Application of untargeted volatile profiling to investigate the fate of aroma compounds during wine oral processing

María Pérez-Jiménez [a], Emma Sherman [b], María Ángeles Pozo-Bayón [a], Carolina Muñoz-González [a], Farhana R. Pinu [b,*]

[a] *Institute of Food Science Research (CIAL), CSIC-UAM, C/Nicolás Cabrera 9, 28049 Madrid, Spain*
[b] *The New Zealand Institute for Plant and Food Research Limited, Private Bag 92169, Auckland 1142, New Zealand*

A B S T R A C T

We employed an untargeted volatile profiling approach in combination with spit off-odorant measurement procedure to investigate the fate of aroma compounds in mouth by determining how oral processing and intrinsic biological variables influence the overall volatile composition. A red wine before and after oral processing (expectorated wine), and control samples (expectorated water) were analyzed using GC-TOF-MS to determine as many volatile compounds as possible. We identified compounds in expectorated wines that originated in mouth from either an endogenous or exogenous source, while confirming that compounds might have metabolized by the action of salivary enzymes. Our data also show the changes in volatiles via hydrolysis from the corresponding molecules and may provide evidence of *de novo* formation of volatiles via transesterification reaction in mouth. While investigating the impact of intrinsic biological variables, we found age and gender specific differences in wine volatile composition due to oral processing and identified the key volatiles.

1. Introduction

During wine consumption, aroma composition is modified by oral processing by different mechanisms that involve dilution with saliva, interactions between aroma compounds and salivary proteins, or interactions with oral epithelial cells and microorganisms (Perez-Jiménez et al., 2020). Additionally, the metabolism of aroma compounds into other related compounds, particularly the formation of new odorant molecules from non-odorant precursors, has also been described previously (Muñoz-González et al., 2015; Muñoz-González, Feron et al., 2018; Parker et al., 2017; Pérez-Jiménez, Muñoz-González et al., 2021)). Recent research has highlighted that the metabolism of aroma compounds during food consumption might have a significant impact on aroma perception by individuals (Ijichi et al., 2019; Muñoz-Gonzalez et al., 2021). Some of the factors that are believed to be involved in the metabolism of odorant molecules during consumption are related to the oral microbiota composition or to the presence of enzymes from salivary glands or oral epithelial cells (Muñoz-Gonzalez et al., 2021). For instance, enzymes produced by oral microbiota can hydrolyze non-aromatic wine glycosidic precursors, thus releasing different aromatic molecules (e.g. terpenes, benzenoid compounds and lipid derivatives)

(Muñoz-González et al., 2015). Salivary enzymes are also responsible for metabolizing different aroma compounds, producing new metabolites with different odour thresholds and aroma nuances. Some examples include: conversion of esters to their correspondent carboxylic acids (Pérez-Jiménez et al., 2019; Pérez-Jiménez et al., 2020); oxidation of thiols to thioesters or thioalcohols; formation of alcohols from aldehydes (Muñoz-González, Feron et al., 2018; Muñoz-González, Vandenberghe-Descamps et al., 2018); and conversion of diketones and monoketones into saturated ketones and alcohols (Muñoz-González, Feron et al., 2018; Muñoz-González, Vandenberghe-Descamps et al., 2018; Ployon et al., 2020).

Intrinsic biological factors of individuals such as age, gender or ethnicity, might also influence oral physiological parameters, thus exerting an impact on wine aroma metabolism during consumption. For instance, sensory studies conducted on Spanish wine consumers showed higher and longer intensity perception of smoked and black pepper aroma attributes among senior adults compared to young adults during oral processing (Criado, Muñoz-González et al., 2021). These differences might be related to oral physiological differences arising from saliva flow and composition (total salivary protein content) that affected the dynamics of aroma release in the mouth. Recently, Criado, Pérez-

---

Jiménez, Muñoz-González, and Pozo-Bayón (Criado, Pérez-Jiménez et al., 2021) confirmed this hypothesis by using an in-mouth head-space sorptive extraction (HSSE) technique. In this study, the authors observed significant differences in the in-mouth release profile of 24 wine volatile compounds depending on the age and gender of participants (n = 32). For instance, senior individuals exhibited a higher release of furanic compounds and C_{13}-norisoprenoids than young adults did, and a similar behavior in the release of aroma compounds between male and females after the red wine intake. In contrast, young participants showed significant differences in aroma release between males and females. Young males released higher amount of esters and alcohols, while young females had the lowest aroma release of all the studied aromas classes (esters, alcohols, furanic acids, terpenes, lactones and C_{13}-nor-isoprenoids). Besides the effect of saliva composition (total protein content, minerals, enzymatic activities, pH, flow) on phenomena such as dilution, interaction with proteins, etc., they also suggested that the differences observed on in-mouth aroma release between age–gender groups could be due to a different metabolism of aroma molecules in the mouth, mainly from saliva.

Saliva composition including volatiles can vary between individuals depending on many factors (e.g. age) (Schipper et al., 2007). Volatiles in saliva can originate from both endogenous and exogenous sources. More than 500 volatiles belonging to different chemical groups have been detected in human breath (Miekisch et al., 2004), while >350 have been detected in saliva (Amann et al., 2014). It is well known that volatiles from saliva develop from the normal metabolism including the microbial activity of the mouth (Milanowski et al., 2017), blood, gingival exudate, nasal cavity or gastrointestinal reflux (Amann et al., 2014). Nonetheless, most of the volatile metabolites that have been previously reported to be found in saliva did not present an endogenous origin (Miekisch et al., 2004). These volatiles could come from the air through normal breathing, as in the case of air pollutants found in saliva, or from the use of health and cosmetic products (Milanowski et al., 2017). Additionally, volatiles could also originate from residues from eating or drinking (Milanowski et al., 2017), or from the first digestion phase that takes place in the mouth. However, the effect of age and gender on saliva volatile composition due to oral processing has been scarcely investigated.

To study the influence of oral components on wine aroma composition different techniques can be employed. For instance, the spit off odorant measurement (SOOM) procedure is one that has been used to compare the volatile profile of wines before and after oral processing to determine the aroma compounds present in the wine and in the expectorated wine sample (a mixture of wine and saliva) (Esteban-Fernández et al., 2016; Muñoz-González, Pérez-Jiménez et al., 2020). Through the employment of this technique adsorption capacities of volatiles to oral mucosa between 6 and 43% have been observed, which depended on the characteristics of the odorant (Esteban-Fernández et al., 2016). For instance, the most adsorbed compound to the oral mucosa was guaiacol (43%), while the least adsorbed compounds was 2-phenylethanol (6.6%) (Esteban-Fernández et al., 2016). Although application of SOOM generated valuable data to elucidate the adsorptive and release capacity of wine aromas in the mouth, most of these published studies were performed with synthetic wines spiked with a few volatiles (Esteban-Fernández et al., 2016; Muñoz-González et al., 2020). Therefore, knowledge gathered from these studies is not comprehensive, as model wines do not represent the concentration and the wide range of aroma compounds present in a real wine. In addition, these studies employed targeted gas chromatography-mass spectrometry (GC–MS) approaches to evaluate the behavior and changes of aroma compounds in the mouth during wine consumption, mainly considering only a small number of major volatiles in wine. Therefore, information about wide range of odorants and related metabolites (known and unknown) that are present in variable concentrations in wine, but have important roles in wine aroma perception, have been largely overlooked (Pinu, 2018).

To overcome this limitation, untargeted GC–MS approaches, which enable the detection and identification of as many metabolites as possible, are often used to fill the existing knowledge gaps on the role of wine aroma metabolites in aroma perception. Particularly, the combination of SOOM and untargeted volatile profiling or volatilomics could be an alternative technique to determine a wide range of volatile metabolites in wine and expectorate samples. Recently, Pérez-Jiménez (Pérez-Jiménez, Sherman et al., 2021) and co-workers reviewed the application of untargeted volatile profiling and how this approach could be used for detecting and identifying large numbers of volatile metabolites in wine flavoromics research. Untargeted approaches using high-resolution analytical instrumentation are gaining more popularity in recent years for determining the wine volatile profile and for elucidating its role in wine flavor (Nicolli et al., 2018). Although the use of untargeted volatile profiling approaches in wine aroma and flavor research offers a multitude of benefits, until now we found no studies that applied such approaches to investigate the fate of aroma compounds during the oral processing of wine.

In this study, our main aim was to develop and apply a comprehensive analytical workflow using a volatilomics approach to determine the influence of oral processing on the wine aroma profile. We combined the SOOM procedure with untargeted volatile profiling using gas chromatography-time of flight-mass spectrometry (GC-TOF-MS) to determine the volatile composition of a red wine before and after oral processing. Additionally, we also investigated the impact of other intrinsic biological variables (gender and age) during wine oral processing. To the very best of our knowledge, this is the first study where the changes of aroma compounds in wine during consumption has been evaluated using an untargeted volatile profiling method. In addition, this research focused on developing a better understanding about the metabolism of wine odorants in the mouth through the formation or degradation of volatiles into different metabolites, providing further insights on wine aroma perception.

2. Materials and methods

2.1. Chemicals

Calcium chloride ($CaCl_2$) was obtained from VWR (Leuven, Belgium), dichloromethane and sodium sulphate (Na_2SO_4) were purchased from Merck (Darmstadt, Germany). Methyl nonanoate and alkane standards were provided by Sigma-Aldrich (St. Louis, MO, USA) and 3-octanol was from Fluka (Steinheim, Switzerland).

2.2. Wine

An oak aged commercial red wine produced in 2014 from the grape varieties Tempranillo (84 %), Graciano (9 %), Mazuelo (5 %) and Garnacha (2 %) was used in this study (provided by Marqués de Murrieta winery, La Rioja, Spain). The chemical characteristics of the wine were determined by following previously published protocols (Pérez-Jiménez et al., 2019): pH of 3.73 ± 0.03, 14 % of ethanol (v/v), total polyphenol content of 1656 ± 85 mg of gallic acid/L, total procyanidins of 962 ± 11 mg of catechin/L, neutral polysaccharides of 4033 ± 716 mg of mannose/L, free amino acids of 358 ± 18 mg of leucine/L, and free amino acids plus peptides of 461 ± 137 mg of leucine/L.

2.3. Individuals

Sixteen young adults (<35 years old) and 16 senior adults (>55 years old) participated in this study. The average age of young and senior adults was 26.6 ± 2.5 and 62.5 ± 7.5, respectively. Both young and senior participants were separated into two groups depending on their gender (female or male): young females (n = 8), young males (n = 8), senior females (n = 7) and senior males (n = 9). The exclusion criteria for the recruitment of volunteers were: pregnancy, food allergies or diseases for which the consumption of wine is contraindicated, oral

diseases and medication that may interact with alcohol consumption.

The participants were instructed not to eat or drink for 1 h prior to the collection of water and wine samples. They were informed about the experimental procedure of the study and provided written consent to participate. Ethical approval was obtained from Bioethical Committee of the Spanish Council of Research (CSIC, Spain).

