samples. Thus, all ions having more than 5 negative areas over all the recorded release curves giving a
387 list of 101 ions were eliminated. In order to avoid effect due to changes in the ionization condition, all
388 experiments exhibiting large variations of the amount of $[H_2O+H]^+$ reactant ions were also removed. After
389 this removing there were not anymore significant differences in the amount of $[H_2O+H]^+$ for all selected
390 files.

391 *2.6.3. Statistical analyses*

392 For each studied ion, its 0-50 s area under the curve was analysed with a repeated mixed model of ANOVA
393 using the procedure MIXED from the SAS software. The model featured wines (6 levels) and sips (3 levels)
394 as fixed effects, while panellist and its interaction with wine and sip were random effects with an instructed
395 covariance matrix between them. The sip factor was declared as repeated within panellist by wine and
396 replication with an unstructured covariance matrix. Estimation of the model was done by restricted
397 maximum likelihood (REML). Sip effect was significant for most ions denoting evolutions over time.
398 However, sip by wine interactions were never significant denoting that these evolutions were the same across
399 wine for every ion. Therefore, sip effects will not be reported here, but contributed to a better estimation of
400 the model. Wine effect was significant at p=0.05 for 8 ions and at p=0.15 for 11 others. However, contrast
401 effects comparing each of the 3 wines to its oxidized version were also investigated, as well as contrast
402 effects comparing each pair composed of two of those 3 oxidation effects. Finally, a list of 23 ions featuring
403 either product or contrast oxidation effects was obtained. To compare TDS to PTR-MS results, the 5 s areas
404 under the curve for the 0-50 s period of the 23 affected ions were submitted to a Student t-test (alpha=0.05)
405 as a function of the condition compared.

406 **3. Results and discussion**

407 *3.1. Effect of oenological tannins on base wine flavour perception*

408 TDS curves of the non-oxidized wines with (BWP, BWE) or without (BW) oenological tannins are presented
409 in Figure 2.A. Dual TDS-analysis of non-oxidized wines reveals that the three samples have a similar pattern
410 of dominant sensations through time. Regarding aromas characteristics, fruity is the dominant attribute for
411 the three wines while the dominant attributes for the in-mouth sensations are astringency and acidity. The
412 fruity attributes (i.e. red berries) correspond to the attribute generally reported for non-oaked Pinot Noir
413 wines from Burgundy. The main difference is observed for BWP that presents a higher dominance of
414 astringency particularly from 20 s, which is typically the time required to reach the maximum of astringency
415 intensity. As a result, BWP sample appears slightly less acidic and fruity than BW. Astringency ratings by
416 the subjects at the end of the TDS evaluation confirmed that BWP has a significantly higher level of
417 astringency than BW and BWE, which are rated with similar intensities (Figure 3). This result might be
418 explained by the fact that BWP contains the highest tannins concentration (200 mg/L of proanthocyanidins
419 addition against 50 mg/L of ellagitannin for BWE in regards to the 300 mg/L of flavanol monomers, dimers
420 and trimers (Table SI 1) determined by HPLC and the 1549.41 mg/L of proanthocyanidin units measured by
421 HPLC after phloroglucinolysis (Table SI2) present in the BW. The decrease of acidity intensity can be at the
422 origin of the slight decrease in the fruity aroma perception, as acidity can impact the perception of fruity
423 sensation (Bonnans & Noble, 1993). Nevertheless, this result indicates that the addition of the two
424 oenological tannins has no major effects on the perception of BW flavour through the period 0-50 s, which is
425 a prerequisite for our subsequent analyses.

426 *3.2. Effect of oxidation on wine flavour perception*

427 TDS curves of the base wine prior (BW) and after oxidation (OW) in the presence of the two kinds of
428 tannins (OWP, OWE) are presented in Figure 2.B. Dual TDS-analysis of the oxidized wines reveals that
429 oxidation has almost no effect on the pattern of dominance of taste and astringency sensations. These
430 observations are confirmed by the astringency ratings (Figure 3). The perception of astringency has been
431 rated significantly more intense in BWP than in BW and BWE; after oxidation, only a trend is observed,
432 with no significant difference among the three oxidized samples. Regarding aroma characteristics, oxidation
433 significantly impacts the pattern of dominant sensations of aroma through time. Non-oxidized wine BW is
434 dominated by the fruity attribute, while OW and OWP are dominated by maderised, prune and fruity

435 attributes. OWE is dominated by only two attributes: prune and fruity. This result agrees with the previously
436 reported effects of oxidation, which leads to a decrease of fruity notes and the appearance of oxidative
437 attributes such as maderised/Porto or prune (Cullere et al., 2007; Escudero et al., 2000; Silva Ferreira et al.,
438 2003; Ugliano, 2013). Oxidation ratings by the subjects at the end of the TDS evaluation confirmed that OW
439 samples have a significant higher level of perceived oxidation than BW (Figure 3). Addition of ellagitannins
440 prior to oxidation induced a decrease of maderised dominance while increasing the fruity one and showed no
441 effect on prune attribute. A possible explanation of the difference observed between proanthocyanidins and
442 ellagitannins could be linked to a different effect of the tannins as a function of their structure. Two other
443 point should also be considered: firstly, the base wine (BW) was not aged in oak-barrels meaning that
444 ellagitannins were not present, with their addition being therefore more impacting compared to the addition
445 of proanthocyanidins, already present in BW due to their origin from grape berries; secondly, ellagitannins
446 are the fastest oxygen consumers of the different oenological tannins (Pascual et al., 2017) and thus could
447 have more impact than proanthocyanidins.

