Stimulation of saliva affects the release of aroma in wine; a study of microbiota, biochemistry and participant origin

Xinwei Ruan[1], Yipeng Chen[1], Aafreen Chauhan[1] and Kate S. Howell[1*]

[1]School of Agriculture, Food and Ecosystem Sciences, Faculty of Science, University of Melbourne, Parkville 3010, Australia

Xinwei Ruan: ORCID: 0000-0002-1963-3807

Yipeng Chen: ORCID: 0009-0002-7511-374X

Aafreen Chauhan: aschauhan@student.unimelb.edu.au

*** Corresponding author: Kate S. Howell**

Email: khowell@unimelb.edu.au; **ORCID:** 0000-0001-6498-0472

Abstract

Saliva influences the release of aroma in the oral cavity. The composition of human saliva varies depending on stimulation and host's origin; however, the compositional differences of saliva and their influences on aroma release have not been fully evaluated. In this study, we recruited 30 healthy adults (15 Australians and 15 Chinese) and collected saliva samples at three stages: before, during, and after stimulation. Salivary samples were characterised by flow rate, total protein concentration, esterase activity, microbiome composition by full-length 16S rRNA gene sequencing, and the ability to release aroma from wine by headspace solid-phase microextraction gas chromatography–mass spectrometry (HS-SPME-GC-MS). Differences in salivary composition and specific wine volatiles were found between Australian and Chinese participants, and amongst the three stimulation stages. Significant correlations between the relative abundance of 3 bacterial species and 10 wine volatiles were observed. Our results confirm the influence of participant's geographic origin and stimulation on salivary composition, highlighting the role of salivary components, especially salivary bacteria, on the release of aroma from wine.

Keywords: Microbiota, Salivary microbiome, Geographic variation, Aroma perception, Preference

28 **1 Introduction**

29 Saliva plays an important role in aroma release from foods and beverages [1-3]. When mixing with saliva
30 in oral cavity, volatile compounds are released from food matrix and then travel to olfactory receptors
31 via a retronasal route. Physiochemically, saliva has a dilution effect on aroma due to continuous
32 salivation [4]. Saliva contains salt, sugar and the proteinaceous fraction of mucin, which may influence
33 how volatiles partition in a solution [5]. Further biochemical effects by degrading odorants or releasing
34 aroma from aroma precursors have been reported [6-8]. Two critical components of saliva, proteins and
35 microbiota, are implicated with release or formation of aroma compounds and thus affect aroma release
36 from food

37 Proteins are able to bind, trap and interact with volatile compounds, thereby influencing the aroma
38 release [9]. Two abundant proteins in whole human saliva, mucin and α-amylase, modify release of aroma
39 compounds through hydrophobic interactions [10]. Pérez-Jiménez, Rocha-Alcubilla and Pozo-Bayón [11]
40 demonstrated the efficacy of esterase in human saliva to degrade esters by comparing whole saliva with
41 enzymatically inhibited saliva. Large inter-individual variations have been observed in the total salivary
42 esterase activity [12]. The chemical structure of esters also influences the extent of enzymatic degradation
43 [6]. In addition, the effect of salivary enzymatic reactions on aldehydes [7] and thiols [6] has also been
44 demonstrated by comparing the aroma release ability of thermal-treated saliva with untreated saliva.

45 In contrast to salivary proteins, the role of oral bacteria in aroma release is not well characterised. The
46 oral microbiota contribute to the ability of human saliva to hydrolyse odourless grape glycosides [8, 13].
47 A previous study [14] identified the differences in salivary microbial profile between obese and normal-
48 weight individuals. However, no association was established between the differences of these two
49 groups in aroma release of wine and the microbial profiles. In recent years, the advances in culture-
50 independent methods for oral microbiome identification have deepened our understanding of
51 microorganisms in the oral cavity [15] and reveal the salivary microbial composition with a stable core
52 bacterial community shared by geographically diverse populations [16]. Increased accuracy of taxonomic
53 identification would facilitate to study the role of salivary bacteria in aroma release.

54 Salivary protein and microbiota are likely to be dynamic during oral processing. Saliva production
55 switches between stimulated and unstimulated depending on the presence of stimuli. During stimulation
56 evoked by taste or mastication, the saliva secretion volume increases [17]. More than 90% of saliva comes
57 from three pairs of major salivary glands, including the submandibular, sublingual, and parotid glands.
58 The saliva produced by these three glands differs in composition and function because of the cells that
59 constitute the glands [18]. For example, the parotid glands secrete watery fluids since they exclusively
60 consist of serous acinar cells, while the sublingual glands produce viscous saliva rich in mucins and
61 other glycoproteins due to the high proportion of mucous acinar cells [19]. Therefore, the salivary
62 composition varies depending on the contribution of different glands with or without stimulation [20]. It
63 has been documented that parotid glands have a higher secretory rate under stimulation than in
64 unstimulated conditions. Meanwhile, the submandibular and sublingual glands produce more saliva
65 under unstimulated conditions [21].

66 Recently, the published literature has offered contradictory findings on whether the microbial diversity
67 in saliva is different between unstimulated and stimulated status [22-24]. Specifically, the alpha- and beta-
68 diversity of salivary microbiome were reported as significantly different by Gomar-Vercher, Simón-
69 Soro, Montiel-Company, Almerich-Silla and Mira [22]. In contrast, the other two studies observed no
70 significance [23, 24]. Significant differences have also been observed by previous studies between saliva
71 collected before and during stimulation in various salivary biochemical parameters, including protein
72 concentration [25], antioxidant activity [25], esterase activity [26]. Meanwhile, the composition of saliva
73 collected after stimulation by food or other stimuli have not been characterised. Since the after-odour
74 and after-taste generated after the swallowing or expectoration of food is also considered an essential
75 sensory parameter [27, 28], studying the salivary composition after stimulation may give insight into
76 understanding this process.

77 Meanwhile, the microbiota and type of protein in saliva varies among populations and is also shaped
78 by the host characteristics, such as age [29], gender [30], body mass index (BMI) [31] and other lifestyle factors
79 [32]. The correlation of a host's ethnicity on the bacterial salivary composition has received considerable
80 critical attention. Mason, Nagaraja, Camerlengo, Joshi and Kumar [33] investigated the oral microbial

81 profile of 192 participants belonging to Chinese, Latinos, non-Hispanic whites, and non-Hispanic
82 blacks, finding the lower alpha diversity of non-Hispanic blacks than the other three groups. Yang,
83 Zheng, Cai, Shrubsole, Pei, Brucker, Steinwandel, Bordenstein, Li and Blot [34] observed the differences
84 in overall microbial composition between African Americans and European Americans. A recent study
85 documented different microbial alpha and beta diversity of supragingival plaque microbiome among 96
86 children from four ethnic groups, including African American, Burmese, Caucasian and Hispanic [35]. A
87 study conducted by our group demonstrated the differences between Australian and Chinese wine
88 experts' salivary protein profile and their sensory responses [36]. The mode of variation between
89 Australian and Chinese saliva has not been established, but our preliminary data suggest that dietary
90 differences might be correlated with these differences (data not shown).

91 Food preference is strongly influenced by a consumer's cultural, geographic and personal background
92 [37, 38]. The preferences of Australian and Chinese consumers for 14 red wines have been compared by
93 Williamson, Robichaud and Francis [37], finding variance in some attributes for liking, such as "alcohol",
94 "sweetness" and "dark fruit". Aroma perception is a prominent factor that influences the acceptability
95 and preference of customers for different wines [39]. However, much uncertainty exists about the
96 relationships between host factors, salivary composition, and aroma. We ask if the functional properties
97 of diverse salivary microbiome communities can provide insight into the differences in sensory
98 perception amongst wine consumers.

99 To enhance our understanding of saliva composition and aroma perception, we analysed samples from
100 30 participants from Australia and China and mixed the saliva samples with wine to observe the ability
101 of human saliva to release aroma compounds from wine. Each participant provided three types of saliva
102 samples collected before, during and after mechanical stimulation to extend our knowledge of reactions
103 that occur in the oral cavity during stages of appreciating wine. Enzymatic activity tests and full-length
104 16S rRNA sequencing highlighted the differences of saliva type and origin of participants on salivary
105 composition. Combining these findings with the wine aroma profile detected by headspace solid-phase
106 microextraction gas chromatography-mass spectrometry (HS-SPME-GC-MS), we demonstrate

107 differences in salivary proteins and microbiome due to the mode of salivary stimulation and how this
108 varies based on geographic origin of the participant.

109

110 ## 2 Material and Methods

111 ### 2.1 Materials and reagents

112 All reagents and chemicals used in this study were of analytical grade. Methanol, citric acid, 4-
113 Nitrophenyl acetate, sodium chloride, sodium phosphate, sodium bicarbonate, potassium chloride,
114 potassium phosphate dibasic trihydrate, calcium chloride dihydrate, mucin from porcine stomach,
115 phosphate-buffered saline, Tris-buffered saline, 4-octanol, amylase activity assay kit, and standards for
116 volatile compounds detected in GC-MS analysis was obtained from Sigma-Aldrich (Castle Hill NSW,
117 Australia). Micro bicinchoninic acid (BCA) protein assay kit was acquired from Thermo Fisher
118 Scientific (Waltham, MA). Pure water was obtained from a Milli-Q system (Millipore Australia,
119 Bayswater, Victoria, Australia). A Shiraz wine (Ladies Who Shoot Their Lunch; vintage 2019; 14.9%
120 v/v alcohol) produced in Victoria, Australia, was purchased from a local wine producer, and used in all
121 GC-MS assays with wine (section 2.6). This wine was selected because different aroma perception were
122 observed between Chinese and Australian panellists in a previous sensory evaluation [36].