2.4. Collection of expectorated samples

For the collection of samples, we followed a previously published protocol (Muñoz-González et al., 2020). All expectorated samples were collected in the same day, during a period of 2 h. Before starting with the wine samples, one rinse with 15 mL of mineral water was collected in order to check the oral aroma background. Then, for the collection of expectorated wine samples, each individual took the whole volume of wine (15 mL) in the mouth in one sip. They performed soft rinses for 30 s and spat the wine into 50 mL centrifuge tubes (VWR, Pennsylvania, PA, USA). Each individual repeated this procedure three times, in order to obtain the samples in triplicates. Each of the three expectorated samples were analyzed in duplicate. Between wine rinses, the participants cleaned their mouths with a pectin water solution (1 g/L) and with tap water and they waited 15 min until the oral processing of the next wine sample. A diagram of the procedure is shown in Supplementary Fig. 1. Immediately after spitting out, 0.5 g of $CaCl_2$ were added to the expectorated water and wine samples in order to stop any enzymatic reaction (Pérez-Jiménez et al., 2020). In total four expectorated samples, three wine expectorated and one water expectorated, were obtained from each individual. Wine expectorated samples were not mixed together. In addition to expectorated samples (water and wine), one sample of the wine was also collected from the bottle. Finally, both wine and expectorated samples (water and wine) were stored immediately after their collection at $-80\ ^\circ C$ until their analysis.

2.5. Untargeted volatile profiling using GC-TOF-MS

2.5.1. Liquid-liquid extraction of volatile compounds

Volatiles from commercial red wine, expectorated water and expectorated wine samples were extracted by liquid–liquid extraction using a previously published protocol (Esteban-Fernández et al., 2016). Briefly, the internal standard methyl nonanoate (1 mg/L) was added to 10 mL of sample. Then, samples were extracted with 1 mL of dichloromethane two times and stirred manually ten times. Then organic phase was separated by ultrasonication in an ice bath and further centrifugation. Extracted samples were then dried with anhydrous Na_2SO_4. After this, solutions were filtered with glass wool, added with the second internal standard (3-octanol 1 mg/L) and made up to 5 mL with dichloromethane. Finally, samples were concentrated up to 2 mL under nitrogen flow. All the extracts were frozen ($-80\ ^\circ C$) until their analysis.

Prior to analysis, sample extracts were defrosted and quality control (QC) samples prepared by pooling 20 μL aliquots of every sample. One QC sample was also spiked with an aliphatic alkane series (47.6 μg/mL, $C_7 - C_{30}$) and analyzed to calculate retention indices.

2.5.2. GC-TOF-MS analysis

The analysis of the volatile composition of the extracts was carried out in duplicate using a Pegasus® BT GC-TOF-MS mass spectrometer (LECO Corporation, St. Joseph, MI, USA) coupled to an Agilent 7890 gas chromatograph (Agilent Technologies, Santa Clara, CA, USA) fitted with a 30 m × 0.25 mm ID × 0.25 μm film thickness DB-Wax polar capillary column (Agilent Technologies, Santa Clara, CA, USA). The method was adapted for liquid samples from Sherman, Coe, Grose, Martin, and Greenwood (Sherman et al., 2020). A volume of 1 μL of sample extract was injected into the GC–MS system using a Gerstel MPS2 autosampler (GERSTEL GmbH & Co.KG, Mülheim an der Ruhr, Germany). The temperature of the injector was 250 °C, and the initial GC temperature was held at 35 °C for 1 min followed by temperature ramps to 136 °C at

4 °C/min and then to 250 °C at 6 °C/min. The final temperature was held for 2 min giving a total analysis time of 47.25 min. The carrier gas was helium with a flow rate of 1 mL/min. Samples were injected using splitless mode with a purge flow of 20 mL/min at 120 s after injection. The transfer line and ion source were both held at 250 °C, and masses were collected between 29 and 450 *m/z* at 30 spectra/s in electron impact (EI) ionization mode at 70 eV. An acquisition delay of 270 s was set where the filament was off to avoid overloading the detector with the solvent front.

2.5.3. Data processing

GC-TOF-MS raw chromatograms were processed by ChromaTOF (version 5.40, LECO Corporation, St. Joseph, MI, USA). Lists of peak heights were generated and aligned using a previously published data processing procedure with some modifications (Sherman et al., 2020). Briefly, a reference peak table was created from the peak lists generated from QC samples and a subset of samples analyzed (including water and wine expectorates, and wine sample extracts). Retention indices were calculated from the analysis of the alkane spiked QC sample and assigning the internal standards and a selection of ubiquitous and abundant identified sample components and phthalate contaminants as retention index markers. Peak detection and deconvolution was performed on the raw data files, and feature identifiers assigned where the peak spectral match factor with a feature in the reference peak list was > 750 and the retention time deviation was <6 s. All mass-to-charge ratios (*m/z*) used to quantitate peak heights were manually checked and assigned to the most abundant ion in the deconvoluted spectrum *m/z* > 45. Sample peak lists were then exported to Microsoft Excel for further processing as per the methods described by Sherman et al. (Sherman et al., 2020). Peak heights normalized to the internal standard methyl nonanoate were used for subsequent data analysis.

2.6. Data analysis

Prior to performing any statistical analyses, data were log-transformed and range scaled (mean-centered and divided by the range of each variable). Statistical analyses commonly used for determining important features in untargeted metabolite data were performed using a web-based platform Metaboanalyst 5.0 (http://www.metaboanalyst.ca) (Pang et al., 2021). These include different unsupervised and supervised statistical analyses including t-tests, principal component analysis (PCA), orthogonal partial least square-discriminant analysis (O-PLSDA) and variance in projection (VIP) scores calculation. Two factor analyses including two-way heat map clustering and visualization and ANOVA-simultaneous component analysis (ASCA) were also carried out to determine interaction between age and gender. This also allowed us to determine the most significant features associated with the interaction. Once we were confident on the data quality and after performing appropriate statistical analyses, differentiating features were putatively identified by following the guidelines provided by the Metabolites Standard Initiatives (Spicer et al., 2017).

3. Results and discussion

As most of the previously published studies on wine oral processing relied on the utilization of targeted metabolite (particularly volatile compounds) analysis (Esteban-Fernández et al., 2018; Muñoz-González, Pérez-Jiménez et al., 2020; Pérez-Jiménez, Sherman et al., 2021), we opted to perform an untargeted volatile profiling method in this study to generate more information about the influence of oral processing on wine volatile composition. The application of an unbiased data driven method allowed us to generate a large amount of data on volatile compounds present in wine, and water and wine expectorates. Using these data, we additionally determined how different factors including age and gender contribute to changes in volatile compounds.

3.1. Overview of the untargeted volatile profile data

A total of 1770 features were detected across all sample types (commercial wine, water expectorate, wine expectorate and pooled quality control samples) after raw data processing by using a typical untargeted volatile profiling protocol (Sherman et al., 2020). Data generated using untargeted metabolite profiling require vigorous data cleaning procedures to avoid biases from sample preparation and instrumental analysis. Therefore, we followed typical workflow of untargeted volatile profiling starting from raw data that underwent data cleaning, duplicate detection, outlier detection, relative standard deviation (RSD) filtering (based on technical replicates, n = 2), and removal of contaminants (e.g. siloxanes, phthalates). This allowed us to reduce the feature list to 248. Then, QC samples, analyzed with each batch run (n = 20), were used to assess data quality. Out of the 248 features, 245 were detected in the QC samples, 183 in controls, 248 in wine expectorates and 215 in the wine. All but four features had RSDs <35 %, and 168 had RSDs <20 %, which indicated that data obtained after cleaning steps were of good quality (Pinu et al., 2016). Metabolite annotation to determine the putative identities of the features was performed after determining the most important features using different univariate and multivariate statistical analysis.

We performed PCA using 248 features to determine the differences among water, wine expectorated and QC samples. As shown in Fig. 1, 89.1 % of the total variance was explained by PC1 (72.2 % of the total variance), PC2 (13 % of the total variance), and PC3 (3.9 % of the total variance). We observed a clear separation between the different types of samples used in this study (Fig. 1a). The PC1 separated the wine expectorate and QC samples on the right side, from expectorated water samples on the left side. A large number of features were positively correlated to PC1 with factor loadings > 0.1. Among them, the 10 main features (putatively identified) were ethylparaben, tyramine derivative, an unknown compound (P1413_RT2350.6_mz100.1), 2-methylbutanoic acid, ethyl methyl succinate, ethyl 2-hydroxy-4-methylpentanoate, isovaleric acid, ethyl 5-oxo-DL-prolinate and 4-ethylphenol. On the other hand, some of the features negatively correlated to PC1. The most relevant were noted as alkanes (P1136_RT2008.7_mz57.1, P0981_RT1825.9_mz57.1, P0271_RT684.9_mz 57.1, P0497_RT1075.4_mz57.1) and dimethyl sulfone. The separation

among samples was related to the higher abundance of most of the volatile metabolites in wine expectorated and QC samples than control (expectorated water) samples.

On the other hand, Fig. 1b showed the PCA biplot from PC1 and PC3, in which PC3 clearly separated between wine and wine expectorated samples. PC3 was negatively correlated to 2-methyl-1-butanol, pantolactone and ethyl 3-hydroxybenzoate (factor loadings < -0.134), and positively (factor loadings > 0.163) with one alkane (P0144_RT451.1_mz57.1) and two branches alkanes (P0263_RT669.6_mz71.1, P0578_RT1189.6_mz57.1).

Fig. 1 shows QC, wine and wine expectorate samples clustered near to each other while control (saliva + water) samples clustered separately. This suggests that wine expectorated samples were highly influenced by the volatile composition of wines and comparatively less impacted by the saliva volatiles. QC samples formed a relatively tight cluster (Fig. 1) compared to the rest of samples (expectorated wines, or control samples), indicating little variability caused by the different timing of running samples.

Once we were confident on the data quality and observed differences among different sample types, we then investigated the effect of oral processing. First, we compared volatile profiles of the wine and the expectorated wine samples. Second, the differentiating features were putatively identified by following the guidelines provided by the Metabolites Standard Initiatives (Spicer et al., 2017) to explore the changes in volatile compounds among sample types.

3.2. Changes in wine volatiles due to oral processing

To investigate the effect of oral processing in a real wine with a complex volatile profile, we compared the abundances of the volatile features found in both wine and expectorated wine samples. Additionally, water expectorates samples (obtained before wine rinses) were analyzed using the same methodology in order to confirm that the observed changes were due to the oral processing of the wine. Table 1 shows 99 putatively identified features that differed in their relative abundance between wine, controls and expectorated wine samples.