448 *3.3. Effect of oenological tannins on in-mouth aroma release*

449 In-mouth aroma release is a dynamic process that impacts the variation of the temporal dominance of
450 sensations. This study aimed at investigating if aroma release can be linked to TDS evaluation. Throughout
451 the dual-TDS experiments, the nasal cavity of the subjects was connected to a PTR-ToF allowing a real-time
452 recording of the release of aroma compounds during the dynamic sensory evaluation of the different wines.
453 Typical release curves are presented in figure SI 2 for the ion at *m/z* 43.02. The figure suggests that the
454 release curves of the ion are similar for the same subject while showing interindividual variability. This
455 appears as an interesting research topic that should require further analysis in the future. The 0-50 s areas
456 under the curve were submitted to a mixed model of ANOVA as described in section 2.4.5, giving a list of
457 23 ions significantly affected by the type of wine. To compare TDS and PTR-MS data, the areas under the
458 curve of the 23 ions were extracted every 5 s from 0 to 80 s and then submitted to a student t-test comparing
459 two different conditions. On the top of Figure 4 the comparisons of BW Vs BWE and BW Vs BWP are
460 presented. Over the 0-50 s period, very few differences are observed, indicating that tannin addition did not
461 affect the release of aroma compounds in that specific analysis timing. The most impacted ions are
462 represented in Figures SI 3 and SI 4. This result agrees with the TDS results, which showed almost no impact

463 on the dominance of sensations over this period (Figure 2). However, regarding the 50-80 s, numerous
464 significant differences are observed, particularly concerning the comparison of BW Vs BWE (cf Figure 4). It
465 is observed that ellagitannin addition increases the release of aroma compounds through this analysis timing,
466 suggesting an enhancing effect of ellagitannins on aroma persistence. This could be explained by the fact that
467 tannins with different nature can differently interact with aroma compounds, affecting their release, as
468 recently reviewed (Pittari et al., 2021). Moreover, aroma compounds can also interact with the oral mucosa
469 (Ployon et al., 2020), and these interactions could be affected by cross-molecular interactions of tannins with
470 the mucosal pellicle, leading to the aggregation of the mucosal pellicle (Ployon et al., 2018). However, as
471 aroma persistence (i.e., 50-80 s) was not evaluated by TDS sensory analysis, further trials to confirm this
472 interesting outcome are necessary.

473

474 3.4. *Effect of wine oxidation on in-mouth aroma release*

475 Figure 5 presents the p-values resulting from the t-test comparing the base wine before (BW) and after
476 oxidation (OW) as a function of time (every 5 s) for the 23 ions, which are significantly affected by the type
477 of wine during the TDS evaluation period (0-50 s). It reveals that for the 0-50 s period only 4 ions (61.03,
478 73.07, 87.05, and 201.19) + 2 isotopes [74.07 (^{13}C isotope of 73.07) and 202.19 (^{13}C isotope of 201.19)] are
479 significantly affected by the oxidation of the base wine when considering areas under the curve for periods of
480 5 s. The mean 5s-areas of the 4 affected ions are also presented as a function of time with the ones of two
481 other ions (*m/z* 73.04 and 76.05), which show significant differences during the 50-80 s period. These curves
482 show that among the 4 ions with significant differences during the TDS evaluation (0-50 s), the release of the
483 ion 201.19 and of its isotope 202.19, is lower during the consumption of OW. The release of the ion 61.03 is
484 lower during the first 20 s of OW tasting, then increasing until 80 s. These two ions (201.19 and 61.03) can
485 be tentatively attributed to the protonated species of ethyl decanoate ($[C_{12}H_{24}O_2+H]^{1+}$) and acetic acid
486 ($[C_2H_4O_2+H]^{1+}$) (Deuscher et al., 2019), respectively. At the opposite, ions with *m/z* 73.07 and 87.05, which
487 can be tentatively attributed to isobutyraldehyde ($[C_4H_8O+H]^{1+}$) (Campbell-Sills et al., 2016) and butane-2,3-
488 dione or isovaleraldehyde ($[C_4H_6O_2+H]^{1+}$ or $[C_5H_{10}O+H]^{+}$) (Deuscher et al., 2019), are more released in
489 OW. Ethyl decanoate is a wine ester contributing to wine aroma. Its organoleptic profile can be described as
490 fruity, apple, grape (Waterhouse et al., 2016). Together with ethyl hexanoate and ethyl octanoate, ethyl