123 ### 2.2 Study cohort

124 We recruited 30 healthy adults to meet the following criteria: (i) self-reported good systemic health (i.e.,
125 no diagnosed diabetes, cardiovascular diseases, endocrine disorders, or hypertension); (ii) no clinically
126 diagnosed oral diseases; (iii) not receiving antibiotic or steroid hormone use within the previous three
127 months; (iv) no smoking habits (less than several times a month); (v) not a regular drinker (less than
128 several times a week); (vi) have a BMI index no higher than 24 kg/m^2. The sample collection was
129 performed at the University of Melbourne, Australia. An online questionnaire was provided to all
130 volunteers to ensure they met the inclusion criteria. Demographics were also acquired using self-
131 reported questionnaires before the collection. Fifteen Chinese and fifteen Australian participants (aged
132 18-28) joined the study. All participants resided in Melbourne, Australia. The participants were grouped

133 according to their country of birth and residence history. The Australian group includes participants
134 born in Australia and have spent most of their lives there. The Chinese group contains participants born
135 in China and have lived in Australia for less than five years. The ethnic origin of the participant's parents
136 or grandparents was not collected nor considered in this study. Participants self-identified as 'Chinese'
137 or 'Australian'. The gender and BMI index of participants were balanced in each group **(Table S1)**.
138 This study was approved by the University of Melbourne Human Research Ethics Committees (Review
139 Reference Number: 2022-24220-29889-3).

140 **2.3 Sample collection**

141 Before collection, sampling procedures were explained in detail to all participants. Participants were
142 asked to refrain from eating and drinking any beverage except water for an hour prior to providing
143 saliva samples. Three types of saliva were collected before (Phase A), during (Phase B), and after (Phase
144 C) stimulation. The collection procedure is illustrated in **Figure S1**. For Phase A, participants naturally
145 expectorated the unstimulated saliva into a 50 mL sterile collection tube until the volume reached 5 mL.
146 For Phase B, participants were instructed to chew a piece of paraffin wax and expectorated the
147 stimulated saliva into another collection tube in 5 minutes. Finally, after removing paraffin wax from
148 the oral cavity, the participant repeated the saliva collection procedure described in Phase A. The saliva
149 samples collected after stimulation were named as Phase C. Time was recorded in Phases A and C to
150 calculate the flow rate as volume collected per minute (mL/min). For the same participant, Phase A, B,
151 and C collections must be completed consecutively on the same day. Immediately after the collection,
152 saliva samples were separated in smaller aliquots and kept frozen at -80 ºC until analysis.

153 **2.4 Biochemical analyses**

154 Salivary total protein content, and enzymatic activities of 90 samples were tested individually to find
155 the differences among different types of saliva. All analyses were performed in triplicates. Protein
156 concentration of whole saliva samples was tested by Micro BCA™ Protein Assay Kit (Thermo
157 Scientific™) using bovine serum albumin as standard for calibration.

158 For the enzymatic activity measurements, saliva samples were centrifuged at 15,000 ×g for 15 min at
159 4 °C to remove insoluble matter. All enzymatic activity assays were performed in triplicate. Total
160 salivary esterase activity was conducted on individual samples accordng to the method from Pérez-
161 Jiménez, Muñoz-González and Pozo-Bayón [26]. α-Amylase activity assay was performed following the
162 user manual of an Amylase Activity Assay Kit (Sigma-Aldrich), we pooled the samples for this assay
163 because of the limited material for some participants.

164 **2.5 Full length 16S rRNA gene sequencing**

165 Bacterial genomic DNA was extracted from 90 whole human saliva samples using the QIAGEN®
166 DNeasy® PowerSoil® Kit. The concentration of genomic DNA was measured using a Qubit HS DNA
167 kit (Thermo Scientific™) and normalised to 1 ng/μL prior to Polymerase Chain Reaction (PCR).
168 Following PacBio's recommended PCR protocol, full-length 16S amplicons were generated using the
169 Platinum™ SuperFi II High-fidelity PCR enzyme (Thermo Scientific™), with 2ng of input DNA. The
170 amplicons generated were visualised on a 2% agarose gel and pooled. SMRTbell™ cleanup
171 beads (PacBio) were used to purify the pooled amplicons according to the PacBio protocol. The
172 amplicon library was contracted with the SMRTbell® prep kit 3.0 library prep kit (PacBio) following
173 the protocol. The library was then polymerase-bounded using the Revio™ Polymerase Kit (PacBio) and
174 loaded on a single-molecule real-time (SMRT) Cell 25M (PacBio). 16S rRNA full-length sequencing
175 was performed for 30 hours on the PacBio Revio instrument.

176 **2.6 Gas chromatography-mass spectrometry (GC-MS) analysis for whole human saliva**

177 Different types of saliva samples were pooled together for the headspace solid-phase microextraction
178 GC-MS analyses (HS-SPEM-GC-MS) to overcome the inter-individual variation in saliva composition
179 and the availability of saliva from each participant. Saliva was pooled based on stimulation phase, by
180 participant origin, resulting in 6 groups of saliva samples: Australia-Phase A, Australia-Phase B,
181 Australia-Phase C, Chinese-Phase A, Chinese-Phase B and Chinese-Phase C. Each sample type was
182 measured in triplicate.

183 Three types of controls were used: the mixture of wine and water, the mixture of wine and artificial
184 saliva, and the mixture of six pooled saliva samples and model wine. The mixture of wine and water
185 were used to control for the dilution effect of saliva in the GC analysis matrix [4, 40]. An artificial saliva
186 solution was spiked with wine to control for the effect of mucin and salts in aroma release. It was
187 prepared according to the method of Genovese, Piombino, Gambuti and Moio [1]. The artificial saliva
188 samples were freshly prepared by dissolving mucin (2.16 g/L), $NaHCO_3$ (5.208g/L), $K_2HPO_4 \cdot 3H_2O$
189 (1.369 g/L), NaCl (0.877 g/L), KCl (0.477 g/L) and $CaCl_2 \cdot 2H_2O$ (0.441 g/L) in Milli-Q water. The
190 mixture of the model wine and six types of saliva were used to control for the confounder effect of the
191 volatiles that may present in saliva itself. The model wine used in this study was prepared by saturating
192 12% ethanol (v/v) with potassium hydrogen tartrate, adjusting to pH 3.0 by 40% (w/v) tartaric acid.
193 Each mixture was measured in triplicate.

194 The static headspace volatile extraction method was conducted following the method of Muñoz-
195 González, Feron, Guichard, Rodríguez-Bencomo, Martín-Álvarez, Moreno-Arribas and Pozo-Bayón [41]
196 with some modifications. The 20 mL GC vial was incubated at 37°C for 20 min to stabilise the
197 temperature of the reaction environment. Then 5 mL of wine and 1 mL of pooled saliva were added to
198 the vial with 10 µL of internal standard (100 mg/L 4-octanol). The wine-saliva mixture was incubated
199 at 37 °C for 12 min. The headspace volatile extraction from the GC vial was performed by a 65 µL
200 polydimethylsiloxane/divinylbenzene (PDMS/DVB) SPME fibre with 1 cm fibre length (Supelco,
201 Bellefonte, PA) at 37 °C for 5 min.

202 GC-MS analysis was carried out with an Agilent 7890A GC system (Agilent Technologies, Mulgrave,
203 VIC, Australia) coupled with an Agilent 5975C MS (Agilent Technologies). The fibre was desorbed at
204 220 °C in splitless mode at 8.7 psi for 30 s. Chromatographic separation of volatile compounds were
205 performed with a DB-Wax polar capillary column (Agilent Technologies; 30 m × 0.25 mm × 0.25 µm
206 film thickness). Helium was used as the carrier gas at a flow rate of 0.7 mL/min. After initially holding
207 the oven temperature at 40°C for 4 mins, it then ramped at 3°C/min to 220 °C, and held at 220 °C for
208 10 min. For the MS system, electron ionisation mode at 70 eV was used to record the electron impact

209 mass spectra. The scanning mass range was from m/z 35 to 350. The temperature of the MS interface,
210 MS source, MS quadrupole was 250, 230 and 150 °C.

211 The volatile compounds identification was based on the retention indices (RIs) with the facility of NIST
212 library (version 11.0). RIs were obtained by calculating the retention times of alkane standards (C_5–C_{30})
213 on the same column. Peaks were semi-quantified using the internal standard, 4-octanol. The
214 concentrations of volatiles were expressed as μg/L 4-octanol equivalence. Among the 27 compounds
215 identified, sixteen compounds have also been quantified using external standards to validate the results
216 of the semi-quantified method, facilitated by the calibration curve of standard compounds as:

217 $$Concentration\ (g/mL) = \left(\frac{peak\ area\ of\ identified\ compound}{peak\ area\ of\ internal\ standard} \times slope\right) + intercept$$

218 **2.7 Statistical analyses**

219 The data analyses of salivary biochemical parameters and the volatile compounds generated from the
220 wine-saliva mixture were conducted in R (version 4.1.0). Student t-tests were used to examinate the
221 significant ($p < 0.05$) differences between samples from Chinese and Australian participants. Two-way
222 analysis of variance (ANOVA) with Tukey's comparison were performed to test the significant ($p <$
223 0.05) differences among saliva samples collected before, during and after stimulation. The Spearman's
224 correlations were calculated with *rcorr* function in the R package *Hmisc* [42].

225 For bioinformatic analyses, the demultiplexed PacBio HiFi full-length 16S rRNA gene sequences are
226 quality filtered using QIIME2 [43]. DADA2 [44] was used to denoise the sequences into amplicon single
227 variants (ASVs) using the function "dada2 denoise-ccs". A phylogenetic tree was generated from the
228 *mafft* alignment. The naïve Bayesian machine learning based classification was performed using the
229 expanded Human Oral Microbiome Database (eHOMD) version 15.23 [45]. If a species-level match was
230 not found, it was further classified based on three other databases that successively refer to the next one.
231 The three databases are GTDB r207, Silva v138 [46] and the NCBI RefSeq 16S rRNA database
232 supplemented by the Ribosomal Database Project (RDP). The resulting objects were imported to R
233 (version 4.1.0) for further analyses. Alpha diversity was calculated with Simpson's diversity index.