As shown in Table 1, 49 volatiles showed a similar abundance without significant differences (NS; p > 0.05) between wine expectorated and water expectorated samples, while 12 volatiles were found at
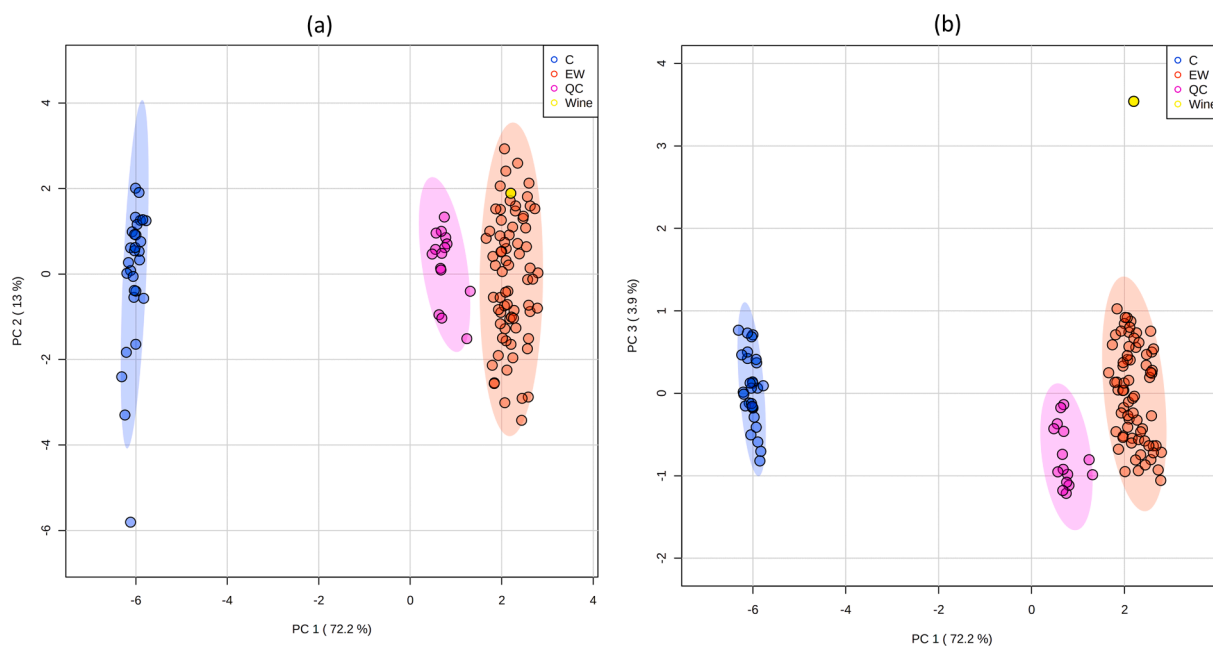
Fig. 1. Principal Component Analysis (PCA) score plot showing the differences in volatile profiles among different orally processed samples. Fig. 1a shows the PCA scoreplot representing PC1 and PC2, and 1b shows the PCA scoreplot representing PC1 and PC3. Here, wine, expectorated wine (saliva + wine) = EW, expectorated water (saliva + water) = C, and pooled quality control samples analyzed with every batch of samples = QC.

Table 1

Relative abundances of putatively identified volatile features detected in wine, expectorated wines and expectorated water (control) samples. Between sample type differences assessed by ANOVA with Fishers LSD and two-sample t-tests.

Feature N°	Feature	Putative identification	Class	RI Exp.	RI Lit.	Detected in			t-test results	
						Wine	Exp. Wine	Control	Wine/ Exp. Wine	Control/ Exp. Wine
1	P0245_RT643_mz57.1	2-Methyl-1-butanol	Alcohol	1189	1191	ND	+++	++	***	***
2	P0372_RT848.8_mz86.1	2-Methyl-2-buten-1-ol	Alcohol	1314	1324	ND	++	+++	***	*
3	P1610_RT2587.2_mz97.1	1-Eicosanol	Alcohol	2714	2717	+	+++	+++	***	NS
4	P1716_RT2723.8_mz107.1	Ethyl 3-hydroxybenzoate	Ester	2894	?	ND	+++	ND	***	***
5	P1319_RT2228.3_mz83.1	Methyl (3-oxo-2-pentylcyclopentyl)acetate	Ester	2283	2274	ND	+++	++	***	**
6	P1326_RT2236.4_mz163.1	Dimethyl phthalate	Ester	2290	2276	ND	+++	++	***	***
7	P1359_RT2286_mz55	Methyl hydrogen succinate	Ester	2335	?	ND	+++	ND	***	***
8	P1566_RT2533.9_mz56.1	Isobutyl stearate	Ester	2643	?	ND	+++	+++	***	NS
9	P0494_RT1066.2_mz88.1	Ethyl octanoate	Ester	1432	1428	+++	++	ND	***	***
10	P1564_RT2533.1_mz285.3	Butyl octadecanoate	Ester	2642	?	ND	+++	+++	***	NS
11	P0984_RT1829.3_mz101	Ethyl isopentyl succinate	Ester	1902	1928	+++	++	ND	***	***
12	P1351_RT2279.6_mz101	Methyl hydrogen succinate	Ester	2335	?	+++	++	ND	***	***
13	P0880_RT1704.9_mz104.1	2-Phenylethyl acetate	Ester	1805	1801	+++	++	ND	***	***
14	P0713_RT1421.1_mz115.1	Ethyl methyl succinate	Ester	1635	1631	+++	++	ND	**	***
15	P0865_RT1682.7_mz101	Ethyl butyl succinate	Ester	1789	?	+++	++	ND	*	***
16	P0186_RT532.4_mz57.1	3-Heptanone	Ketone	1140	1124	ND	+++	+++	***	NS
17	P0976_RT1809.5_mz79	Dimethyl sulfone	Ketone	1890	1895	ND	++	+++	***	***
18	P1181_RT2079.6_mz99.1	2-Piperidinone	Ketone	2153	2060	ND	++	+++	***	***
19	P1624_RT2601.2_mz57.1	7,9-Di-tert-butyl-1-oxaspiro [4.5]deca-6,9-diene-2,8-dione	Ketone	2733	?	++	+++	+	**	***
20	P1739_RT2751.4_mz182.1	Syringaldehyde	Aldehyde	2929	2904	++	+++	ND	*	***
21	P0586_RT1206_mz77.1	Benzaldehyde	Aldehyde	1509	1508	++	+++	ND	*	***
22	P0451_RT982_mz98.1	Nonanal	Aldehyde	1387	1391	+	++	+++	*	*
23	P0613_RT1244.7_mz74	Propanoic acid	Carboxylic acid	1532	1525	+	+++	++	***	***
24	P1721_RT2727.1_mz73	n-Hexadecanoic acid	Carboxylic acid	2897	2900	++	+++	+	***	***
25	P1217_RT2114.5_mz85	2-Ethyl-2-hydroxybutyric acid	Carboxylic acid	2158	?	++	+++	+	***	***
26	P0911_RT1750.7_mz60	Hexanoic acid	Carboxylic acid	1838	1842	+++	++	+	**	***
27	P0702_RT1405.7_mz60	Butanoic acid	Carboxylic acid	1626	1626	++	+++	ND	*	***
28	P1308_RT2216.5_mz60	n-Decanoic acid	Carboxylic acid	2268	2268	+++	++	+	*	***
29	P1134_RT2005.8_mz60	Octanoic acid	Carboxylic acid	2062	2060	+++	++	+	*	***
30	P1495_RT2457.4_mz91.1	Benzeneacetic acid	Carboxylic acid	2545	2250	++	+++	ND	*	***
31	P1103_RT1967.7_mz71.1	Pantolactone	Lactone	2027	2033	ND	+++	ND	***	***
32	P1042_RT1885.5_mz99.1	5-Butyl-4-methyldihydro-2(3H)-furanone	Lactone	1953	1953	+++	++	+	***	***
33	P0723_RT1429.1_mz87.1	N-Ethylacetamide	Amide	1641	1608	+++	++	ND	***	***
34	P0970_RT1799_mz99.1	trans-3-Methyl-4-octanolide	Amide	1876	?	+++	++	ND	*	***
35	P1229_RT2120.4_mz107.1	4-Ethylphenol	Volatile phenol	2164	2167	+++	++	ND	***	***
36	P1426_RT2361.8_mz117.1	Indole	Nitrogen-containing volatile	2430	2448	ND	++	+++	***	***
37	P0579_RT1192.8_mz83	Tetrachloroethane	Halogen-containing volatile	1501	1516	ND	+++	++	***	*
38	P0416_RT916.4_mz57.1	Unknown branched alkane	Alkane	1353	?	ND	+++	++	***	***
39	P1058_RT1913_mz57.1	Unknown branched alkane	Alkane	1972	?	ND	+++	+++	***	NS
40	P0556_RT1156.5_mz57.1	Unknown alkane	Alkane	1478	?	ND	+++	+++	***	NS
41	P0292_RT716.8_mz85.1	Unknown alkane	Alkane	1231	?	ND	+++	+++	***	NS
42	P0625_RT1263_mz85.1	Unknown alkane	Alkane	1541	?	ND	+++	+++	***	NS
43	P0614_RT1245.1_mz71.1	Unknown alkane	Alkane	1531	?	ND	+++	+++	***	NS
44	P0608_RT1234.6_mz57.1	Unknown branched alkane	Alkane	1528	?	ND	++	+++	***	**
45	P0966_RT1797_mz71.1	Unknown alkane	Alkane	1868	?	ND	+++	+++	***	NS
46	P0964_RT1793.6_mz57.1	Unknown alkane	Alkane	1866	?	ND	+++	+++	***	NS
47	P1183_RT2081_mz68.1	Unknown cyclic alkane	Alkane	2133	?	ND	+++	+++	***	NS
48	P0995_RT1835.3_mz85.1	Unknown alkane	Alkane	1910	?	ND	+++	+++	***	NS
49	P1136_RT2008.7_mz57.1	Unknown alkane	Alkane	2060	?	ND	++	+++	***	***
50	P0678_RT1365.3_mz57.1	Unknown alkane	Alkane	1603	?	+	+++	+++	***	NS
51	P0789_RT1522.5_mz57.1	Unknown alkane	Alkane	1693	?	+	+++	+++	***	NS
52	P0801_RT1553.2_mz57.1	Unknown alkane	Alkane	1711	?	+	+++	+++	***	NS
53	P0903_RT1736.6_mz57.1	Unknown branched alkane	Alkane	1820	?	+	+++	+++	***	NS
54	P0785_RT1511.2_mz71.1	Unknown alkane	Alkane	1683	?	+	+++	+++	***	NS
55	P0894_RT1725.6_mz57.1	Unknown alkane	Alkane	1817	?	+	+++	+++	***	NS
56	P0588_RT1206.8_mz57.1	Unknown alkane	Alkane	1505	?	+	+++	+++	***	NS
57	P0574_RT1184_mz57.1	Unknown alkane	Alkane	1495	?	+	+++	+++	***	NS
58	P0735_RT1439.5_mz57.1	Unknown branched alkane	Alkane	1645	?	+	+++	+++	***	NS
59	P0974_RT1804.1_mz57.1	Unknown alkane	Alkane	1878	?	+	+++	+++	***	NS
60	P0473_RT1026.3_mz57.1	Unknown alkane	Alkane	1410	?	+	+++	+++	***	NS
61	P0918_RT1753.3_mz57.1	Unknown alkane	Alkane	1840	?	+	+++	+++	***	NS
62	P0978_RT1815.3_mz57.1	Unknown alkane	Alkane	1888	?	+	+++	+++	***	NS
63	P1152_RT2028_mz57.1	Unknown alkane	Alkane	2071	?	+	+++	+++	***	NS