491 decanoate is considered as being a highly positive aroma compound of young wine “bouquet”, introducing
492 fruity flavour notes (Waterhouse et al., 2016). Thus, the decrease of the fruity attribute in OW compared to
493 BW in TDS experiment could be linked to the decrease of ethyl decanoate during the process of wine
494 oxidation. During the parallel *in-vitro* experiment (no saliva) conducted by Headspace - Solid Phase
495 Microextraction – Gas Chromatography - Mass Spectrometry (HS-SPME – GC-MS) analyses (data not
496 shown), a similar result has been obtained, confirming a significant lower concentration of ethyl decanoate,
497 together with other important wine esters (e.g., ethyl butanoate, 2- and 3-methylbutyrate, hexanoate,
498 octanoate, isoamyl and hexyl acetate), in OW compared to BW. However, as ethyl decanoate is a highly
499 volatile compound~~s~~ with a low affinity for the aqueous phase due to its high hydrophobicity ($logP_{octanol/water}$
500 value 4.86), it is possible that the decrease observed here was due to evaporation of this compound~~s~~ during
501 the procedure of oxidation. But, ion at m/z 145.14, which can be attributed to ethyl hexanoate, was not
502 affected by the oxidation procedure and no difference was observed in the *in-vitro* experiment between the
503 different matrices for the oxidized condition indicating no evaporation for this hydrophobic ester
504 ($logP_{octanol/water}$ value 2.82) (cf paragraph 3.6. and figure 6.D.). At the opposite, the higher perception of
505 maderised attribute is probably linked to the increase of aldehydes such as isobutyraldehyde or
506 isovaleraldehyde during wine oxidation (Figure 5) as previously observed (Bueno et al., 2016). Indeed,
507 aldehydes are the main cause of the development of oxidation-related off-odours and wine aroma
508 deterioration (Bueno et al., 2016; Ugliano, 2013). Isobutyraldehyde (2-methylpropanal) and isovaleraldehyde
509 (3-methylbutanal) are Strecker aldehydes. A recent study showed that the presence of Strecker aldehydes
510 (including isobutyraldehyde and isovaleraldehyde) induced the reduction of fruitiness in young wines and of
511 woody notes in oaked wines as well as the appearance of the typical attributes that define wine oxidation
512 (Marrufo-Curtido et al., 2021). Thus, the decrease in fruitiness may rather be a perceptual effect caused by
513 aroma suppression induced by Strecker aldehydes. Strecker aldehydes can be formed i) from the
514 corresponding precursor alcohols by peroxidation (Juan et al., 2012) and ii) via Strecker degradation of the
515 corresponding precursor amino acid as secondary reactions of the *ortho*-quinone derivatives formed through
516 the oxidation of wine polyphenols by polyphenoloxidases and/or molecular oxygen (Rizzi, 2006). The
517 Strecker degradation of amino acids is described as a result of the Maillard reaction and involves the
518 interaction of sugar-derived α-dicarbonyl compounds with free amino acids. In presence of α-dicarbonyl

519 compounds, the amino acid is decarboxylated and deaminated, forming an aldehyde with one carbon atom
520 less than the amino acid and known as "Strecker aldehyde" (Singleton, 1987). Carbonyl compounds exist in
521 all types of wines, particularly in red wines and in wines that undergo malolactic fermentation. Glyoxal,
522 methylglyoxal, diacetyl and pentane-2,3-dione are the principal α-dicarbonyl compounds found in wine but
523 only α-diketones are relatively abundant in wine. Typically, α-dicarbonyls with n=0 are reported as Strecker
524 degradation reagents but, in principle, any dicarbonyl compound with extended conjugation (n>0) can be
525 used (Rizzi, 2006). The latter structural category can be extended to include *ortho*-quinones, particularly
526 abundant during oxidation processes (Rizzi, 2006). However, looking at the initial patterns of ions putatively
527 corresponding to Strecker aldehydes (*m/z* 73.07 and 87.05), their increase could not be attributed to a "de
528 novo" formation from amino acids. They could be already there as complexes with SO_2 and be at higher
529 levels in oxidised samples because SO_2 has been oxidised (Bueno et al., 2016).

530 *3.5. Effect of oenological tannins on in-mouth aroma release of oxidized wine*

531 On the bottom of Figure 4 are also presented the p-values resulting from the t-test comparing the oxidised
532 wine prior (OW) and after the addition of the types of tannins (OWP or OWE) as a function of time (every 5
533 s) for the 23 ions that are significantly affected by the type of wine during the TDS evaluation period (0-50
534 s). While sensory analysis revealed that the addition of ellagitannins preserves the fruity attribute dominance
535 and decreases the maderised one, the only chemical evidence that could be linked to this effect is a
536 significant increase of ethyl decanoate release during the 0-5 s interval of the consumption of the wine
537 containing ellagitannins. Considering that non-attentively perceived odours may impact on cognitive
538 processing (Mas et al., 2020), it cannot be excluded that this difference impacted on the cognitive processing
539 of flavour perception. The impact of tannins on the *in-vivo* release of ethyl decanoate has been previously
540 reported (Muñoz-Gonzalez et al., 2019). It should be kept in mind that the response times of human subjects
541 and the PTR-MS are not the same. It is expected that the description of wine flavour quality by human
542 subjects requires few seconds of delay between the activation of the olfactory receptors and their sensory
543 evaluation, while the response time of PTR-MS is expected to be less than 100 ms. Another limit of the
544 present study is that the ionization efficiency of PTR-MS is based on VOC proton affinity while ionization
545 competition may occur in the drift tube, where VOC ionization occurs. These phenomena may prevent the
546 obtention of the full picture of the composition in VOCs of the subject's nose space. For instance, thiols,