234 Wilcoxon rank-sum tests were used to determine the statistically significant ($p < 0.05$) differences. Beta
235 diversity was assessed using Bray-Curtis dissimilarly. Permutational multivariate analysis of variance
236 (PERMANOVA) with 999 permutations was conducted to examine the statistically significant ($p <$
237 0.05) difference in beta-diversity using the *adonis2* function of *vegan* package [47]. The differential taxa
238 were defined from phylum level to species level by *metacoder* package [48] with false discovery rate-
239 adjusted Wilcoxon rank-sum test. ANCOM-BC2 [49] was performed to identify the differential species
240 with the default parameters.

241

242 **3 Results**

243 **3.1 Simulated saliva (Phase B) has a distinguishable protein and biochemical composition**

244 *3.1.1 Differences in saliva composition amongst the three phases*

245 To determine whether human saliva produced before, during and after stimulation is different, the
246 biochemical properties and microbial composition of human saliva samples were tested. The salivary
247 flow rates were calculated as volume collected per minute, reflecting the speed of participants to
248 produce saliva. *Ex vivo* measurements of α-amylase and esterase were conducted to assess enzymatic
249 activity of the centrifuged saliva. The flow rate observed in Phase B and total salivary esterase activity
250 were significantly higher than the other two phases, while Phase A and C saliva were not significantly
251 different **(Figure 1A, E)**. For the total protein concentration of saliva samples, the value of Phase A
252 was significantly higher than that of the other two phases **(Figure 1C)**. No significant differences were
253 found among the three groups in the amylase activity **(Table S2)**.

254 Full-length 16S rRNA gene sequencing was used to detect the differences in the salivary microbiome.
255 In contrast to the protein concentration and enzymatic activities, no significant differences were
256 detected among Phases A, B and C regarding the alpha-diversity and beta-diversity of the microbial
257 communities ($p > 0.05$). Although several species have been identified as differential taxa **(Figure**
258 **S2)**. Most differential species had the lowest relative abundance in Phase B **(Figure S2A)**. The

259 differential species identified in Phase A and C were clustered together in the heatmap **(Figure S2B)**,
260 suggesting the microbial profile is distinct in Phase B at species level.

261 As the volatiles may also have originated from the human oral cavity, the volatiles in the saliva samples
262 were assessed by mixing each pooled saliva sample with model wine. Among the detected volatiles, (–)
263 – clovene was found in the mixture of saliva and wine and the mixture of saliva and model wine.
264 Therefore, this compound was excluded from further analysis.

265 *3.1.2 Effect of stimulation on the release of aroma from wine*

266 To evaluate the ability of different saliva samples to release aroma compounds from wine, HS-SPME-
267 GC-MS analyses were performed on the same wine sample spiked with saliva samples. Saliva samples
268 were pooled into six groups: Australia-Phase A, Australia-Phase B, Australia-Phase C, Chinese-Phase
269 A, Chinese-Phase B and Chinese-Phase C. After mixing the six pooled saliva samples with wine,
270 twenty-seven volatile compounds were identified, consisting of 12 esters, 6 alcohols, 3 acids, 2
271 benzenoids and 4 terpenes **(Table 1)**. The wine sample was also mixed with water and artificial saliva
272 as the control groups **(Figure S3A)**. For many compounds, especially esters, the addition of artificial
273 saliva induced a significant decrease in their concentration compared to the wine-water mixture.
274 Depending on the type of pooled saliva added, the decrease caused by artificial saliva could either be
275 counteracted or further decreased **(Figure S3B)**. It suggests the whole human saliva induces other
276 reactions with the wine matrix other than the effect of mucin.

277 For the 26 included volatiles, twenty-one volatiles were constantly detected across all six groups, while
278 the other 5 volatiles were only occasionally found **(Table 1)**. Methyl hexanoate is the only ester that
279 was not constantly detected in all groups. The other occasionally detected compounds are all benzenoids
280 and terpenes. These compounds were mostly absent in Phases A and C samples except limonene, which
281 was not detected in the Australian-Phase B group. The one-way ANOVA conducted with the
282 concentration of 16 volatiles quantified using external standards is compatible with the results
283 calculated using semi-quantification method **(Table 1; Table S3)**.

284 The dataset was divided into two subgroups, Australian **(Figure 2A)** and Chinese **(Figure 2B)**, to show
285 the changing trend for 21 constant-detected compounds among Phases A, B and C. The statistical
286 analyses suggest that the Australian and Chinese groups individually have 9 and 8 compounds with
287 significantly higher concentrations in Phase B than in the other two phases ($p < 0.05$). Most of the
288 distinguishing compounds are esters, while octanoic acid and 1,3-ditertiary butyl benzene show the
289 same trend in either the Australian or both groups. In addition, several compounds demonstrated
290 significant differences between Phase A and C, including diethyl succinate, 3-methylbutyl acetate, 3-
291 ethyl-2-pentanol and acetic acid. 3-Ethyl-2-pentanol and acetic acid only showed a significant
292 difference among phases in the Australian group, with significantly lower concentration in Phase A (p
293 < 0.05). Further, a significant difference was observed between the Australian and Chinese groups of
294 these two compounds in Phase A **(Figure 2C)**.

295 To better understand the relationship between three types of saliva and the aroma released from wine,
296 a principal component analysis (PCA) was performed on the 21 detected compounds shared amongst
297 all samples **(Figure 2D)**. The internal standard equivalent concentration of the aroma compounds,
298 (expressed as μg/L 4-octanol equivalents), distinguished the saliva samples from different phases. The
299 first two principal components explained 68.57% of the variance. The first principal component (PC1)
300 separated the samples of Phase B from those of Phase A and C. The samples of Phase B were highly
301 associated with most esters, 1,3−ditertiarybutylbenzene and 3−ethyl−2−pentanol, positioned in the
302 space defined by negative PC1. On the contrary, 1-butanol was positively related to PC1. The second
303 principal component (PC2) was negatively associated with most alcohols, including 2-methyl-1-
304 propanol, 3-methyl-1-butanol, 1-hexanol and 2,3-butanediol. Phase A and C samples were separated on
305 PC2 with some overlap retained, suggesting the interaction between the two investigated covariates,
306 phase, and subject origin **(Table S2)**. In addition, PCAs were conducted using a total 27 compounds
307 and 16 compounds quantified using external standards, separately **(Figure S4)**. These results agree with
308 the PCA constructed by the semi-quantified compounds.

309 **3.2 The composition of saliva from Australian and Chinese participants is different**

310 *3.2.1 Differences in salivary proteins between two groups*

311 The flow rate, total protein concentration, and activities of alpha-amylase and esterase of the saliva
312 samples collected from Australian and Chinese participants were compared to investigate the influence
313 of the host's cultural background on salivary composition. Although no differences were found between
314 the flow rate of Australian and Chinese participants' saliva **(Figure 1B)**, the saliva of Australian
315 participants demonstrated higher total protein concentration **(Figure 1D)**. For the enzymatic activities,
316 the two groups demonstrated no statistical differences in either amylase or esterase activity **(Table S2;**
317 **Figure 1F)**.

318 *3.2.2 Differences in salivary microbiome profiles*

319 For the comparison of the salivary microbial profile of participants from Australia and China, the full-
320 length 16rRNA gene was sequenced. After denoising by DADA2, the samples were rarefied to 5,232,
321 resulting in 11,052 amplicon sequence variants (ASVs). The Australian and Chinese groups differ in
322 the alpha-diversity characterised by Simpson Index **(Figure 4A)**. Beta-diversity analysis conducted
323 using Bray-Curtis dissimilarity also demonstrated the significant differences between these two groups
324 (PERMANOVA, $p = 0.001$). The principal coordinate analysis (PCoA) result showed the separation
325 between the microbial composition of samples from two groups **(Figure 4B)**.

326 When investigating the taxa that discriminate saliva samples from Australian and Chinese participants,
327 we first compared the relative abundance of the two groups at phyla **(Figure 3A)** and genus
328 levels **(Figure 3B)**. Firmicutes, the phylum with the highest relative abundance in both groups, showed
329 higher relative abundance in the saliva samples from Australian participants. A higher relative
330 abundance of *Veillonella* in Australian saliva was observed. We performed a Wilcoxon Rank Sum test
331 with a false discovery rate correction to each taxon, spanning all taxonomic levels **(Figure**
332 **3C)**. Genus *Neisseria*, *Lautropia*, *Porphyromonas*, *Capnocytophaga*, *Cardiobacterium*, *Filifactor* and
333 *Parvimonas* were higher in Chinese participants' saliva, with several species of these genre. All taxa
334 belong to phyla Synergistetes and Spirochaetes, except the species were more prevalent in the Chinese

335 group. In contrast, the saliva microbiota of Australian participants was distinguished mainly by taxa
336 under the order *Veillonellales*, especially the genus *Veillonella* and *Megasphasera*. The genus with
337 high abundances, such as *Streptococcus*, *Prevotella* and *Haemonphilus*, contain both species identified
338 as biomarkers of Chinese and Australian participants.

339 We also used ANCOM-BC2 to identify the differential species between Australian and Chinese saliva
340 to accomplish more precise results at the species level. We found 8 differential species for comparing
341 two groups, mostly under the phylum *Bateroidetes* and *Firmicutes* **(Figure 4C)**. Among
342 them, *Porphyromonas pasteri* and *Neisseria subflava* have higher relative abundance in the Chinese
343 group, which agrees with the results of the Wilcoxon Rank Sum tests **(Figure 3C)**. Similarly, the six
344 differential species enriched in Australian samples identified by ANCOM-BC2 are also found in the
345 Wilcoxon Rank Sum tests. In the Australian group, the differential species of Phase A and C were
346 clustered together, suggesting they are closer to each other than Phase B. Meanwhile, the differential
347 species in the Chinese Phase A and B samples were clustered.