(continued on next page)

Table 1 (*continued*)

Feature N°	Feature	Putative identification	Class	RI Exp.	RI Lit.	Detected in			t-test results	
						Wine	Exp. Wine	Control	Wine/ Exp. Wine	Control/ Exp. Wine
64	P0886_RT1712.5_mz57.1	Unknown alkane	Alkane	1812	?	+	++	+++	***	**
65	P0850_RT1638.6_mz57.1	Unknown branched alkane	Alkane	1760	?	+	+++	+++	***	NS
66	P0920_RT1754.7_mz85.1	Unknown alkane	Alkane	1842	?	+	+++	+++	***	NS
67	P0714_RT1421.7_mz57.1	Unknown alkane	Alkane	1634	?	+	+++	+++	**	NS
68	P0792_RT1537.3_mz57.1	Unknown alkane	Alkane	1703	?	+	+++	+++	**	NS
69	P0542_RT1126.1_mz57.1	Unknown alkane	Alkane	1464	?	+	+++	+++	**	NS
70	P0554_RT1152.8_mz71.1	Unknown alkane	Alkane	1478	?	+	+++	+++	**	NS
71	P1120_RT1991.1_mz57.1	Unknown alkane	Alkane	2056	?	+	+++	+++	**	NS
72	P0495_RT1068.9_mz57.1	Unknown alkane	Alkane	1435	?	+	++	+++	**	**
73	P0538_RT1118.9_mz71.1	Unknown alkane	Alkane	1460	?	+	+++	+++	**	NS
74	P0987_RT1832.5_mz57.1	Unknown alkane	Alkane	1901	?	++	+++	+	*	***
75	P0848_RT1630.2_mz57.1	Unknown branched alkane	Alkane	1756	?	+	+++	+++	*	NS
76	P0550_RT1139.2_mz57.1	Unknown alkane	Alkane	1471	?	+	+++	+++	*	NS
77	P0523_RT1105.1_mz57.1	Unknown alkane	Alkane	1448	?	++	+	+++	*	*
78	P1051_RT1895.5_mz57.1	Unknown branched alkane	Alkane	1959	?	+	+++	+++	*	NS
79	P0472_RT1021.7_mz57.1	Unknown branched alkane	Alkane	1408	?	++	+++	+++	*	NS
80	P0467_RT1006_mz57.1	Unknown alkane	Alkane	1400	?	++	+	+++	*	*
81	P0770_RT1498.1_mz57.1	Unknown alkane	Alkane	1679	?	+	+++	+++	*	NS
82	P1553_RT2522.7_mz55.1	Unknown	-	2622	?	ND	+++	++	***	*
83	P1429_RT2366_mz108.1	Unknown	-	2429	?	ND	+++	ND	***	***
84	P0339_RT785.4_mz59.1	Unknown	-	1275	?	ND	++	+++	***	***
85	P0194_RT545.4_mz71.1	Unknown	-	1144	?	ND	+++	+++	***	NS
86	P0563_RT1171_mz85.1	Unknown	-	1487	?	ND	+++	+++	***	NS
87	P0295_RT718.4_mz57.1	Unknown	-	1240	?	ND	+++	+++	***	NS
88	P1018_RT1842.4_mz205.2	Unknown	-	1919	?	ND	+++	+++	***	NS
89	P0390_RT868.1_mz69.1	Unknown	-	1322	?	+	+++	+++	***	NS
90	P0898_RT1727.9_mz85.1	Unknown	-	1817	?	+	+++	+++	***	NS
91	P0761_RT1483.5_mz71.1	Unknown	-	1668	?	+	+++	+++	***	NS
92	P1478_RT2445.4_mz194.1	Unknown	-	2529	?	+++	++	ND	***	***
93	P1337_RT2261.1_mz85	Unknown	-	2315	?	+++	++	+	**	***
94	P0831_RT1588.7_mz138.1	Unknown	-	1733	?	+	+++	+++	**	NS
95	P1608_RT2585.5_mz135.1	Unknown	-	2712	?	++	+++	ND	*	***
96	P0429_RT921.5_mz71.1	Unknown	-	1353	?	++	+++	+++	*	NS
97	P1311_RT2220.3_mz91.1	Unknown	-	2271	?	+++	++	ND	*	***
98	P0882_RT1705.7_mz87	Unknown	-	1802	?	+++	++	+	*	***
99	P1339_RT2262.7_mz101	Unknown	-	2316	?	+++	++	ND	*	***

RI Exp.: retention index experimental; RI Lit.: retention index literature; Exp. Wine: expectorated wine; Exp. Water: expectorated water; ND: not detected; +: low abundance; ++: medium abundance; +++: highest abundance; NS: no significant differences; *: significance level $p < 0.05$; **: significance level $p < 0.01$; ***: significance level $p < 0.001$.

significantly higher abundance in expectorated water samples compared to wine and wine expectorated samples. This observation indicated that all these compounds (in total, 61 compounds) were already present in the mouth at similar or even higher concentrations before the wine oral processing. Thus, their presence in the expectorated wine could not be attributed to oral metabolism of wine components. These compounds most probably originated from saliva or residues from healthcare/cosmetic products (Milanowski et al., 2017). Among those 61 volatiles, features were tentatively identified and belonged to different chemical classes including. alkanes (42 features), three ketones (2-piperidinone, 3-heptanone, dimethyl sulfone), two alcohols (1-eicosanol, 2-methyl-2-buten-1-ol), two esters (isobutyl stearate, butyl octadecanoate), one aldehyde (nonanal), one nitrogen compound (indole), and 10 unknown compounds (Table 1).

A total of 18 volatile compounds were found at the highest abundance in wine samples compared to expectorated wine and control (expectorated water) samples ($p < 0.05$) (Table 1). Among them, 12 volatiles (six esters, two amides, one volatile phenol and three unknown volatiles) were not detected in control samples, indicating that these compounds were not previously present in the mouth and may have originated from the wine. Traces of six volatiles (three carboxylic acids, one lactone and two unknown compounds) were present in control samples, indicating a very small abundance of these compounds in the mouth before wine tasting. The lower abundance of the same volatile compound in the expectorated wine compared to the wine could be due to a slight dilution effect or to the metabolism of these compounds during the oral processing by the action of enzymes from saliva or oral

mucosa. The enzymatic activities would lead to a reduction in the substrates, and an increase in the abundance of volatile products in the expectorated wine. For instance, lower abundances of esters (ethyl octanoate, ethyl isopentyl succinate, methyl hydrogen succinate, 2-phenylethyl acetate, ethyl methyl succinate and ethyl butyl succinate) in the expectorated wine than in the wine indicates that these compounds were transformed in the mouth due to salivary enzymes (esterases). This observation was in agreement with previously published studies that provided evidence of degradation of esters (ethyl hexanoate, ethyl octanoate, ethyl decanoate) into their correspondent carboxylic acids (hexanoic, octanoic and, decanoic acids) by saliva esterases (Pérez-Jiménez, Muñoz-González et al., 2021). Other explanation of the lower abundance of volatiles in the expectorated wine compared to the wine could be related to the adsorption of these compounds by oral conditions. For example, lower abundance of a lactone (5-butyl-4-methyl-dihydro-2(3H)-furanone) and a volatile phenol (4-ethylphenol) in expectorated wines compared to wine samples could be explained by their retention in the oral cavity by proteins from oral mucosa. Previous research also reported higher retention of other lactones (β-ionone) and volatile phenols (guaiacol) by oral mucosa compared to other volatile compounds (e.g. esters, alcohols) (Esteban-Fernández et al., 2016). Higher retention of volatiles by oral mucosa can be related to both the hydrophobicity of the compound and the molecular structure (e.g. cyclic structure) (Esteban-Fernández et al., 2016; Pérez-Jiménez, Sherman et al., 2021).

In addition, our data also suggest that the impact of oral processing on the metabolism of carboxylic acids and amides. As shown in Table 1,

some carboxylic acids (hexanoic, *n*-decanoic and octanoic acids) and amides (ethylacetamide, *trans*-3-methyl-4-octanolide) were in very low concentrations in the controls (expectorated water) while being more abundant in the wine compared to the expectorated wine. This suggests a possible metabolism of these compounds by salivary enzymes, specifically from the oxidoreductase family (e.g. alcohol dehydrogenases) and carbonic anhydrases, or in the case of amides, also by carboxyl esterase enzymes, which have been described in both saliva and oral mucosa (Schwartz et al., 2021). It is noteworthy that in some cases the esters and the related carboxylic acid (e.g. ethyl octanoate and octanoic acid) both showed a lower abundance in expectorated wine than wine, indicating an impact from oral processing. As previously mentioned, the dilution of these compounds in the mouth, their metabolism or their retention by oral physiology could explain this reduction.

While comparing volatile profiles of wine, expectorated wines and control samples, we found that 16 features were present in significantly higher abundance ($p < 0.05$) in expectorated wines than in both wine and control samples (Table 1). Since these 16 volatiles were already present in the mouth or in the wine, their higher abundance in expectorated wine samples could be due to the sum of these volatiles (volatiles originally present in the wine + those already found in the mouth before wine rinses). However, this could also be due to the production of these volatiles during the oral processing of wine. For example, carboxylic acids (*n*-hexadecanoic, 2-ethyl-2-hydroxybutyric, butanoic, benzeneacetic and propanoic acids) could have been generated from the hydrolysis of esters by the action of esterase enzymes, as previously explained (Pérez-Jiménez, Muñoz-González et al., 2021). The hydrolysis of esters by salivary esterases has been reported for different carboxylic acids (hexanoic, octanoic and, decanoic acids) (Pérez-Jiménez, Muñoz-González et al., 2021). Therefore, our observation on higher abundance of carboxylic acids in expectorated wine samples could be explained by production of these compounds during wine oral processing. Carboxylic acids usually have higher perception thresholds than their corresponding esters, and contribute to different odorant qualities, thus impacting wine aroma perception (Sumby et al., 2010).