547 which may also take part in the fruity notes of red wine, are poorly ionized with PTR-MS. Finally, the results
548 do not take into account the interindividual differences which may affect both the sensory evaluation and *in-*
549 *vivo* aroma release that may have been impacted by physiological parameters(Muñoz-González et al., 2021).
550 Comparing the 4 patterns, it is interesting to notice that while the addition of proanthocyanidins to both BW
551 and OW has almost no effect on aroma release, the addition of ellagitannins, on the contrary, influences
552 aroma release in the 50-80 s time in both BW and OW. It is interesting to observe that while ellagitannins
553 increase aroma persistence in the non-oxidized wine, they have a lower effect after oxidation (Figures 4 and
554 SI 5). A hypothesis is that the oxidized structures of ellagitannins interact differently with the oral mucosa
555 and aroma compounds decreasing the adsorption/desorption of aroma compounds at the surface of the oral
556 mucosa. These results suggest that ellagitannins could differently impact aroma persistence during red wine
557 tasting, which represents an interesting outcome from an oenological point of view, therefore deserving
558 further investigations.

559 *3.6. Effect of oenological tannins on wine aroma*

560 Ions showing the most significant differences in *in-vivo* experiments, were also monitored through *in-vitro*
561 analysis. Figure 6 represents the behaviour of the ions with *m/z* 61.03, 73.07, 87.05 and 201.19, detected by
562 PTR-ToF-MS (no saliva) in all the analysed wine matrices, including the base wine (BW) and the
563 corresponding oxidized wine (OW) spiked with two concentrations of ellagitannins (BWE and BWE2, OWE
564 and OWE2) and proanthocyanidins (BWP and BWP2, OWP and OWP2), as well as the base wine oxidized
565 under nitrogen (OWN). The four ions, as already exposed above, are tentatively attributed to acetic acid,
566 isobutyraldehyde, isovaleraldehyde and ethyl decanoate, respectively. The first three compounds are volatile
567 markers of wine oxidation (Ugliano, 2013)**.** While significant trends are not observed for the ions with *m/z*
568 61.03 and 87.05, significant increase and decrease are observed after oxidation for ions at *m/z* 73.07 and
569 201.19 respectively (t-test; p-value=0.05). However, whatever the added tannin, no significant difference is
570 observed for both the oxidized and the non-oxidized conditions. The difference of significance observed
571 between in vitro and in vivo data, can be explained by the lower number of observations by condition for the
572 in vitro experiments. Ion at *m/z* 73.07 is significantly higher in OW compared to BW, suggesting that it is
573 formed during wine air exposition and its formation seems to be contrasted by nitrogen (OWN). The
574 formation of this ion seems not to be prevented by the addition of tannins, independently from their nature

575 and concentration. The ion at *m/z* 201.19 is significantly affected by oxidation but, according to t-test, OW is
576 not significantly different from BW. This ion is also not significantly affected by the presence of tannins,
577 whatever the condition.

578

579 ## 4. Conclusion

580 By coupling the evaluation of temporal dominance of sensation with nose space analysis by proton-transfer-
581 reaction mass spectrometry, this study investigated the effect of the addition of oenological tannins on wine
582 perception before and after oxidation. The addition of either proanthocyanidins or ellagitannins had almost
583 no impact on both the temporal dominance of sensations and the *in-vivo* release of aroma of the non-oxidized
584 wines during the first 50s. After 50s, this study demonstrates for the first time that the addition of
585 ellagitannins significantly increased the release of VOCs during wine consumption for the non-oxidized
586 wine, while the persistence of aroma compounds was not evaluated by sensory analysis. Regarding wine
587 oxidation, it induced a decrease of the fruity attribute while the dominance of maderised and prune notes
588 increased. In parallel, significant changes in the composition of subject's nose space were observed with a
589 decrease of ethyl decanoate and an increase of Strecker aldehydes. Strecker aldehydes can be responsible for
590 the appearance of oxidative notes, while ethyl decanoate is an ester with fruity notes. However, its
591 contribution to wine fruitiness is less relevant than those of other ethyl esters, such as ethyl hexanoate, which
592 was not affected by the oxidative procedure. Addition of ellagitannins before oxidation leads to the
593 preservation of the dominance of fruity attribute and to the decrease of the maderised one, while addition of
594 proanthocyanidins did not. The composition of the subject's nose space poorly explains this effect as the
595 only significant effect is an increase of ethyl decanoate release during the 0-5 s interval of the consumption
596 of the wine containing ellagitannins. It suggests probably the occurrence of perceptual interactions that need
597 to be further explored. It should be also indicated that the present study presents some limits, as the
598 interindividual differences and response time differences between human subjects and PTR-MS were not
599 taken into account and the temporal of dominance of sensations records an analogical signal while PTR-MS
600 experiments a digital one.