348 *3.2.3 Differences in saliva composition between groups are associated with aroma release*

349 To investigate the aroma consequences of compositional differences in the saliva of saliva samples
350 collected from Australian and Chinese participants, the volatile profile was collected by HS-SPME-
351 GC-MS and compared. The result of two-way ANOVA showed that 11 compounds were released
352 differently after mixing with saliva samples from two groups. Different concentrations due to the saliva
353 samples collected at different phases were observed **(Figure 4E)**. All differential compounds showed
354 higher concentration in the Chinese group, especially in unstimulated Phases A and C. Meanwhile, ethyl
355 heptanoate released after mixing with Chinese saliva samples demonstrated higher concentration in
356 Phases A and B.

357 We suspected that the salivary bacteria contributed to aroma release, and so we calculated the
358 Spearman's rank correlation between the relative abundance of the species in pooled saliva samples and
359 the aroma compounds released after mixing the wine samples with those saliva samples. We found
360 three species significantly correlated to wine aroma profile, including *Streptococcus sp.*

361 *HMT006*, *Prevotella shahii* and an unclassified species belongs to *Selenomonas* **(Figure 4D)**. The
362 concentration of three differential esters, ethyl propanoate, 3-methylbutyl acetate and ethyl hexanoate,
363 are positively associated with the relative abundance of *Streptococcus sp. HMT006*, while negatively
364 associated with the relative abundance of the unclassified species of *Selenomonas* **(Figure 4F)**. The
365 higher concentration of 3-ethyl-2-pentanol and several esters in the Chinese group has been positively
366 correlated with the increased relative abundance of *Streptococcus sp. HMT006* in saliva samples from
367 Chinese participants in Phase A. As the only benzenoid identified, the concentration of 1,3-
368 ditertiarybutylbenzene showed a negative correlation with the unclassified species
369 of *Selenomonas.* Although two-way ANOVA concluded that the concentration of 2,3-butanediol was
370 not different between the Chinese and Australian groups, it was positively associated with the higher
371 relative abundance of *Prevotella shahii* in the Chinese saliva

372 **4 Discussion**

373 In the current study, we observed the differences in salivary composition between saliva samples collect
374 at different stimulation stages and from participants with different geographic origin. This study further
375 demonstrates the association between host factors, salivary composition, and aroma release from wine.
376 For the two parameters controlled in this study, the phase of saliva production and the host's ethnic
377 background, we simultaneously observed the differences in salivary composition and the aroma profiles
378 of wine after mixing with saliva samples. The salivary stimulation stages and subject origin both
379 influence aroma release ability as the composition of saliva is different. These findings shed light on
380 the role of human saliva in immediate and prolonged retronasal perception of aroma and has introduced
381 unstimulated saliva as a control in *ex vivo* experiments.

382 Overall, saliva samples collected before, during and after stimulation were different in composition. We
383 observed a higher total protein concentration in the saliva samples produced in Phase A. The lower
384 protein concentration in Phase B and C may be due to the dilution of the substantial water content
385 secreted in Phase B [50]. Interestingly, even though the flow rate dropped significantly after removing the
386 stimulus from the oral cavity, the total protein concentration in saliva did not return to the level when
387 the stimulus was not received. Further analyses, such as proteomics should be applied to assess the

388 decreasing rate of individual proteins. By observing whether the decreasing rate of different proteins
389 are even, it is possible to infer if dilution is the main reason for the lower protein concentration in Phase
390 C. In contrast, a higher total salivary esterase activity was found in Phase B, while the esterase activity
391 of Phases A and C had no significant difference. The esterase in saliva is an important enzyme for
392 hydrolysing esters into carboxylic acids and alcohols [6]. The higher esterase activity in stimulated saliva
393 than in unstimulated saliva has also been observed by Pérez-Jiménez, Muñoz-González and Pozo-
394 Bayón [26]. Esterase did not follow a consistent trend with total protein concentration, suggesting the
395 mechanisms beyond dilution that may be responsible for modulating the concentration of different
396 proteins after stimulation. Meanwhile, no significant differences were observed in the current study
397 between the microbial diversities of saliva samples collected at different stimulation stages. The
398 association between oral microbiota and the type of saliva has been widely investigated by previous
399 studies but remains controversial. A previous study suggested a significant difference between
400 unstimulated and stimulated saliva in both alpha and beta diversity [22]. On the contrary, another two
401 studies [23, 24] reported no significant differences between the Shannon diversity index of bacteria in
402 unstimulated and stimulated saliva samples. Several species have been identified as differential among
403 the mouth-rinse water, unstimulated saliva, and stimulated saliva by Jo, Nishimoto, Umezawa, Yama,
404 Aita, Ichiba, Murakami, Kakizawa, Kumagai, Yamada and Fukuda [24], which agrees with our results.
405 However, no intersection has been found between the differential species defined by the previous work
406 and our study. The reason could be the different statistical tools used. It is possible that the differences
407 could be too subtle to detect. Further study with higher resolution, such as metagenomic sequencing,
408 need to be applied to monitor the sub-species level or community-level differences.

409 The relationship between the stimulation stages and aroma release ability was found to be focused on
410 the esters in wine and the esterase activity in human saliva. Esters are an essential group of aromatic
411 compounds to contribute fruitiness to wine [55]. Most esters detected in this study have the odour activity
412 values greater than 1 **(Table S3)**, meaning that these compounds are likely to have a sensory impact.
413 Saliva samples produced in Phase B demonstrated the highest esterase activity and induced the highest
414 concentration of esters released from wine samples. However, the presence of esterase was previously

415 associated with the hydrolysis of esters [3] and the generation of carboxylic acids [11]. Esters are relatively
416 less persistent in the oral cavity after swallowing, probably due to the modification by oral microbiota
417 directly or with enzymatic action [56]. This rather contradictory result may be explained by the presence
418 of reactions other than enzymatic hydrolysis of the esters. In addition, the total salivary esterase activity
419 could be attributed to different types of enzymes, such as carboxylesterases, carbonic anhydrases,
420 trypsin, and cholesterol esterases [26]. The catalytic effect of specific enzymes could be accompanied by
421 concurrent synthesis of esters. Interestingly, the same phenomenon was reported in our recent study [36],
422 which strengthens our assumption that perhaps more than one type of reaction involving esterases
423 occurs when human saliva mixes with esters in wine.

424 Notably, several volatiles showed differences between Phase A and Phase C in the Australia group,
425 including 3-methylbutyl acetate, 3-ethyl-2-pentanol and acetic acid. 3-Methylbutyl acetate is an ester
426 with fruity, banana-like aroma (**Figure S3**). In contrast, 3-ethyl-2-pentanol and acetic acid may provide
427 an unpleasant flavour to wine [57]. Those volatiles may play a role in the after-stimulation stage. During
428 retronasal perception of wine, two distinct types of aroma release are perceived by consumers: the
429 immediate impression produced when swallowing and the prolonged perception after swallowing [58].
430 By comparing the saliva samples collected during and after stimulation with the control, our study
431 demonstrates that it is important to specify the type of saliva collected and used in *ex vivo* experiments.
432 Stimulated saliva needs to be specified for the research targeting the reactions with stimulus. These
433 findings could also shed light on understanding the differences between immediate and prolonged
434 perception of wine aroma. Meanwhile, although we demonstrated the different aroma release capacities
435 of saliva samples collected during and after the mechanical stimulation, more complex mechanisms are
436 introduced in aroma perception occurs during the wine tasting experience of human beings. Mixing the
437 saliva samples collected after the mechanical stimulation with wine could not completely reflect the
438 complexity and dynamics of "after-odour" occurring in human oral cavity. We set the stopping point
439 for the collection of Phase C as the time that volume reaches 5 mL to allow enough time for saliva to
440 change from "stimulated" to "unstimulated". However, after swallowing the wine, various amounts of
441 odorants will be captured by oral mucosa and keep working as "stimulus" in mouth [28]. Therefore, the

442 changes of saliva from “stimulated” to “unstimulated” could not be as abruptly as what we mimicked
443 using paraffin chewing. The proportion of wine and saliva used in our static headspace SPME method
444 does not reflect all the consequences of oral processing to explain aroma production in the ‘after-taste’
445 period. Further analytic approaches to simulate the relationships between post-stimulated saliva and
446 wine, include modelling the oral mucosa [60], and should be used to further understand the role of saliva
447 in the after-taste phase.

448 Moreover, the chemical composition of wine also influences the aroma release of wine aroma volatiles.
449 For example, the phenolic compounds impact the aroma release depending on the chemical structure [61,
450 62]. Specifically, phenolic acids facilitate the intra-oral release of β-phenyl ethanol, linalool and β-ionone,
451 while flavonoids in wine decrease the release of some esters [61]. Meanwhile, ethanol in wine may also
452 affect the salivary proteins [63]. Future research is required to explore the influences of host factors on the
453 aroma release ability of saliva on other food matrices, such as non-alcoholic beverages and solid foods.

454 The influence of the host’s origin on salivary composition, especially the oral microbiome, has also
455 been observed in the current study. To control the confounder effect of other host factors, we applied a
456 series of selection criteria to ensure the two groups of participants have balanced gender and BMI.
457 Although significant difference was identified in age of two groups, all participants in this study were
458 aged between 18 to 28. The differences induced by host origin have been attributed to genetic or external
459 factors, such as diet [51] and altitude [52]. Differences in cultural background and lifestyle habits between
460 Asian countries and Western countries have led to interest in understanding the differences in salivary
461 composition between different countries [53]. Our previous study collected salivary microbiome data from
462 47 publicly available datasets, reporting the differences in beta diversity of saliva samples from three
463 geographic locations, including North America, Europe, and China [16]. Notably, the variance explained
464 in the previous study could be confounded by external factors other than the differences among three
465 populations, such as climate and collection methods. To better understand the differences between two
466 participant groups, the current study effectively eliminated the variance caused by geographic location
467 by collecting all the saliva samples from the same location within a short period. *Veillonella* spp.,
468 including *Veillonella atypica* and *Veillonella dispar*, were identified as the differential taxa that were

469 higher in Australian samples, which was consistent with the result of our previous study. Among
470 them, *Veillonella atypica* has also been identified as a biomarker to differentiate people with obesity
471 from normal-weight individuals [54], underlying a potential association among diet, body weight and
472 ethnicity.