As shown in Table 1, we found higher abundance of 2-methyl-1-butanol in expectorated wine samples than controls and wine (Table 1). We assume that this alcohol could also be a metabolism product from the degradation of esters by esterases, since transformation of esters (e.g. benzyl acetate) into alcohols (benzyl alcohol) has been previously observed (Ijichi et al., 2019). However, this transformation has not been reported before for this volatile compound. Other possible explanation of the higher abundance of 2-methyl-1-butanol in wine expectorated samples was its production due to the reduction of carbonyl compounds (ketones or aldehydes) into alcohols (Ijichi et al., 2019; Muñoz-González, Feron et al., 2018; Muñoz-González, Vandenberghe-Descamps et al., 2018). Enzymes from the aldoketoreductase family have been proposed to contribute to these metabolic reactions (Schwartz et al., 2021). Similarly, ketones (7,9-Di-*tert*-butyl-1-oxaspiro[4.5]deca-6,9-diene-2,8-dione, syringaldehyde and benzaldehyde) could have been formed from the reduction of other mono or diketones. In previous works, the conversion of ketones into different ones (e.g. conversion of 2,3-pentanedione into 3-hydroxypentan-2-one and 2-hydroxypentan-2-one) in the presence of saliva has been observed (Muñoz-González, Feron et al., 2018; Muñoz-González, Vandenberghe-Descamps et al., 2018; Ployon et al., 2020). Several enzyme candidates have been identified that carried out those reactions including carbonyl reductases, aldoketoreductases or short chain dehydrogenase/reductase (Schwartz et al., 2021).

We also found higher concentrations of some aldehydes (syringaldehyde and benzaldehyde) in wine expectorated samples than controls and wines (Table 1). Although the reduction of aldehydes into alcohols in the presence of saliva has been previously reported (Ijichi et al., 2019; Muñoz-González, Feron et al., 2018; Muñoz-González, Vandenberghe-Descamps et al., 2018), no previous studies provided evidence of an increase in the abundance of aldehydes in the presence of human saliva.

Therefore, this is the first study in which the formation of aldehydes due to wine oral processing was observed. This transformation could be explained by the action of alcohol dehydrogenase enzymes present in the mouth (Schwartz et al., 2021). A higher abundance of aldehydes due to oral processing could have an impact on wine sensory perception since they present very different aroma nuances (e.g. almond, smoky, wood aromas) (Ferreira et al., 2014).

We observed different change patterns of different esters during wine oral processing. For example, concentration of methyl (3-oxo-2-pentyl cyclopentyl) acetate was comparatively higher in expectorated wines than control and wine samples. However, methyl hydrogen succinate and ethyl 3-hydroxybenzoate were only present in expectorated wines while being not detected in control and wines, indicating formation of esters due to the wine oral processing. Formation of esters in the mouth by transesterification reactions has been reported previously. In the presence of acidic pH and ethanol, as is the case of wine, esters can be produced from fatty and other carboxylic acids in wine (Christoph & Bauer-Christoph, 2007). Although the formation of esters in the mouth has not been proven yet, this transformation has been already suggested by different authors by *in vitro* approaches as they observed an increase in ester release in the headspace when these compounds were incubated with saliva (Pérez-Jiménez, Muñoz-González et al., 2021). Since transesterification reactions require an acid medium and presence of alcohol, the mouth environment during the oral processing of wine might favor this type of reaction. In addition, the temperature of the mouth (36 °C) and the kinetic energy of swirling during wine rinses could favor this reaction in the oral cavity. Transesterification reactions have been reported for human esterases from different tissues than saliva (liver esterases) (Dean et al., 1991). Within salivary enzymes, the hydrolase family (e.g. lipases, esterases) have the ability to catalyze the formation of esters from carboxylic acids (Nelson et al., 1977).

Interestingly, one lactone (pantolactone) and one unknown compound (P1429_RT2366_mz108.1) were exclusively present in the expectorated wine samples, while not being detected in the controls and the wine (Table 1). This observation suggests that these compounds were not present in the mouth before wine rinses, thus indicating a possibility of a *de novo* generation of these compounds during the oral processing of wine from odourless precursors. The ability of enzymes from oral microbiota to hydrolyze odourless wine glycoside precursors leading to the formation of odor impact compounds has been reported previously (Muñoz-González et al., 2015). Pantolactone is produced in both white (Chardonnay, Gewürztraminer) and red (Tempranillo, Merlot) wines from glycosidic precursors by the action of yeasts hydrolytic enzymes during winemaking (Hernandez-Orte et al., 2015). Some lactones are also known to be produced from the hydrolysis of galloyl odorless glucosides during wine aging (Parker et al., 2017). Additionally, the odor of pantolactone has been described as licorice, smoky, toasted bread and its perception threshold (2000 µg/L) (Qian et al., 2020) is higher than that of some lactones (e.g. β-ionone = 0.09 µg/L), but lower than that of others (γ-butyrolactone = 35,000 µg/L) (Juan et al., 2012). Therefore, its formation during the oral processing of wine could have implication for the wine aroma perception. However, the ability of oral microbiota or salivary enzymes to metabolize lactone precursors has not been reported yet. Our data provide an indication that lactones could also be generated during wine oral processing by yet unknown molecular mechanisms. This also warrants further investigation while reinforcing the potential of untargeted volatile profiling data on generating new insights that were not known before.

We found higher abundance of a few features (tetrachloroethane, dimethyl phthalate, alkane (P0987_RT1832.5_mz57.1), and branched alkane (P0416_RT916.4_mz57.1)) in expectorated wine than wine samples (Table 1). These could be potential environmental pollutants that were adsorbed to the oral surfaces, which could have been removed by the ethanol contained in the wine during the mouth rinsing with the wine (Milanowski et al., 2017). This observation led us to suggest an important change in step for further studies where control (wash with

water) should be replaced by ethonolic water solution or model wines.

3.3. Effect of biological individual variability on the volatile profile after the oral processing of wine

After determining how wine oral processing influenced the wine volatile composition, we investigated the role of different biological variables associated with the participants, such as their age and gender. We performed orthogonal partial least squares discriminant analysis (O-PLSDA), a supervised chemometric tool used for the analysis of untargeted metabolomics data (Tinnevelt et al., 2020), by grouping the individuals according to their gender: male or female to investigate differences based on gender.

The O-PLSDA scores plot (Supplementary Fig. 2) did not show a good discrimination pattern between male and female groups, which was evident from the lack of separate clusters. This suggests that gender might have little influence on the overall volatile composition of wine after oral processing and there might be other variables hindering the separation. These results are in agreement with previous *in vivo* studies where no differences in the in-nose release of odorants were found between male and female subjects using PTR-ToF-MS (Muñoz-González, Feron et al., 2020). Despite seeing no clustering pattern in OPLS-DA

scoreplots depending on gender, we carefully investigated the untargeted volatile profiling data so that we did not overlook any specific pattern among features that might be distinguishable between male and female participants. As a next step, we performed a *t*-test to determine the volatile compounds and found observed 48 features that were significantly different (*p*<0.05) between male and female participants. Among these, 38 were alkanes (branched and straight chain) and were higher in abundance in males than females. Fig. 2 shows the boxplots from the six selected features (methyl (3-oxo-2-pentylcyclopentyl) acetate, benzaldehyde, 2,4-di-ter-butylphenol, butyrolactone) that were different between male and female participants (*p* = <0.05). Higher abundances of alkanes (alkane, branched alkane), alcohol (2,4-di-*tert*-butylphenol) and aldehyde (benzaldehyde) were found in male than female expectorated wine samples.

Interestingly, one ester (methyl (3-oxo-2-pentylcyclopentyl) acetate) and one lactone (butyrolactone) were higher in the expectorated wines from females than from males (Fig. 2), suggesting differences in salivary enzymatic activities between males and females. As previously explained (section 3.2) these compounds could have been generated *de novo* during the oral processing of wine by transesterification enzymatic reactions in the case of esters, or by the hydrolysis from odourless precursors by the action of microbial enzymes. Although no previous
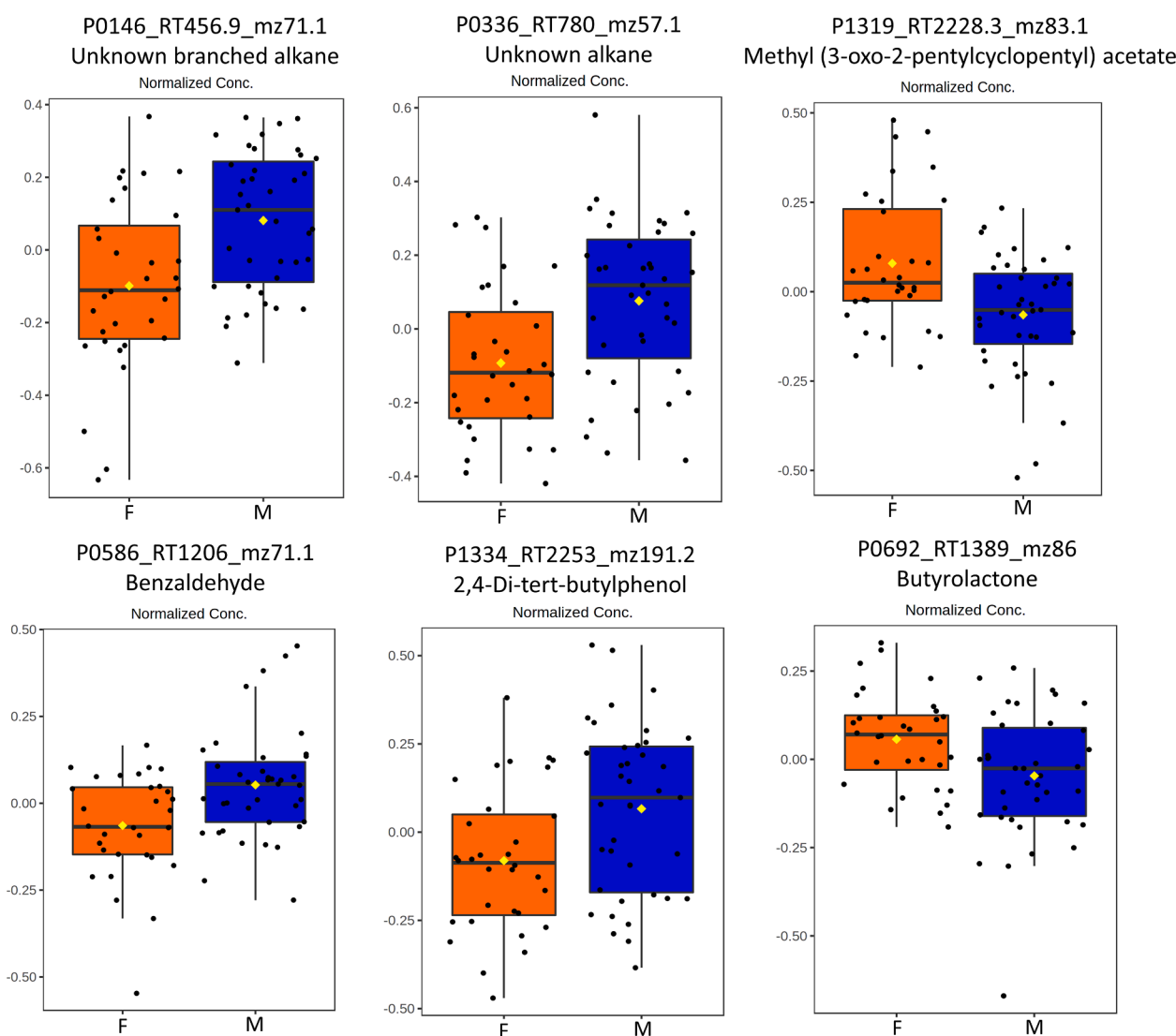

Fig. 2. Boxplots showing the selected volatile features significantly (*p*<0.05) different depending on gender based on *t*-test. Yellow diamond symbol represents the mean relative abundance of the metabolites. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

differences by gender were found in other salivary activities (amylase or protease) (Criado, Muñoz-González et al., 2021), previous research have noted differences in protein content and type due to gender differences (Melis et al., 2021). Nonetheless, little research has been conducted on salivary enzymatic activities and none of them is large enough to extract straightforward conclusions about gender effects. Our untargeted volatile data, therefore, provided a new insight on the gender difference in aroma composition due to oral processing, which was not found in previous studies that looked only into 20–30 aroma compounds. As our study aimed to determine if this type of analytical methodology is suitable for wine oral processing experiments, there is a need for studies to investigate this further using larger number of participants.