601 Nevertheless, these results provide new information for the use of oenological tannins in winemaking and
602 their potential impact on wine perception. More specifically, it evidences that the presence of ellagitannins

can have a positive impact on wine perception, and both on the aroma persistence in young red wine and on the perception of the fruity aroma after oxidation. Therefore, they can be useful for winemakers to better understand and manage red wines' oak-barrel ageing. Indeed, according to our results, wood-barrel ageing of young fruity red wines, which corresponds to a storage in the presence of ellagitannins (extracted from the wood to wine) and oxygen (permeated through the wood into the wine), could be a way to preserve fruitiness and smooth astringency. This preservation of fruity aromas could potentially help to counterbalance the contribution of aromas extracted from wood and in masking the appearance of oxidative notes with a positive impact on the sensory shelf-life. Further investigations and new methodological developments are required to determine more clearly the origin of the preservation of fruity aromas and the increase of aroma persistence observed in this study when the fraction of ellagitannins was added.

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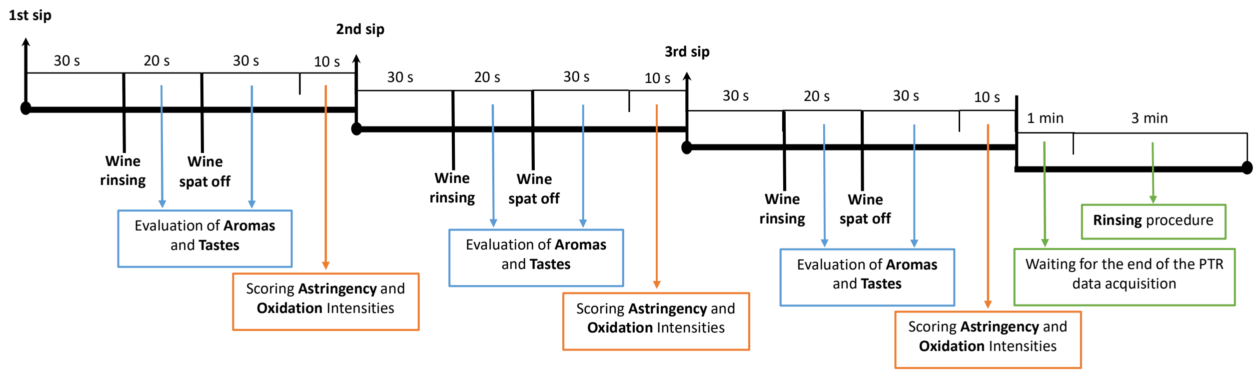
Conflicts of Interests:

The authors declare no conflict of interest

622 **Figures:**

623 **Figure 1**



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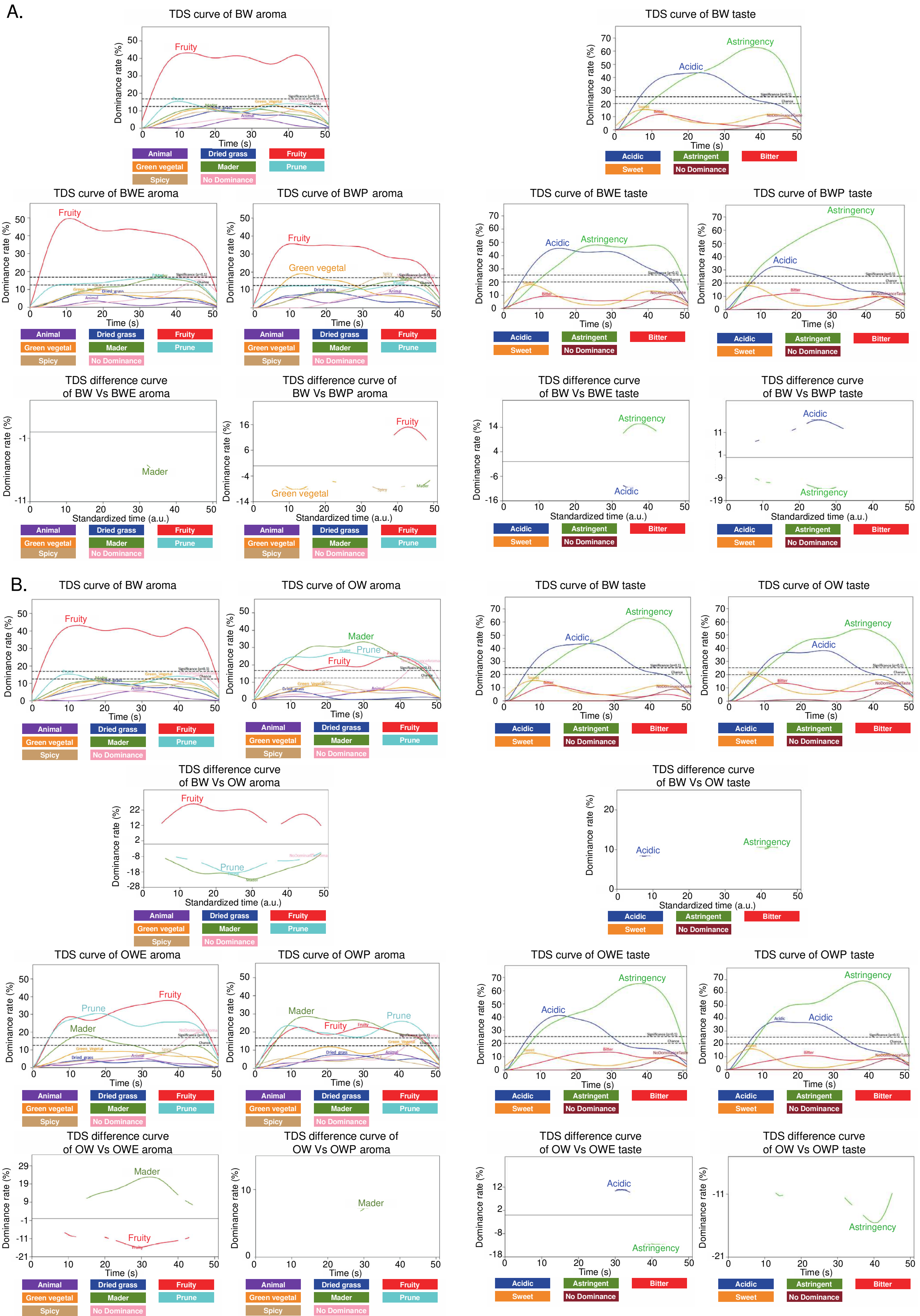
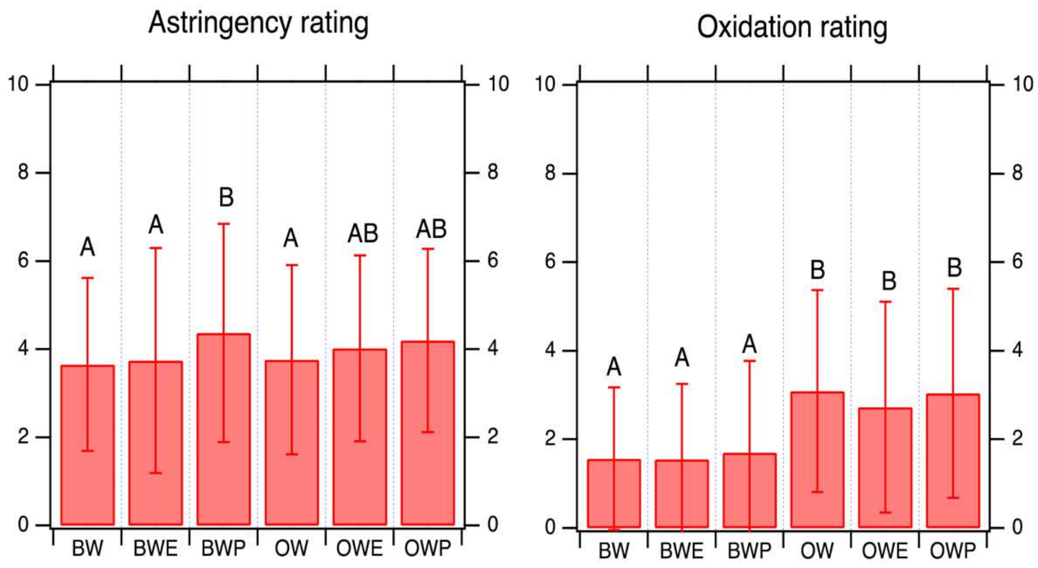
628 **Figure 2**