473 Although the differences between the Chinese and Australia group have been observed in our study,
474 association between ethnicity and salivary protein composition is far from conclusive. A previous study
475 [29] compared protein concentration, flow rate, amylolytic activity, and lipolytic activity of unstimulated
476 saliva samples from 15 Chinese and 15 Caucasian participants living in the Netherlands. Like the
477 current study, they focused on the difference in saliva samples between Chinese and residents of a
478 country defined as "Western countries". However, they observed higher total salivary protein
479 concentrations in Chinese than in Caucasian participants. This discrepancy between two studies could
480 be attributed to the differences between the Western countries selected. Australian and Dutch residents
481 could have specific differences in diets and lifestyles, leading to the different biochemical parameters
482 of saliva. Meanwhile, no significant differences in flow rate and amylolytic activity was detected by
483 Mosca et al. [29] , which is consistent with our findings. Further proteomic analyses on samples collected
484 in the current study would facilitate the observation of the differences on specific protein concentration;
485 we suspect that differences in published studies might also be due to the collection of stimulated or
486 unstimulated saliva (or merging of these two classes).

487 Our *ex vivo* results show that Australian and Chinese have different aroma compounds released in the
488 oral cavity. Our previous study reported that people from Australia and China have different sensory
489 responses to the same wine [36] and reported increased levels of esters in the mixture of Chinese saliva
490 and wine. According to the results of current study, the prevalence of *Streptococcus* sp. HMT 066 has
491 been associated with the concentration of esters released. It has been reported that *Streptococcus* spp.
492 have the ability to release aroma from odourless precursors [8]. 2,3-butanediol is the second most
493 abundant by-product generated during the alcoholic fermentation of wine, contributing to its bitterness
494 [64]. The higher 2,3- butanediol concentration found in our study using wine and saliva from the Chinese
495 group positively correlates with the relative abundance of *Prevotella shahii* **(Figure 4F)**. *Prevotella* spp.

496 has been described as a potential contributor to aroma perception by enzymatic activity [13]. Nevertheless,
497 within genera like *Streptococcus* and *Prevotella*, the function of different species varies. Functional
498 annotation and taxonomic profiling with higher resolution at special or even stain level could be done
499 to differentiate the bacteria belongs to these two genera. Further experiments, such as strain isolation,
500 are required to verify the function of specific species. *Veillonella* spp. have been found different in
501 relative abundance between hosts from China and Western countries in both a previous study [16] and the
502 current study. Although no correlation was found between *Veillonella* spp. and any aroma compounds
503 in this study, more research on *Veillonella* could help understand the differences in aroma perception
504 between Chinese and Western people.

505 In summary, this study demonstrated that saliva secreted before, during and after stimulation differs in
506 composition and the ability of the three types of saliva to release aroma compounds from wine varies.
507 Although Phase A and C have more in common than Phase B, there are still some differences in their
508 composition and the role in aroma release. The enzymatic activity of esterase may explain the variation
509 in the ability of human saliva to release aroma compounds attributed to stimulation. Another important
510 finding of this study was the difference in salivary composition, especially salivary microbiota, between
511 Australian and Chinese participants. Several differential species were identified by full-length 16S
512 rRNA sequencing. We took samples from 30 people in this study, which is a relatively small number,
513 yet our findings mirror data collected in other studies. For example, our findings of a differential relative
514 abundance of *Veillonella* spp. contributes additional evidence to support this genus as a biomarker to
515 differentiate people originating in different geographic areas. The volatiles released from the wine-
516 saliva mixture of these two groups highlight the associations between salivary microbiota and aroma
517 release. Our findings serve as a basis for future studies targeting the relationship between salivary
518 composition, aroma perception, and wine preference. A better understanding of the variations in wine
519 preference gives insights into designing products for specific consumer groups and should be
520 complemented in the future by sensory tests to verify the findings in the human oral cavity.

521 **Figure Legends**

522 **Figure 1. The differences of salivary composition amongst three phases of collection and two**
523 **participant groups.** Boxplots showing the flow rate of (**A**) Phase A, B, C and (**B**) Australian and
524 Chinese participants; the total protein concentration of (**C**) Phase A, B, C and (**D**) Australian and
525 Chinese participants; the total salivary esterase activity of (**E**) Phase A, B, C and (**F**) Australian and
526 Chinese participants. Significant differences ($p < 0.05$, Student's t-test; $p < 0.05$, one-way ANOVA)
527 were tested and are marked with "*"; with NS denoting no significant differences observed.

528

529 **Figure 2. The aroma released from wine with saliva samples from three collection phases are**
530 **different.** Point plots with lines showing the change of each volatile released from (**A**) wine-Australia
531 saliva mixture and (**B**) wine-Chinese saliva mixture. The green lines represent the compounds showing
532 highest concentration in Phase B and have no significant differences between Phase A and C (one-way
533 ANOVA, $p < 0.05$). Among them, light green lines represent compounds only significantly higher than
534 Phase A, while dark green lines represent compounds significantly higher than both Phase A and C.
535 The pink lines represent the compounds that also demonstrate significant differences between Phase A
536 and C. The dark pink lines showing the compounds have significant differences among all three
537 comparisons. The light pink lines showing the compounds have differential concentration between
538 Phase A and B, and Phase A and C. The volatiles with significantly changes are indicate by letters: a)
539 Ethyl acetate; b) Diethyl succinate; c) Ethyl hexanoate; d) 3-Methylbutyl acetate; e) Ethyl butanoate; f)
540 Ethyl 3-methylbutanoate; g) Ethyl propanoate; h) Ethyl 2-methylbutanoate; i) Hexyl acetate; j) 3-Ethyl-
541 2-pentanol; k) Acetic acid; l) 1,3-Ditertiarybutylbenzene; m) Octanoic acid. (**C**) Bar plots showing the
542 concentration of 3-Ethyl-2-pentanol and Acetic acid in six subgroups. Significant differences ($p < 0.05$,
543 Student's t-test) between Australian and Chinese group in Phase A are marked with "*". (**D**) Principal
544 component analysis (PCA) plot demonstrating the wine aroma release after spiking with samples from
545 three groups.

546

547 **Figure 3. The microbial profiles of Australian and Chinese participants' saliva samples are**
548 **different.** Comparison of the salivary microbial composition between two groups in stacked bar plots
549 at (**A**) phylum level and (**B**) genus level. (**C**) Taxonomic hierarchies showing the enrichment of taxa
550 from two groups from phylum to species level. The taxa with significant differences (false discovery
551 rate-adjusted Wilcoxon rank-sum $q < 0.05$) were coloured with green (higher in Australian group) and
552 pink (higher in Chinese group).

553

554 **Figure 4. The differences in salivary microbial composition between Australian and Chinese**
555 **participants associate with the differences in aroma released after mixing with wine.** (**A**)
556 Comparison of the alpha-diversity between two groups, representing by Simpson's Diversity Index
557 (Wilcoxon rank-sum test; $p = 0.029$). (**B**) Principal coordinate analysis (PCoA) with Bray-Curtis
558 dissimilarity showing the difference between two groups (PERMANOVA; $p = 0.001$). (**C**) and (**D**)
559 Heatmap represents the mean relative abundance of (**C**) eight differential species identified by
560 ANCOM-BC2 and (**D**) three species that significantly correlated ($p < 0.01$, FDR-adjusted Spearman's
561 rank correlation) with aroma compounds in six pooled saliva groups. (**E**) Bar plots showing the log-
562 transformed concentration (μg/L 4-octanol equivalence) of aroma compounds detected in two groups.
563 The coloured squares on the bottom indicate the result of two-way ANOVA. (**F**) Spearman's correlation
564 heatmap between aroma compounds and relative abundance of species. Significant correlated pairs
565 ($p < 0.01$, FDR-adjusted Spearman's rank correlation) are labelled with "*".

566

Funding

This study was funded by the Faculty of Science at the University of Melbourne. XR and YC are supported by a Melbourne Research Scholarship administered by the University of Melbourne.

Acknowledgements

The authors would like to acknowledge all participants. The authors would also like to acknowledge the assistance of Zijian Liang to the sample collection process. The work of Dr Pangzhen Zhang to set up methodologies and verify volatile analysis is gratefully acknowledged.

Ethics approval and consent to participate

The study is under the approval of the University of Melbourne Human Research Ethics Committees (Review Reference Number: 2022-24220-29889-3). All participants provided written informed consent.

Data availability

The sequencing data generated and analysed in the present study are available in NCBI BioProject database, PRJNA1077481(https://www.ncbi.nlm.nih.gov/bioproject/1077481).

Declaration of Competing Interest

The authors declare that they have no competing financial interest.

587 **Supporting Information**

588 **Supplementary Table 1** Background information of participants from Chinese and Australia

589 **Supplementary Table 2** The influence of two factors, phase and ethnicity on the amylase activity and
590 aroma compounds detected by HS-SPME-GC-MS from the mixture of six types of pooled saliva
591 samples and wine, evaluated by two-way ANOVA and Tukey's pairwise comparison.

592 **Supplementary Table 3** The concentration and odour activity values (OAV) of aroma compounds
593 detected by headspace solid-phase microextraction gas chromatography mass spectrometry (HS-SPME-
594 GC-MS) from the mixture of six types of pooled saliva samples and wine, quantified using external
595 standards.

596 **Supplementary Figure 1** Flow chart showing the sample collection procedure of three types of saliva
597 from each participant: before, during and after stimulation.

598 **Supplementary Figure 2 Thirteen species identified as differential among three phases by**
599 **ANCOM-BC. A)** Point plots with lines showing the log-transformed relative abundance of differential
600 species in three phases. The green lines indicate the species with the lowest relative abundance in Phase
601 A, and the pink lines indicate the species with the lowest relative abundance in Phase B; **B)** Heatmap
602 showing the log-transformed mean relative abundance of differential species.