We performed another O-PLSDA by grouping the individuals according to their age (young = <35 years old or senior = >55 years old) to determine any age specific differences in wine volatile profiles after oral processing. As shown in Fig. 3, some discrimination between young and senior samples was observed (R^2 = 0.531 with p = 0.001 and Q^2 = 0.266 with p<0.001) in the O-PLSDA scores plot (Fig. 3). This indicates that age is a factor that may have a role in changing wine volatile fractions after wine consumption. However, five samples from the young group overlapped with samples from seniors. Interestingly, these five samples came from three young female participants, thus indicating that the combination of both factors (age and gender) simultaneously may also have an effect on the wine volatile profiles obtained after wine oral processing.

Fig. 3 also shows that samples from senior individuals were clustered more closely together in the scoreplot than samples from young individuals, which were more spread out. This indicates that the volatile profiles after wine oral processing was more similar among senior individuals, while young volunteers were more variable. This is also in agreement with results from a previous study based on *in vivo* aroma release by using in-mouth HSSE where smaller differences between senior individuals were observed in their oral aroma release profile compared to young individuals (Criado, Pérez-Jiménez et al., 2021). The smaller differences among senior individuals may be related to smaller differences in oral physiological parameters, saliva composition or to a

slower metabolism of volatiles in the oral cavity (Muñoz-González, Feron et al., 2018; Muñoz-González, Vandenberghe-Descamps et al., 2018).

Additionally, the O-PLSDA analysis also identified the features that contribute significantly to the variation in volatile profiles between two age groups. Table 2 shows the list of the most important volatile features (VIP > 1) and their putative identification. These features belonged to diverse chemical groups of wine volatiles, including 22 alkanes, 14 esters, nine alcohols, seven carboxylic acids, five ketones, three lactones, three amides, two aldehydes, one furan and one halogen-containing compound. In addition, 10 more features showing also VIP scores >1 were labeled as unknown as they were not found from the metabolite database search and warrants further investigation (Table 2).

Interestingly, the relative abundances of all the features (except ethyl octanoate) were higher in expectorate wines from senior individuals than young (Table 2). These differences could be attributed from different oral behavior (including metabolism) of wine volatiles during oral processing in senior more than young individuals. Among these, nine (ethyl octanoate, ethyl hexanoate, methyl (3-oxo-2-pentyl cyclopentyl acetate), methyl hydrogen succinate, benzophenone, propanoic acid, tetrachloroetane and branched alkane) are volatiles that are susceptible to be metabolized in the oral cavity (as discussed in Section 3.2 and in Table 1). This suggests a higher metabolic conversion by seniors than young adults, which could be due to a higher esterase activity or a higher retention of this compound in the mouth of senior participants. A higher esterase activity would lead to a greater degradation of ethyl octanoate into different volatiles (e.g. octanoic acid) with different odour thresholds and quality. Higher abundance of ethyl octanoate in the mouth of seniors can also be explained by higher salivary protein content in seniors compared to young individuals (Criado, Muñoz-González et al., 2021). Relatively high hydrophobicity of ethyl octanoate (log P = 3.5) might have favored its hydrophobic interactions with proteins in the mucosal pellicle (Esteban-Fernández et al., 2016), and therefore, a lower recovery of this compound in the expectorated wine from senior individuals.

The differences observed between age groups in the expectorated wines could be attributed to differences in salivary composition and flow, oral enzymes, or differences in overall oral physiological parameters. However, it cannot be overruled that some of these differences are due to a different saliva volatilome between young and senior adults, since saliva composition depends on many factors, including age (Schipper et al., 2007). However, the effect of age on saliva volatile composition has been scarcely investigated.

3.4. Effect of both age and gender on wine oral processing

After we determined the effect of age and gender separately based on changes in the volatile profiles during wine oral processing, we further investigated the combined effect (interaction) of age and gender to develop more insights. We performed a two-way analysis of variance (ANOVA), a two-factor comparison, using untargeted volatile profile data. Results showed significant differences in 46 volatiles (p<0.05) when the interaction between gender and age was considered simultaneously (Supplementary Fig. 3) and gender also seemed to contribute in distinguishing wine expectorate samples. Among those significant features, we tentatively identified compounds belonging to alkanes, higher alcohols, furans and esters, along with a few unknown features. Variations in the release of wine volatiles in the oral cavity among age and gender groups have also been studied previously by targeted *in vivo* analysis (Criado, Pérez-Jiménez et al., 2021).

To narrow down this list, we carried out an ANOVA – Simultaneous Component Analysis (ASCA) to identify the major patterns associated with each factor (age or gender) and also from the interaction between the factors (age × gender). We identified significant variables based on the leverage and the Squared Prediction Errors (SPE) associated with each variable. Nine features had SPE < 0.01, indicating that these
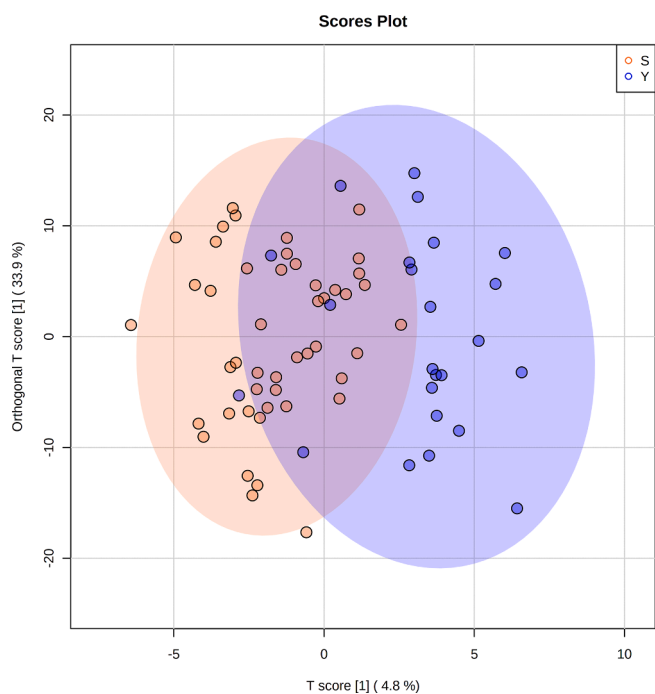

Fig. 3. Orthogonal partial least squares discriminant analysis (O-PLSDA) scores plot showing the difference between expectorated wine volatile profiles based on age. S: Senior individuals (>55 years); Y: Young individuals (<35 years).

Table 2

List of volatile features that contributed to the discrimination of expectorated wines from senior (>55 years) and young (<35 years) individuals.