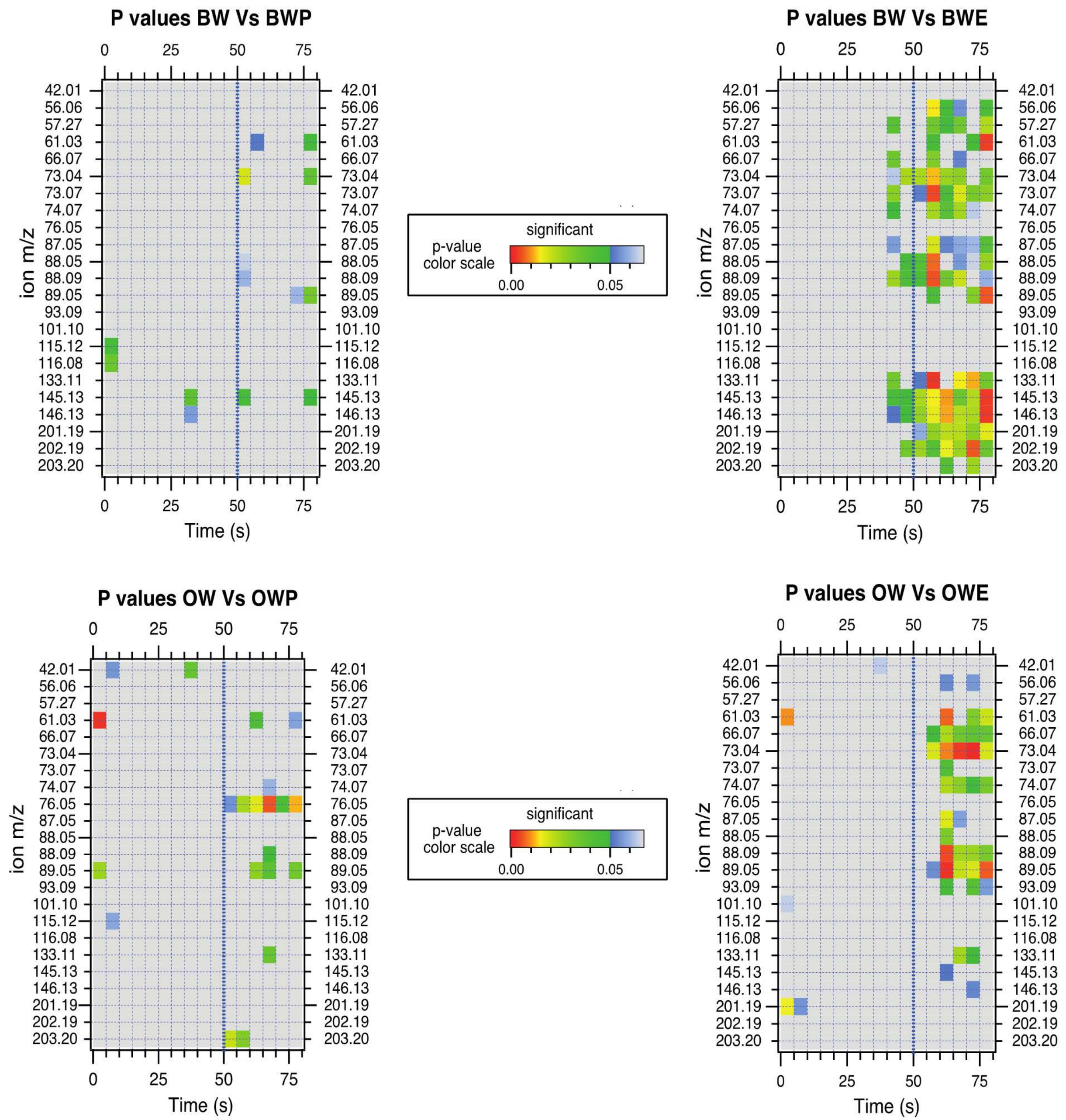
629

630 **Figure 3**



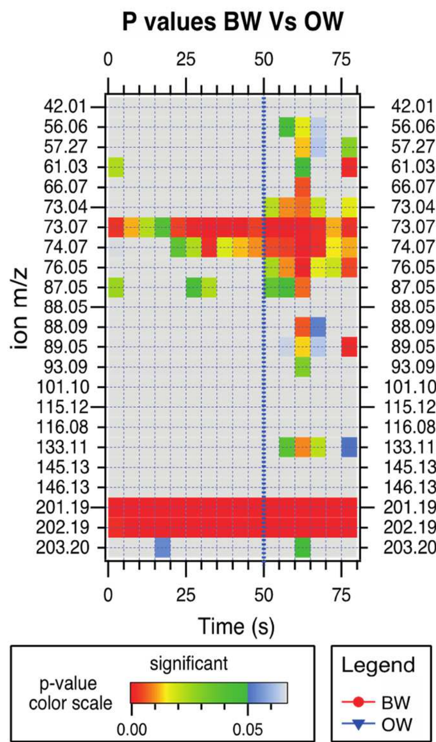
631

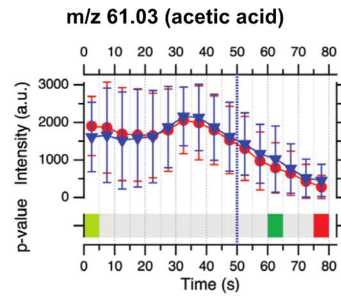
632 **Figure 4**

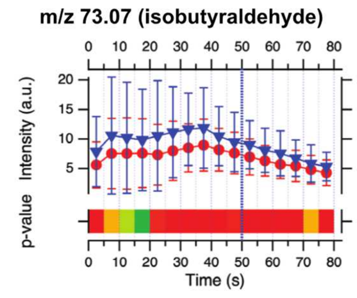


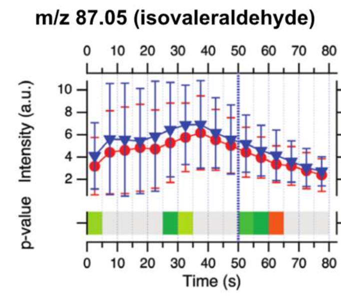
633

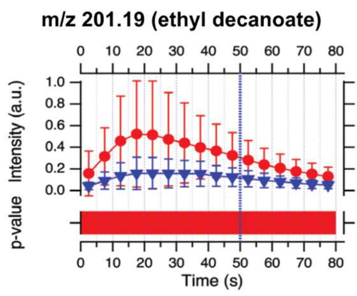
634 **Figure 5**











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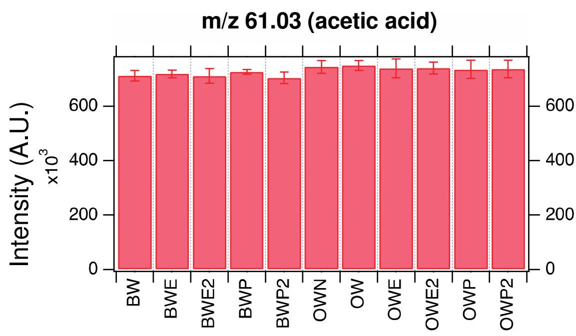
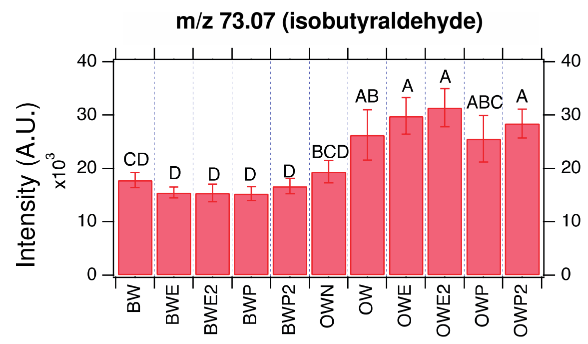
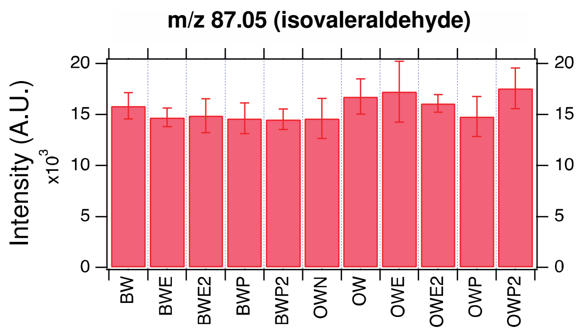
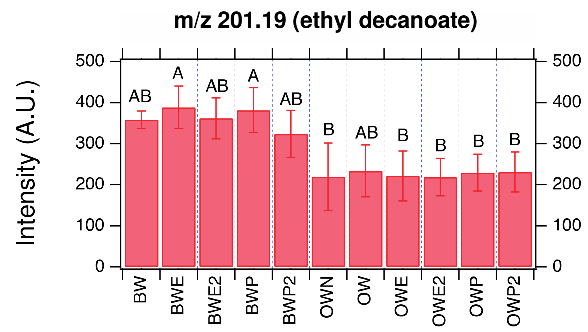
636 **Figure 6**









637

638 **Figure Caption:**

639 **Figure 1.** Dual-TDS-Multi Sips protocol followed by the panellists for products ‘evaluation.

640 **Figure 2. A.** Dominance evolution of the sensory perceptions of aroma and taste/astringency sensations for
641 BW, BWE and BWP. **B.** Dominance evolution of the sensory perceptions of aroma and taste/astringency
642 sensations for BW, OW, OWE and OWP.

643 **Figure 3.** Astringency and oxidation ratings of the different wines using two continuous intensity scales
644 (from very low to very high). Significant differences are marked with different letters ($p < 0.05$).

645 **Figure 4.** Comparison of aroma release of BW vs BWP, BW vs BWE, OW vs OWP and OW vs OWE.
646 Matrix of the t-test of BW Vs BWP, BW vs BWE, OW vs OWP and OW vs OWE of the areas under the
647 curve every 5 s from 0 to 80 s of the 23 significantly affected ions.

648 **Figure 5.** Comparison of aroma release of BW Vs OW. Matrix of the t-test of BW Vs OW of the areas under
649 the curve every 5 s from 0 to 80 s of the 23 significantly affected ions. Average areas under the curve every 5
650 s for the main significantly affected ions with the respective standard deviations.

651 **Figure 6.** Release of m/z 61.03, 73.07, 87.05 and 201.19 detected by PTR-ToF-MS (no saliva) in all the
652 analysed wine matrices, including the base wine (BW) and the corresponding oxidised wine (OW) spiked
653 with two concentrations of ellagitannins (BWE and BWE2, OWE and OWE2) and proanthocyanidins (BWP
654 and BWP2, OWP and OWP2), as well as the base wine oxidised under nitrogen (OWN). Significant
655 differences are marked with different letters ($p < 0.05$).

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