603 **Supplementary Figure 3 The aroma released from wine after mixing with water, artificial saliva,**
604 **and pooled saliva samples. A)** The table showing the concentration of volatiles released from wine-
605 water and wine-artificial saliva mixture. Superscripts indicate significant differences ($p < 0.05$;
606 Student's t test) between the two groups. **B)** The coloured squares indicate the comparison among wine-
607 water, wine-artificial saliva, and wine-saliva mixtures. The green squares represent the higher
608 concentration of released aroma after mixing with the pooled saliva than mixing with water or artificial
609 saliva. The pink squares represent the lower concentration of released aroma after mixing with the
610 pooled saliva than mixing with water or artificial saliva. The grey squares represent the volatiles that
611 have not been detected. The significant differences ($p < 0.05$; one-way ANOVA) were indicated by "*".

612 **Supplementary Figure 4** Principal component analysis (PCA) plots of A) 27 semi-quantified
613 compounds and B) 17 compounds quantified using external standards showing the similar results with
614 the PCA plots of 21 semi-quantified compounds detected constantly in all subgroups.

615

References

(1) Genovese, A.; Piombino, P.; Gambuti, A.; Moio, L. Simulation of retronasal aroma of white and red wine in a model mouth system. Investigating the influence of saliva on volatile compound concentrations. *Food Chem.* **2009**, *114* (1), 100-107. DOI: 10.1016/j.foodchem.2008.09.022.

(2) Feron, G.; Ayed, C.; Qannari, E. M.; Courcoux, P.; Laboure, H.; Guichard, E. Understanding Aroma Release from Model Cheeses by a Statistical Multiblock Approach on Oral Processing. *PLoS One* **2014**, *9* (4), e93113. DOI: 10.1371/journal.pone.0093113.

(3) Ployon, S.; Morzel, M.; Canon, F. The role of saliva in aroma release and perception. *Food Chem.* **2017**, *226*, 212-220. DOI: 10.1016/j.foodchem.2017.01.055.

(4) Taylor, A. J.; Roozen, J. P. Volatile flavor release from foods during eating. *Crit. Rev. Food Sci. Nutr.* **1996**, *36* (8), 765-784. DOI: 10.1080/10408399609527749.

(5) Friel, E. N.; Taylor, A. J. Effect of Salivary Components on Volatile Partitioning from Solutions. *J. Agric. Food. Chem.* **2001**, *49* (8), 3898-3905. DOI: 10.1021/jf010371e.

(6) Buettner, A. Influence of Human Salivary Enzymes on Odorant Concentration Changes Occurring in Vivo. 1. Esters and Thiols. *J. Agric. Food. Chem.* **2002**, *50* (11), 3283-3289. DOI: 10.1021/jf011586r.

(7) Buettner, A. Influence of Human Saliva on Odorant Concentrations. 2. Aldehydes, Alcohols, 3-Alkyl-2-methoxypyrazines, Methoxyphenols, and 3-Hydroxy-4,5-dimethyl-2(5H)-furanone. *J. Agric. Food. Chem.* **2002**, *50* (24), 7105-7110. DOI: 10.1021/jf020714o.

(8) Muñoz-González, C.; Cueva, C.; Ángeles Pozo-Bayón, M.; Victoria Moreno-Arribas, M. Ability of human oral microbiota to produce wine odorant aglycones from odourless grape glycosidic aroma precursors. *Food Chem.* **2015**, *187*, 112-119. DOI: 10.1016/j.foodchem.2015.04.068.

(9) Guichard, E. Flavour retention and release from protein solutions. *Biotechnol. Adv.* **2006**, *24* (2), 226-229. DOI: 10.1016/j.biotechadv.2005.11.003.

(10) Pagès-Hélary, S.; Andriot, I.; Guichard, E.; Canon, F. Retention effect of human saliva on aroma release and respective contribution of salivary mucin and α-amylase. *Food Res. Int.* **2014**, *64*, 424-431. DOI: 10.1016/j.foodres.2014.07.013.

(11) Pérez-Jiménez, M.; Rocha-Alcubilla, N.; Pozo-Bayón, M. Á. Effect of saliva esterase activity on ester solutions and possible consequences for the in-mouth ester release during wine intake. *J. Texture Stud.* **2019**, *50* (1), 62-70. DOI: 10.1111/jtxs.12371.

(12) María, P.-J.; Carolina, M.-G.; María Ángeles, P.-B. Understanding human salivary esterase activity and its variation under wine consumption conditions. *RSC Advances* **2020**, *10* (41), 24352-24361, 10.1039/D0RA04624H. DOI: 10.1039/D0RA04624H.

(13) Parker, M.; Onetto, C.; Hixson, J.; Bilogrevic, E.; Schueth, L.; Pisaniello, L.; Borneman, A.; Herderich, M.; de Barros Lopes, M.; Francis, L. Factors Contributing to Interindividual Variation in Retronasal Odor Perception from Aroma Glycosides: The Role of Odorant Sensory Detection Threshold, Oral Microbiota, and Hydrolysis in Saliva. *J. Agric. Food. Chem.* **2020**, *68* (38), 10299-10309. DOI: 10.1021/acs.jafc.9b05450.

(14) Piombino, P.; Genovese, A.; Esposito, S.; Moio, L.; Cutolo, P. P.; Chambery, A.; Severino, V.; Moneta, E.; Smith, D. P.; Owens, S. M.; et al. Saliva from Obese Individuals Suppresses the Release of Aroma Compounds from Wine. *PLoS One* **2014**, *9* (1), e85611. DOI: 10.1371/journal.pone.0085611.

(15) Wade, W. G. The oral microbiome in health and disease. *Pharmacol. Res.* **2013**, *69* (1), 137-143. DOI: 10.1016/j.phrs.2012.11.006.

(16) Ruan, X.; Luo, J.; Zhang, P.; Howell, K. The salivary microbiome shows a high prevalence of core bacterial members yet variability across human populations. *NPJ Biofilms Microbi.* **2022**, *8* (1), 85. DOI: 10.1038/s41522-022-00343-7.

(17) Emmelin, N. Control of Salivary Glands. In *Oral Physiology*, Emmelin, N., Zotterman, Y. Eds.; Pergamon, 1972; pp 1-16.

(18) Humphrey, S. P.; Williamson, R. T. A review of saliva: Normal composition, flow, and function. *The Journal of Prosthetic Dentistry* **2001**, *85* (2), 162-169. DOI: https://doi.org/10.1067/mpr.2001.113778.

(19) Jasim, H.; Olausson, P.; Hedenberg-Magnusson, B.; Ernberg, M.; Ghafouri, B. The proteomic profile of whole and glandular saliva in healthy pain-free subjects. *Sci. Rep.* **2016**, *6* (1), 39073. DOI: 10.1038/srep39073.

(20) Proctor, G. B.; Carpenter, G. H. Regulation of salivary gland function by autonomic nerves. *Auton. Neurosci.* **2007**, *133* (1), 3-18. DOI: 10.1016/j.autneu.2006.10.006.
(21) Shannon, I. L.; Suddick, R. P.; Chauncey, H. H. Effect of atropine-induced flow rate depression on the composition of unstimulated human parotid fluid. *Arch. Oral Biol.* **1969**, *14* (7), 761-770. DOI: 10.1016/0003-9969(69)90167-8.
(22) Gomar-Vercher, S.; Simón-Soro, A.; Montiel-Company, J. M.; Almerich-Silla, J. M.; Mira, A. Stimulated and unstimulated saliva samples have significantly different bacterial profiles. *PLoS One* **2018**, *13* (6), e0198021. DOI: 10.1371/journal.pone.0198021.
(23) Belstrøm, D.; Holmstrup, P.; Bardow, A.; Kokaras, A.; Fiehn, N. E.; Paster, B. J. Comparative analysis of bacterial profiles in unstimulated and stimulated saliva samples. *J. Oral Microbiol.* **2016**, *8*, 30112. DOI: 10.3402/jom.v8.30112.
(24) Jo, R.; Nishimoto, Y.; Umezawa, K.; Yama, K.; Aita, Y.; Ichiba, Y.; Murakami, S.; Kakizawa, Y.; Kumagai, T.; Yamada, T.; et al. Comparison of oral microbiome profiles in stimulated and unstimulated saliva, tongue, and mouth-rinsed water. *Sci. Rep.* **2019**, *9* (1), 16124. DOI: 10.1038/s41598-019-52445-6.
(25) Neyraud, E.; Palicki, O.; Schwartz, C.; Nicklaus, S.; Feron, G. Variability of human saliva composition: Possible relationships with fat perception and liking. *Arch. Oral Biol.* **2012**, *57* (5), 556-566. DOI: 10.1016/j.archoralbio.2011.09.016.
(26) Pérez-Jiménez, M.; Muñoz-González, C.; Pozo-Bayón, M. Á. Specificity of Saliva Esterases by Wine Carboxylic Esters and Inhibition by Wine Phenolic Compounds Under Simulated Oral Conditions. *Front. Nutr.* **2021**, *8*, Original Research. DOI: 10.3389/fnut.2021.761830.
(27) Carvalho, F. M.; Moksunova, V.; Spence, C. Cup texture influences taste and tactile judgments in the evaluation of specialty coffee. *Food Qual.* **2020**, *81*, 103841. DOI: https://doi.org/10.1016/j.foodqual.2019.103841.
(28) Esteban-Fernández, A.; Rocha-Alcubilla, N.; Muñoz-González, C.; Moreno-Arribas, M. V.; Pozo-Bayón, M. Á. Intra-oral adsorption and release of aroma compounds following in-mouth wine exposure. *Food Chem.* **2016**, *205*, 280-288. DOI: 10.1016/j.foodchem.2016.03.030.
(29) Mosca, A. C.; Stieger, M.; Neyraud, E.; Brignot, H.; van de Wiel, A.; Chen, J. How are macronutrient intake, BMI, ethnicity, age, and gender related to the composition of unstimulated saliva? A case study. *J. Texture Stud.* **2019**, *50* (1), 53-61. DOI: 10.1111/jtxs.12362.
(30) Bhuptani, D.; Kumar, S.; Vats, M.; Sagav, R. Age and gender related changes of salivary total protein levels for forensic application. *J. Forensic Odontostomatol.* **2018**, *36* (1), 26-33.
(31) Burcham, Z. M.; Garneau, N. L.; Comstock, S. S.; Tucker, R. M.; Knight, R.; Metcalf, J. L.; Miranda, A.; Reinhart, B.; Meyers, D.; Woltkamp, D.; et al. Patterns of Oral Microbiota Diversity in Adults and Children: A Crowdsourced Population Study. *Sci. Rep.* **2020**, *10* (1), 2133. DOI: 10.1038/s41598-020-59016-0.
(32) Morita, E.; Narikiyo, M.; Yokoyama, A.; Yano, A.; Kamoi, K.; Yoshikawa, E.; Yamaguchi, T.; Igaki, H.; Tachimori, Y.; Kato, H.; et al. Predominant presence of Streptococcus anginosus in the saliva of alcoholics. *Oral Microbiol. Immunol.* **2005**, *20* (6), 362-365. DOI: 10.1111/j.1399-302X.2005.00242.x.
(33) Mason, M. R.; Nagaraja, H. N.; Camerlengo, T.; Joshi, V.; Kumar, P. S. Deep Sequencing Identifies Ethnicity-Specific Bacterial Signatures in the Oral Microbiome. *PLoS One* **2013**, *8* (10), e77287. DOI: 10.1371/journal.pone.0077287.
(34) Yang, Y.; Zheng, W.; Cai, Q.; Shrubsole, M. J.; Pei, Z.; Brucker, R.; Steinwandel, M.; Bordenstein, S. R.; Li, Z.; Blot, W. J. Racial differences in the oral microbiome: data from low-income populations of African ancestry and European ancestry. *mSystems* **2019**, *4* (6). DOI: 10.1128/msystems. 00639-19.
(35) Premaraj, T. S.; Vella, R.; Chung, J.; Lin, Q.; Hunter, P.; Underwood, K.; Premaraj, S.; Zhou, Y. Ethnic variation of oral microbiota in children. *Sci. Rep.* **2020**, *10* (1), 14788. DOI: 10.1038/s41598-020-71422-y.
(36) Luo, J.; Ruan, X.; Ang, C.-S.; Nolvachai, Y.; Marriott, P. J.; Zhang, P.; Howell, K. Variation of wine preference amongst consumers is influenced by the composition of salivary proteins. *NPJ Sci. Food* **2023**, *7* (1), 51. DOI: 10.1038/s41538-023-00222-1.
(37) Williamson, P. O.; Robichaud, J.; Francis, I. L. Comparison of Chinese and Australian consumers' liking responses for red wines. *Aust. J. Grape Wine Res.* **2012**, *18* (3), 256-267. DOI: 10.1111/j.1755-0238.2012.00201.x.