Feature ID	Putative identification	Chemical family	RI experimental	RI literature	VIP	Relative abundance	
						Senior	Young
P0240_RT638_mz55.1	3-Methyl-1-butanol	Alcohol	1198	1197	1.75	+	−
P0999_RT1837_mz91.1	Phenylethyl alcohol	Alcohol	1913	1912	1.63	+	−
P0372_RT848.8_mz86.1	2-Methyl-2-buten-1-ol	Alcohol	1314	1324	1.6	+	−
P1766_RT2807.2_mz107.1	4-Hydroxyphenethyl alcohol	Alcohol	2998	3008	1.47	+	−
P0424_RT919_mz56.1	1-Hexanol	Alcohol	1351	1357	1.31	+	−
P0245_RT643_mz57.1	2-Methyl-1-butanol	Alcohol	1189	1191	1.25	+	−
P0381_RT854.7_mz71.1	2-Methyl-2-buten-1-ol	Alcohol	1314	1324	1.19	+	−
P0373_RT849.6_mz71.1	2-Methyl-2-buten-1-ol	Alcohol	1314	1324	1.03	+	−
P1610_RT2587.2_mz97.1	1-Eicosanol	Alcohol	2714	2717	2.12	+	−
P0394_RT880.9_mz57.1	Unknown branched alcohol	Alcohol	1329	?	2.36	+	−
P1770_RT2827.8_mz104.1	2-Hydroxyethyl hexadecanoate	Ester	3025	?	2.41	+	−
P0597_RT1222.2_mz71	Ethyl 3-hydroxybutanoate	Ester	1515	1513	2.11	+	−
P0397_RT887_mz75	Ethyl 2-hydroxypropanoate	Ester	1336	1334	2.11	+	−
P1359_RT2286_mz55	Methyl hydrogen succinate	Ester	2335	?	2.07	+	−
P0592_RT1216_mz87.1	Ethyl 3-hydroxybutanoate	Ester	1515	1513	2.01	+	−
P1566_RT2533.9_mz56.1	Isobutyl stearate	Ester	2643	?	1.99	+	−
P1612_RT2590.3_mz57.1	Fatty acetate ester	Ester	2715	?	1.93	+	−
P1467_RT2423.7_mz83.1	Octadecyl acetate	Ester	2503	2521	1.72	+	−
P1189_RT2090.3_mz99	Tributyl phosphate	Ester	2135	2157	1.64	+	−
P1564_RT2533.1_mz285.3	Butyl octadecanoate	Ester	2642	?	1.64	+	−
P0768_RT1495_mz101	Diethyl butanedioate	Ester	1680	1675	1.4	+	−
P1319_RT2228.3_mz83.1	Methyl (3-oxo-2-pentylcyclopentyl)acetate	Ester	2283	2274	1.24	+	−
P0494_RT1066.2_mz88.1	Ethyl octanoate	Ester	1432	1428	1.21	−	+
P1351_RT2279.6_mz101	Methyl hydrogen succinate	Ester	2335	?	1.11	+	−
P0326_RT768_mz88.1	Acetoin	Ketone	1268	1270	2.16	+	−
P1450_RT2395_mz105.1	Benzophenone	Ketone	2470	2470	1.78	+	−
P1181_RT2079.6_mz99.1	2-Piperidinone	Ketone	2153	2060	1.4	+	−
P0186_RT532.4_mz57.1	3-Heptanone	Ketone	1140	1124	1.16	+	−
P1624_RT2601.2_mz57.1	7,9-Di-tert-butyl-1-oxaspiro[4.5]deca-6,9-diene-2,8-dione	Ketone	2733	?	1	+	−
P1459_RT2408.1_mz219.2	3,5-di-tert-Butyl-4-hydroxybenzaldehyde	Aldehyde	2485	?	2.55	+	−
P1745_RT2755.7_mz121.1	4-Hydroxybenzaldehyde	Aldehyde	2934	?	1.41	+	−
P0642_RT1301.3_mz73	Isobutyric acid	Carboxylic acid	1565	1565	2.23	+	−
P0499_RT1080_mz60	Acetic acid	Carboxylic acid	1441	1439	1.79	+	−
P0613_RT1244.7_mz74	Propanoic acid	Carboxylic acid	1532	1525	1.71	+	−
P0702_RT1405.7_mz60	Butanoic acid	Carboxylic acid	1626	1626	1.65	+	−
P0758_RT1480.6_mz74	2-Methylbutanoic acid	Carboxylic acid	1670	1674	1.63	+	−
P0754_RT1477.5_mz60	Isovaleric acid	Carboxylic acid	1669	1665	1.61	+	−
P1217_RT2114.5_mz85	2-Ethyl-2-hydroxybutyric acid	Carboxylic acid	2158	?	1.04	+	−
P1449_RT2394.2_mz85	5-(Hydroxymethyl)dihydrofuran-2(3H)-one	Lactone	2468	?	2.23	+	−
P1483_RT2447_mz71.1	dl-Mevalonic acid lactone	Lactone	2531	?	1.62	+	−
P0692_RT1389_mz86	Butyrolactone	Lactone	1617	1618	1.37	+	−
P1666_RT2663.6_mz87.1	Unknown amide	Amide	2813	?	2.45	+	−
P0723_RT1429.1_mz87.1	N-Ethylacetamide	Amide	1641	1608	1.51	+	−
P1203_RT2103.9_mz72.1	N-Acetylglycine	Amide	2149	?	1.18	+	−
P1500_RT2463.4_mz61	2-(Isopropylthio)pentane	Sulfur-containing volatile	2553	?	1.22	+	−
P0579_RT1192.8_mz83	Tetrachloroethane	Halogen-containing volatile	1501	1516	1.7	+	−
P0416_RT916.4_mz57.1	Unknown branched alkane	Alkane	1353	?	1.72	+	−
P0235_RT634.4_mz71.1	Unknown alkane	Alkane	1190	?	1.71	+	−
P0230_RT627.4_mz57.1	Unknown alkane	Alkane	1190	?	1.64	+	−
P0473_RT1026.3_mz57.1	Unknown alkane	Alkane	1410	?	1.43	+	−
P0662_RT1330.2_mz57.1	Unknown branched alkane	Alkane	1581	?	1.34	+	−
P0578_RT1189.6_mz57.1	Unknown branched alkane	Alkane	1501	?	1.31	+	−
P0328_RT771.6_mz57.1	Unknown branched alkane	Alkane	1270	?	1.3	+	−
P0495_RT1068.9_mz57.1	Unknown alkane	Alkane	1435	?	1.27	+	−
P0588_RT1206.8_mz57.1	Unknown alkane	Alkane	1505	?	1.2	+	−
P0164_RT477.1_mz57.1	Unknown branched alkane	Alkane	1108	?	1.17	+	−
P0608_RT1234.6_mz57.1	Unknown branched alkane	Alkane	1528	?	1.17	+	−
P0664_RT1340.1_mz57.1	Unknown alkane	Alkane	1588	?	1.15	+	−
P0478_RT1044_mz57.1	Unknown alkane	Alkane	1421	?	1.1	+	−
P1024_RT1846.2_mz57.1	Unknown alkane	Alkane	1917	?	1.09	+	−
P0542_RT1126.1_mz57.1	Unknown alkane	Alkane	1464	?	1.09	+	−
P0222_RT594.8_mz57.1	Unknown branched alkane	Alkane	1174	?	1.07	+	−
P0500_RT1079.1_mz57.1	Unknown alkane	Alkane	1437	?	1.06	+	−
P0152_RT461.4_mz69.1	Unknown alkane	Alkane	1100	?	1.05	+	−
P0265_RT671.9_mz57.1	Unknown alkane	Alkane	1216	?	1.04	+	−
P0280_RT699.2_mz71.1	Unknown alkane	Alkane	1230	?	1.01	+	−

*RI: retention index; -: lower abundance; +: higher abundance; ? indicates no literature reference retention index was found.

features were well modelled by the interaction between gender and age, showing their importance in determining the variation in wine expectorate profiles. Fig. 4 presents the boxplots showing differences in the four groups of participants (young, senior, male and female) of the most important volatiles obtained from ASCA. We also observed variable changed patterns in different features, which indicate that there are more factors apart from the age and gender affecting the volatile profile of expectorated wines.

Interestingly, while looking at the change patterns, we found that gender related differences were greater in young individuals than seniors for both male and female participants (Fig. 4). These results were in agreement with previous studies where gender differences in the *in vivo* wine aroma release were more evident in young than senior participants. These differences were related to compositional variation of saliva among age groups (Criado, Pérez-Jiménez et al., 2021).

As shown in Fig. 4, within the four groups of subjects (senior female, young female, senior male, young male), the young male group showed the lowest abundance of ketones (acetoin), alcohols (2-methyl-1-butanol), esters (ethyl-2-hydroxypropanoate, diethyl butanedioate) and acids (acetic acid, isobutyric acid) (Fig. 4) in comparison to other groups. Samples from senior participants, on the other hand, had a higher abundance of alcohol (2-methyl-1-butanol) and esters (ethyl-2-hydroxypropanoate, diethyl butanedioate) in senior female group, and a higher abundance of alkane and unknown compounds (unknown and

unknown alcohol) in senior male group (Fig. 4). None of these volatile features were previously identified as susceptible to oral metabolism, thus providing new knowledge on the impact of gender and age interaction on wine volatile profiling after oral processing.

4. Conclusions

Here, we showed that the SOOM procedure coupled with untargeted volatile analysis by GC-TOF-MS, is a suitable tool to determine the influence of oral processing on volatile profile of wine. While previously published studies on wine oral processing generated data on 20–30 targeted aroma compounds, our approach described here provided information on 248 volatile features. Apart from the endogenous (normal body metabolism) or exogenous origin (residues from food or cosmetic products) of volatiles, we observed the formation of different alcohols and carboxylic acids because of oral metabolism. Salivary enzymes (esterases, peroxidases) may have a role in these transformations. While exploring the impact of intrinsic biological variables on the volatile profile during the oral processing of wine, we found both age and gender impact the aroma composition of wines during oral processing. Additionally, the interaction of both factors (age and gender) showed that gender related differences were greater in young individuals than seniors. We also identified the most important features responsible for this variation depending on age and gender. Data generated in this study also
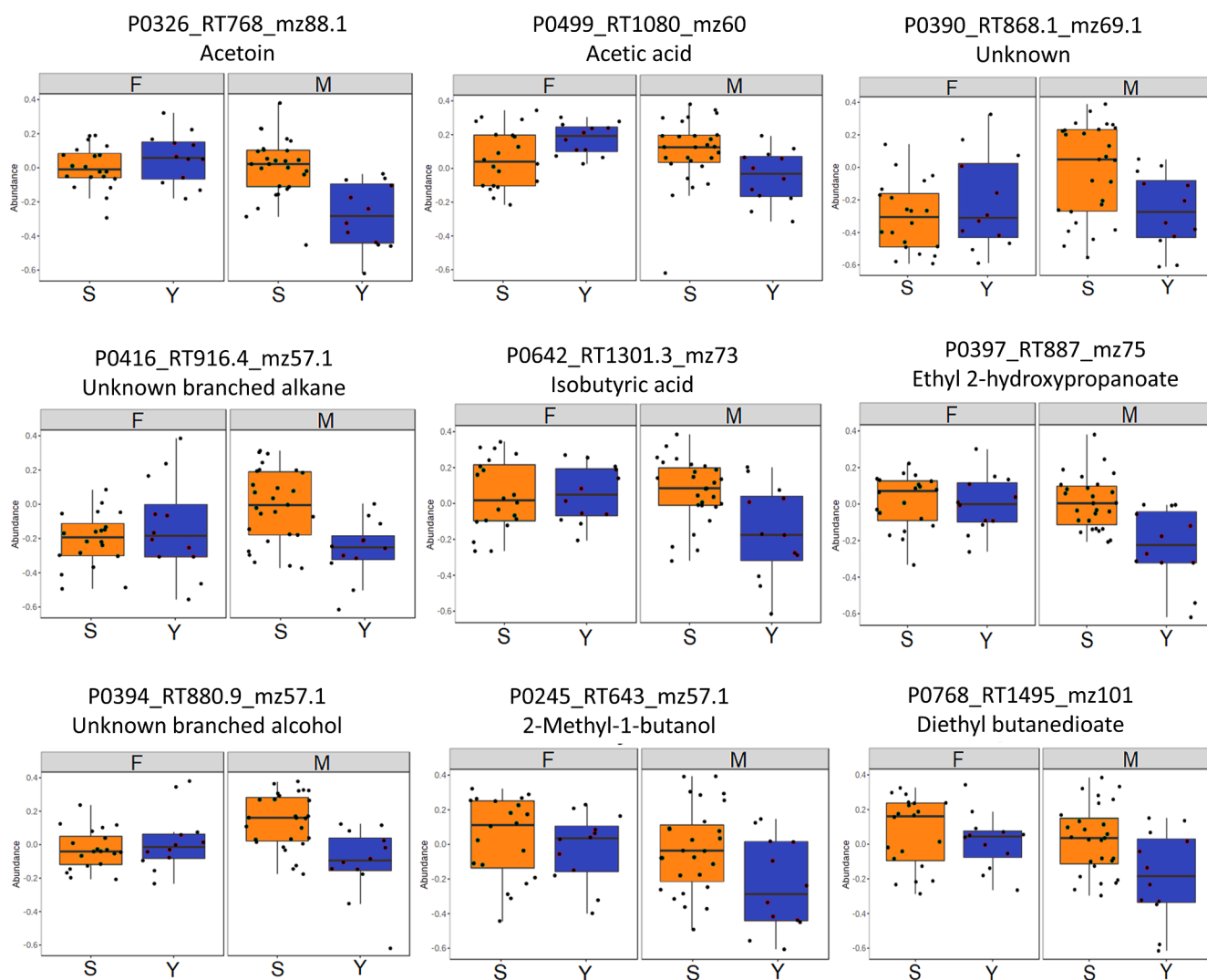

Fig. 4. Boxplots showing the differences in nine important volatile metabolites in expectorated wines collected from male and female participants depending on age and gender. Features were determined using ANOVA - Simultaneous Component Analysis (ASCA). Here, F = Female, M = Male, S = Senior and Y = Young.

allowed us to expand our understanding on the evolution of wine aroma compounds in the mouth and confirmed changes in volatile profiles in wine expectorated samples, generating new knowledge about the effect of oral processing of wine on its volatile composition. We also identified a few new areas that require further investigation to determine the sensory relevance of this process.