(38) Kim, S.; Petard, N.; Hong, J. What is lost in translation: A cross-cultural study to compare the concept of nuttiness and its perception in soymilk among Korean, Chinese, and Western groups. *Food Res. Int.* **2018**, *105*, 970-981. DOI: 10.1016/j.foodres.2017.12.002.
(39) Sáenz-Navajas, M.-P.; Avizcuri, J. M.; Echávarri, J. F.; Ferreira, V.; Fernández-Zurbano, P.; Valentin, D. Understanding quality judgements of red wines by experts: Effect of evaluation condition. *Food Qual.* **2016**, *48*, 216-227. DOI: 10.1016/j.foodqual.2015.10.001.
(40) Benjamin, O.; Silcock, P.; Beauchamp, J.; Buettner, A.; Everett, D. W. Tongue Pressure and Oral Conditions Affect Volatile Release from Liquid Systems in a Model Mouth. *J. Agric. Food. Chem.* **2012**, *60* (39), 9918-9927. DOI: 10.1021/jf3028232.
(41) Muñoz-González, C.; Feron, G.; Guichard, E.; Rodríguez-Bencomo, J. J.; Martín-Álvarez, P. J.; Moreno-Arribas, M. V.; Pozo-Bayón, M. Á. Understanding the Role of Saliva in Aroma Release from Wine by Using Static and Dynamic Headspace Conditions. *J. Agric. Food. Chem.* **2014**, *62* (33), 8274-8288. DOI: 10.1021/jf503503b.
(42) Harrell, F. E. J. *Hmisc: Harrell Miscellaneous*. 2024. https://CRAN.R-project.org/package=Hmisc (accessed 2024-06-05).
(43) Bolyen, E.; Rideout, J. R.; Dillon, M. R.; Bokulich, N. A.; Abnet, C. C.; Al-Ghalith, G. A.; Alexander, H.; Alm, E. J.; Arumugam, M.; Asnicar, F.; et al. Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. *Nat. Biotechnol.* **2019**, *37* (8), 852-857. DOI: 10.1038/s41587-019-0209-9.
(44) Callahan, B. J.; McMurdie, P. J.; Rosen, M. J.; Han, A. W.; Johnson, A. J. A.; Holmes, S. P. DADA2: High-resolution sample inference from Illumina amplicon data. *Nat. Methods* **2016**, *13* (7), 581-583. DOI: 10.1038/nmeth.3869.
(45) Escapa, I. F.; Chen, T.; Huang, Y.; Gajare, P.; Dewhirst, F. E.; Lemon, K. P. New Insights into Human Nostril Microbiome from the Expanded Human Oral Microbiome Database (eHOMD): a Resource for the Microbiome of the Human Aerodigestive Tract. *mSystems* **2018**, *3* (6), 10.1128/msystems.00187-00118. DOI: 10.1128/msystems.00187-18.
(46) Quast, C.; Pruesse, E.; Yilmaz, P.; Gerken, J.; Schweer, T.; Yarza, P.; Peplies, J.; Glöckner, F. O. The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. *Nucleic Acids Res.* **2013**, *41*, D590-596. DOI: 10.1093/nar/gks1219.
(47) Oksanen, J.; Blanchet, F. G.; Friendly, M.; Kindt, R.; Legendre, P.; McGlinn, D.; Minchin, P. R.; O'Hara, R. B.; Simpson, G. L.; Solymos, P.; et al. *vegan: Community Ecology Package. R package version 2.5-7.* 2020. https://CRAN.R-project.org/package=vegan (accessed 2024-06-05).
(48) Foster, Z. S. L.; Sharpton, T. J.; Grünwald, N. J. Metacoder: An R package for visualization and manipulation of community taxonomic diversity data. *PLoS Comp. Biol.* **2017**, *13* (2), e1005404. DOI: 10.1371/journal.pcbi.1005404.
(49) Lin, H.; Peddada, S. D. Multigroup analysis of compositions of microbiomes with covariate adjustments and repeated measures. *Nat. Methods* **2024**, *21* (1), 83-91. DOI: 10.1038/s41592-023-02092-7.
(50) Lee, V.; Linden, R. An olfactory-submandibular salivary reflex in humans. *Exp. Physiol.* **1992**, *77* (1), 221-224. DOI: 10.1113/expphysiol.1992.sp003578.
(51) Liu, K.; Chen, S.; Huang, J.; Ren, F.; Yang, T.; Long, D.; Li, H.; Huang, X. Oral Microbiota of Children Is Conserved across Han, Tibetan and Hui Groups and Is Correlated with Diet and Gut Microbiota. *Microorganisms* **2021**, *9* (5), 1030. DOI: 10.3390/microorganisms9051030.
(52) Hao, Y.; Tang, C.; Du, Q.; Zhou, X.; Peng, X.; Cheng, L. Comparative analysis of oral microbiome from Zang and Han populations living at different altitudes. *Arch. Oral Biol.* **2021**, *121*, 104986. DOI: 10.1016/j.archoralbio.2020.104986.
(53) Kim, T. S.; Kang, N. W.; Lee, S.-B.; Eickholz, P.; Pretzl, B.; Kim, C. Differences in subgingival microflora of Korean and German periodontal patients. *Arch. Oral Biol.* **2009**, *54* (3), 223-229. DOI: 10.1016/j.archoralbio.2008.10.005.
(54) López-Dávalos, P. C.; Requena, T.; Pozo-Bayón, M. Á.; Muñoz-González, C. Decreased retronasal olfaction and taste perception in obesity are related to saliva biochemical and microbiota composition. *Food Res. Int.* **2023**, *167*, 112660. DOI: 10.1016/j.foodres.2023.112660.
(55) Ferreira, V.; López, R.; Escudero, A.; Cacho, J. F. The aroma of Grenache red wine: hierarchy and nature of its main odorants. *J. Sci. Food Agric.* **1998**, *77* (2), 259-267. DOI: 10.1002/(SICI)1097-0010(199806)77:2<259::AID-JSFA36>3.0.CO;2-Q.

(56) Goodstein, E. S.; Bohlscheid, J. C.; Evans, M.; Ross, C. F. Perception of flavor finish in model white wine: A time-intensity study. *Food Qual.* **2014**, *36*, 50-60. DOI: 10.1016/j.foodqual.2014.02.012. Muñoz-González, C.; Pérez-Jiménez, M.; Pozo-Bayón, M. Á. Oral persistence of esters is affected by wine matrix composition. *Food Res. Int.* **2020**, *135*, 109286. DOI: 10.1016/j.foodres.2020.109286.

(57) Guo, C.; Deng, H.; Li, E. Removal of acetic acid in mulberry wine by co-inoculating Saccharomyces cerevisiae with indigenous non-Saccharomyces yeast. *Food Bioscience* **2024**, *58*, 103658. DOI: https://doi.org/10.1016/j.fbio.2024.103658.