Funding sources

CRediT authorship contribution statement

María Pérez-Jiménez: Conceptualization, Methodology, Investigation, Data curation, Writing – original draft, Writing – review & editing. **Emma Sherman:** Methodology, Investigation, Formal analysis, Visualization, Data curation, Writing – original draft, Writing – review & editing. **María Ángeles Pozo-Bayón:** Conceptualization, Supervision, Project administration, Funding acquisition, Writing – review & editing. **Carolina Muñoz-González:** Writing – review & editing. **Farhana R. Pinu:** Conceptualization, Methodology, Investigation, Visualization, Supervision, Project administration, Funding acquisition, Writing – original draft, Writing – review & editing.

Declaration of Competing Interest

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

Data availability

Data will be made available on request.

Acknowledgements

Authors greatly thank the volunteers for their participation in this study.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodchem.2022.134307.

References

Amann, A., de Lacy Costello, B., Miekisch, W., Schubert, J., Buszewski, B., Pleil, J., … Risby, T. (2014). The human volatilome: Volatile organic compounds (VOCs) in exhaled breath, skin emanations, urine, feces and saliva. *Journal of breath research, 8* (3), Article 034001.

Christoph, N., & Bauer-Christoph, C. (2007). In *Flavour of spirit drinks: Raw materials, fermentation, distillation, and ageing* (pp. 219–239). Springer.

Criado, C., Muñoz-González, C., & Pozo-Bayón, M.Á. (2021). Differences in salivary flow and composition between age groups are correlated to dynamic retronasal aroma perception during wine consumption. *Food Quality and Preference, 87*, Article 104046.

Criado, C., Pérez-Jiménez, M., Muñoz-González, C., & Pozo-Bayón, M.Á. (2021). Understanding the effect of saliva composition depending on gender and age on wine aroma perception: Oral aroma release, dynamics of sensory perception and consumer preferences and liking. *Proceedings of the 16th Weurman Flavour Research Symposium*.

Dean, R. A., Christian, C. D., Sample, R. B., & Bosron, W. F. (1991). Human liver cocaine esterases: Ethanol-mediated formation of ethylcocaine. *The FASEB Journal, 5*(12), 2735–2739.

Esteban-Fernández, A., Muñoz-González, C., Jiménez-Girón, A., Pérez-Jiménez, M., & Pozo-Bayón, M.Á. (2018). Aroma release in the oral cavity after wine intake is influenced by wine matrix composition. *Food Chemistry, 243*, 125–133.

Esteban-Fernández, A., Rocha-Alcubilla, N., Muñoz-González, C., Moreno-Arribas, M. V., & Pozo-Bayón, M.Á. (2016). Intra-oral adsorption and release of aroma compounds following in-mouth wine exposure. *Food Chemistry, 205*, 280–288.

Ferreira, V., Bueno, M., Franco-Luesma, E., Cullere, L., & Fernandez-Zurbano, P. (2014). Key changes in wine aroma active compounds during bottle storage of Spanish red wines under different oxygen levels. *Journal of Agricultural and Food Chemistry, 62* (41), 10015–10027.

Hernandez-Orte, P., Concejero, B., Astrain, J., Lacau, B., Cacho, J., & Ferreira, V. (2015). Influence of viticulture practices on grape aroma precursors and their relation with wine aroma. *Journal of the Science of Food and Agriculture, 95*(4), 688–701.

Ijichi, C., Wakabayashi, H., Sugiyama, S., Ihara, Y., Nogi, Y., Nagashima, A., … Touhara, K. (2019). Metabolism of odorant molecules in human nasal/oral cavity affects the odorant perception. *Chemical Senses, 44*(7), 465–481.

Juan, F. S., Cacho, J., Ferreira, V., & Escudero, A. (2012). Aroma chemical composition of red wines from different price categories and its relationship to quality. *Journal of Agricultural and Food Chemistry, 60*(20), 5045–5056.

Melis, M., Mastinu, M., Pintus, S., Cabras, T., Crnjar, R., & Tomassini Barbarossa, I. (2021). Differences in salivary proteins as a function of PROP taster status and gender in normal weight and obese subjects. *Molecules, 26*(8), 2244.

Miekisch, W., Schubert, J. K., & Noeldge-Schomburg, G. F. (2004). Diagnostic potential of breath analysis—focus on volatile organic compounds. *Clinica Chimica Acta, 347* (1–2), 25–39.

Milanowski, M., Pomastowski, P., Ligor, T., & Buszewski, B. (2017). Saliva–volatile biomarkers and profiles. *Critical Reviews in Analytical Chemistry, 47*(3), 251–266.

Muñoz-Gonzalez, C., Brulé, M., Martin, C., Feron, G., & Canon, F. (2021). Molecular mechanisms of aroma persistence: From noncovalent interactions between aroma compounds and oral mucosa to metabolization of aroma compounds by saliva and oral cells. *Food Chemistry, 373*, Article 131467.

Muñoz-González, C., Cueva, C., Pozo-Bayon, M. A., & Moreno-Arribas, M. V. (2015). Ability of human oral microbiota to produce wine odorant aglycones from odourless grape glycosidic aroma precursors. *Food Chemistry, 187*, 112–119.

Muñoz-González, C., Feron, G., Brulé, M., & Canon, F. (2018). Understanding the release and metabolism of aroma compounds using micro-volume saliva samples by ex vivo approaches. *Food Chemistry, 240*, 275–285.

Muñoz-González, C., Feron, G., & Canon, F. (2020). Physiological and oral parameters contribute prediction of retronasal aroma release in an elderly cohort. *Food Chemistry, 128355*.

Muñoz-González, C., Pérez-Jiménez, M., & Pozo-Bayón, M.Á. (2020). Oral persistence of esters is affected by wine matrix composition. *Food Research International, 135*, Article 109286.

Muñoz-González, C., Vandenberghe-Descamps, M., Feron, G., Canon, F., Labouré, H., & Sulmont-Rossé, C. (2018). Association between salivary hypofunction and food consumption in the elderlies. A systematic literature review. *Journal of Nutrition, Health and Aging, 22*(3), 407–419.

Nelson, J., Jensen, R., & Pitas, R. (1977). Pregastric esterase and other oral lipases—a review. *Journal of Dairy Science, 60*(3), 327–362.

Nicolli, K. P., Biasoto, A. C., Souza-Silva, É. A., Guerra, C. C., Dos Santos, H. P., Welke, J. E., & Zini, C. A. (2018). Sensory, olfactometry and comprehensive two-dimensional gas chromatography analyses as appropriate tools to characterize the effects of vine management on wine aroma. *Food Chemistry, 243*, 103–117.

Pang, Z., Chong, J., Zhou, G., de Lima Morais, Chang, L., & Xia, J. (2021). MetaboAnalyst 5.0: narrowing the gap between raw spectra and functional insights. *Nucleic acids research, 49*(W1), W388–W396.

Parker, M., Capone, D. L., Francis, I. L., & Herderich, M. J. (2017). Aroma precursors in grapes and wine: Flavor release during wine production and consumption. *Journal of Agricultural and Food Chemistry, 66*(10), 2281–2286.

Perez-Jiménez, M., Esteban-Fernández, A., Muñoz-González, C., & Pozo-Bayón, M. A. (2020). Interactions among odorants, phenolic compounds, and oral components and their effects on wine aroma volatility. *Molecules, 25*(7), 1701.

Pérez-Jiménez, M., Muñoz-González, C., & Pozo-Bayón, M. A. (2020). Understanding human salivary esterase activity and its variation under wine consumption conditions. *RSC Advances, 10*(41), 24352–24361.

Pérez-Jiménez, M., Muñoz-González, C., & Pozo-Bayón, M. A. (2021). Oral Release behavior of wine aroma compounds by using in-mouth headspace sorptive extraction (HSSE) method. *Foods, 10*(2), 415.

Pérez-Jiménez, M., Rocha-Alcubilla, N., & Pozo-Bayón, M. A. (2019). Effect of saliva esterase activity on ester solutions and possible consequences for the in-mouth ester release during wine intake [Article]. *Journal of Texture Studies, 50*(1), 62–70.

Pérez-Jiménez, Sherman, E., Pozo-Bayón, M. A., & Pinu, F. R. (2021). Application of untargeted volatile profiling and data driven approaches in wine flavoromics research. *Food Research International, 145*, Article 110392.

Pinu, F. R. (2018). Grape and wine metabolomics to develop new insights using untargeted and targeted approaches. *Fermentation, 4*(4), 92.

Pinu, F. R., Carvalho-Silva, D., Trovatti Uetanabaro, A. P., & Villas-Boas, S. G. (2016). Vinegar metabolomics: An explorative study of commercial balsamic vinegars using gas chromatography-mass spectrometry. *Metabolites, 6*(3), 22.

Ployon, S., Brulé, M., Andriot, I., Morzel, M., & Canon, F. (2020). Understanding retention and metabolization of aroma compounds using an in vitro model of oral mucosa. *Food Chemistry, 318*, Article 126468.

Qian, X., Lan, Y., Han, S., Liang, N., Zhu, B., Shi, Y., & Duan, C. (2020). Comprehensive investigation of lactones and furanones in icewines and dry wines using gas chromatography-triple quadrupole mass spectrometry. *Food Research International, 137*, Article 109650.

Schipper, R. G., Silletti, E., & Vingerhoeds, M. H. (2007). Saliva as research material: Biochemical, physicochemical and practical aspects. *Archives of oral biology, 52*(12), 1114–1135.

Schwartz, M., Neiers, F., Charles, J. P., Heydel, J. M., Muñoz-González, C., Feron, G., & Canon, F. (2021). Oral enzymatic detoxification system: Insights obtained from proteome analysis to understand its potential impact on aroma metabolization. *Comprehensive Reviews in Food Science and Food Safety*.

Sherman, E., Coe, M., Grose, C., Martin, D., & Greenwood, D. R. (2020). Metabolomics approach to assess the relative contributions of the volatile and non-volatile composition to expert quality ratings of pinot noir wine quality. *Journal of agricultural and food chemistry, 68*(47), 13380–13396.

Spicer, R. A., Salek, R., & Steinbeck, C. (2017). A decade after the metabolomics standards initiative it's time for a revision. *Scientific Data, 4*(1), 1–3.

Sumby, K. M., Grbin, P. R., & Jiranek, V. (2010). Microbial modulation of aromatic esters in wine: Current knowledge and future prospects. *Food Chemistry, 121*(1), 1–16.

Tinnevelt, G. H., Engelke, U. F., Wevers, R. A., Veenhuis, S., Willemsen, M. A., Coene, K. L., & Jansen, J. J. (2020). Variable selection in untargeted metabolomics and the danger of sparsity. *Metabolites, 10*(11), 470.