(58) Buettner, A. Investigation of Potent Odorants and Afterodor Development in Two Chardonnay Wines Using the Buccal Odor Screening System (BOSS). *J. Agric. Food. Chem.* **2004**, *52* (8), 2339-2346. DOI: 10.1021/jf035322b.

(59) Mayr, C. M.; Parker, M.; Baldock, G. A.; Black, C. A.; Pardon, K. H.; Williamson, P. O.; Herderich, M. J.; Francis, I. L. Determination of the Importance of In-Mouth Release of Volatile Phenol Glycoconjugates to the Flavor of Smoke-Tainted Wines. *J. Agric. Food. Chem.* **2014**, *62* (11), 2327-2336. DOI: 10.1021/jf405327s.

(60) Ployon, S.; Brulé, M.; Andriot, I.; Morzel, M.; Canon, F. Understanding retention and metabolization of aroma compounds using an in vitro model of oral mucosa. *Food Chem.* **2020**, *318*, 126468. DOI: 10.1016/j.foodchem.2020.126468.

(61) Esteban-Fernández, A.; Muñoz-González, C.; Jiménez-Girón, A.; Pérez-Jiménez, M.; Pozo-Bayón, M. Á. Aroma release in the oral cavity after wine intake is influenced by wine matrix composition. *Food Chem.* **2018**, *243*, 125-133. DOI: https://doi.org/10.1016/j.foodchem.2017.09.101.

(62) Perez-Jiménez, M.; Esteban-Fernández, A.; Muñoz-González, C.; Pozo-Bayón, M. A. Interactions among Odorants, Phenolic Compounds, and Oral Components and Their Effects on Wine Aroma Volatility. *Molecules* **2020**, *25* (7), 1701.

(63) Waszkiewicz, N.; Szajda, S. D.; Jankowska, A.; Zwierz, P.; Czernikiewicz, A.; Szulc, A.; Zwierz, K. The Effect of Acute Ethanol Intoxication on Salivary Proteins of Innate and Adaptive Immunity. *Alcoholism: Clinical and Experimental Research* **2008**, *32* (4), 652-656. DOI: https://doi.org/10.1111/j.1530-0277.2007.00613.x.

(64) Romano, P. Metabolic characteristics of wine strains during spontaneous and inoculated fermentation. In *Food Technology and Biotechnology*, 1997; Vol. 35, pp 255-260.

Table 1 Aroma compounds detected by headspace solid-phase microextraction gas chromatography mass spectrometry (HS-SPME-GC-MS) from the mixture of six types of pooled saliva samples and wine.

No.	Aroma Compound	RI-NIST *	RI-Actual	Internal standard (4-octanol) equivalence (μg/L)					
				Phase A		Phase B		Phase C	
				Australian	Chinese	Australian	Chinese	Australian	Chinese
	Esters								
1	Ethyl acetate	888	887	4646.99 ± 691.59^{c}	7449.38 ± 35.97^{a}	8822.61 ± 158.22^{a}	8606 ± 759.79^{a}	5935.32 ± 842.17^{bc}	7368.83 ± 103.25^{ab}
2	Ethyl propanoate	960	958	60.23 ± 4.97^{bc}	74.47 ± 2.46^{ab}	89.82 ± 2.16^{a}	87.58 ± 10.23^{a}	56.58 ± 7.30^{c}	71.78 ± 2.21^{bc}
3	Ethyl isobutyrate	962	959	153.31 ± 37.03^{a}	171.05 ± 54.71^{a}	120.65 ± 38.81^{a}	128.48 ± 38.89^{a}	123.29 ± 34.79^{a}	70.75 ± 61.53^{a}
4	Ethyl butanoate	1039	1035	49.91 ± 43.35^{d}	121.16 ± 3.14^{b}	151.59 ± 5.06^{a}	149.92 ± 17.20^{a}	93.75 ± 11.98^{cd}	120.11 ± 4.30^{bc}
5	Ethyl 2-methylbutanoate	1055	1050	41.49 ± 6.72^{c}	79.85 ± 2.41^{b}	102.68 ± 4.39^{a}	102.34 ± 11.04^{a}	56.79 ± 8.78^{c}	76.28 ± 2.18^{b}
6	Ethyl 3-methylbutanoate	1072	1067	48.24 ± 9.48^{d}	107.18 ± 5.42^{b}	137.85 ± 2.7^{a}	134.99 ± 11.07^{a}	72.17 ± 13.35^{c}	101.04 ± 4.98^{b}
7	3-Methylbutyl acetate	1126	1122	545.84 ± 89.42^{d}	1084.34 ± 9.03^{bc}	1302.7 ± 21.06^{ab}	1347.1 ± 143.51^{a}	765.84 ± 108.53^{d}	1066.07 ± 59.63^{c}
8	Methyl hexanoate	1190	1184	n.d.	2.88 ± 0.57^{bc}	3.58 ± 0.37^{ab}	4.06 ± 0.39^{a}	0.96 ± 0.32^{d}	2.36 ± 0.25^{c}
9	Ethyl hexanoate	1235	1233	851.85 ± 223.93^{d}	1977.03 ± 53.02^{bc}	2391.76 ± 66.67^{ab}	2478.48 ± 252.05^{a}	1259.94 ± 253.43^{d}	1830.77 ± 80.92^{c}
10	Hexyl acetate	1235	1232	7.02 ± 6.64^{c}	15.17 ± 3.00^{abc}	20.62 ± 0.86^{a}	21.65 ± 1.20^{a}	10.06 ± 2.16^{bc}	16.05 ± 1.80^{ab}
11	Ethyl heptanoate	1334	1332	0.09 ± 0.01^{b}	4.29 ± 3.68^{ab}	2.7 ± 4.18^{ab}	8.01 ± 2.27^{a}	0.2 ± 0.11^{b}	0.21 ± 0.16^{b}
12	Diethyl succinate	1681	1681	2752.88 ± 136.55^{a}	2256.51 ± 215.43^{b}	2471.91 ± 151.36^{ab}	2314.02 ± 68.01^{ab}	2557.77 ± 315.12^{ab}	2695.89 ± 66.46^{ab}
	Alcohols								
13	2-Methyl-1-propanol	1098	1096	493.94 ± 24.95^{a}	468.86 ± 20.24^{a}	521.16 ± 20.25^{a}	492.19 ± 50.54^{a}	491.19 ± 35.89^{a}	515.29 ± 17.78^{a}

14	1-Butanol	1144	1141	12.36 ± 0.42[a]	7.74 ± 6.67[a]	8.97 ± 7.73[a]	12.93 ± 1.23[a]	12.3 ± 0.37[a]	12.59 ± 0.86[a]
15	3-Methyl-1-butanol	1204	1205	10562.24 ± 491.79[a]	9444.18 ± 259.00[a]	10666.84 ± 311.22[a]	10051.84 ± 1014.17[a]	10159.14 ± 712.50[a]	10557.02 ± 315.42[a]
16	1-Hexanol	1348	1346	319.34 ± 15.53[a]	305.42 ± 12.75[a]	346.57 ± 13.21[a]	331.82 ± 31.04[a]	315.04 ± 19.65[a]	335.08 ± 13.11[a]
17	3-Ethyl-2-pentanol	N/A	1531	19.18 ± 1.58[b]	32.62 ± 2.77[a]	40.21 ± 6.14[a]	39.85 ± 5.86[a]	29.76 ± 4.30[ab]	36.01 ± 4.12[a]
18	2,3-Butanediol	1542	1540	43.59 ± 1.18[a]	43.61 ± 13.28[a]	37.71 ± 5.61[a]	47.51 ± 6.63[a]	41.67 ± 6.48[a]	51.4 ± 14.36[a]
	Acids								
19	Acetic acid	1461	1454	94.23 ± 21.35[c]	165.63 ± 34.94[b]	221.29 ± 12.79[ab]	205.82 ± 25.96[ab]	228.81 ± 13.09[ab]	230.26 ± 23.85[a]
20	Pentanoic acid	1744	1732	8.81 ± 0.10[a]	7.26 ± 0.85[a]	8.37 ± 0.78[a]	8.39 ± 0.49[a]	7.94 ± 0.63[a]	8.4 ± 1.55[a]
21	Octanoic acid	2087	2079	3.91 ± 0.82[a]	5.9 ± 2.16[a]	7.74 ± 0.66[a]	8.52 ± 2.42[a]	4.63 ± 1.53[a]	7.33 ± 3.15[a]
	Benzenoid								
22	Styrene	1256	1254	n.d.	2.56 ± 0.23[b]	4.47 ± 0.42[a]	4.79 ± 0.77[a]	n.d.	1.19 ± 1.03[b]
23	1,3-Ditertiarybutylbenzene	N/A	1424	17.57 ± 1.55[bc]	32.37 ± 3.73[b]	56.56 ± 4.08[a]	69.43 ± 4.61[a]	14.76 ± 9.41[c]	29.05 ± 7.84[bc]
	Terpenes								
24	D-Limonene	1199	1196	4.5 ± 3.55[a]	n.d.	n.d.	0.92 ± 0.70[a]	n.d.	n.d.
25	p-&m-Cymene	1269	1272	n.d.	n.d.	5.16 ± 0.65[a]	6.96 ± 1.21[a]	n.d.	n.d.
26	(-)-Clovene	N/A	1304	190.44 ± 38.62[a]	40.73 ± 9.73[b]	45.69 ± 19.52[b]	65.47 ± 12.48[b]	16.35 ± 0.60[b]	27.51 ± 3.92[b]
27	1,1,6-Trimethyl-1,2-dihydronaphthalene (TDN)	1712	1704	22.88 ± 5.95[a]	15.8 ± 1.76[ab]	15.54 ± 1.19[ab]	22.88 ± 5.95[a]	n.d.	9.23 ± 1.57[b]

Superscripts letters for the same compound indicate significant differences ($p < 0.05$; Tukey test) on the aroma release from wine after spiking with different saliva samples.

RI = Retention index.

*RI-NIST was obtained from NIST Webbook (2018).