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Insights on the effect of age and gender on in-mouth volatile release during wine tasting

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ABSTRACT

This study focus for the first time, in looking for age-gender effects on *in vivo* volatile release during wine consumption, also considering oral physiological differences (e.g. saliva composition). To do so, the *in-mouth* Head Space Sorptive Extraction technique was used, which allowed monitoring the oral release of twenty-four different types of volatile compounds from white and red wines. Thirty-two individuals (n = 32) males and females, belonging to two different age groups: young (18–35 y.o) and senior (>55 y.o.) participated in this analytical *in vivo* study. Results showed differences in volatile release among age-gender groups, which also depended on the volatile compound and wine type. Senior groups (SM, SF) showed a similar release behaviour among them. Contrarily, young males showed a higher release (between 10 and 29%) of alcohols and esters indistinctly of the wine type, while young females showed the lowest oral volatile release among the four age-gender groups. Gender differences in volatile release were more evident in young than in seniors. A higher release of furanic compounds (furfural and 5-methyl furfural) in seniors was likely related to differences in their saliva composition (total protein content, minerals (Mg, Zn) and α-amylase activity).

1. Introduction

The influence of age and gender on food and beverage odour perception has been investigated in different works (Doty & Kamath, 2009; Gamsakhurdashvili, Antov, Lübke, Pause, & Stockhorst, 2021; Sorokowski et al., 2019; Wang, Zhang, Xia, Yang, & Zhou, 2019; Xu, Liu, Wroblewski, McClintock, & Pinto, 2020). In some of these works, it was shown that women have greater odour detection (Sorokowski et al., 2019), identification (Doty & Kamath, 2009; Sorokowski et al., 2019; Wang et al., 2019), discrimination (Doty & Kamath, 2009; Gamsakhurdashvili et al., 2021; Sorokowski et al., 2019) and memory (Doty & Kamath, 2009) than men. Differences in sex hormones might be one of the main influencing factors that could explain the different odour performance by sex/gender (Doty & Kamath, 2009; Sorokowski et al., 2019; Wang et al., 2019). In this way, it has been shown that there are variations in the odour perception of women during their menstrual cycle or pregnancy, with a higher odour perception during the ovulation phase (Navarrete-Palacios, Hudson, Reyes-Guerrero, & Guevara-Guzmán, 2003) or the later phases of pregnancy (Cameron, 2014). On the other hand, in the case of age, a lower aroma intensity perception by seniors than young adults has been shown (Doty & Kamath, 2014; Lester, Taylor, Corbier, Cornacchia, & Fisk, 2020; Xu et al., 2020). This age-

Abbreviations: **YF**, young-female; **YM**, young-male; **SF**, senior-female; **SM**, senior-male; **IsoAce**, isoamyl acetate; **E.But**, ethyl butanoate; **E.Pen**, ethyl pentanoate; **E.Hex**, ethyl hexanoate; **E.Oct**, ethyl octanoate; **E.Dec**, ethyl decanoate; **DieSuc**, diethyl succinate; **PheAce**, phenethyl acetate; **E.Cin**, ethyl cinnamate; **Btol**, butanol; **Pntol**, pentane; **Hxol**, hexanol; **E-3-Hxol**, E-3-hexenol; **BnzAlch**, benzyl alcohol; **Phethol**, phenyl ethanol; **Fur**, furfural; **MethFur**, 5-methyl furfural; **FurAlch**, furfuryl alcohol; **α-Pin**, α-pinene; **β-Pin**, β-pinene; **Lim**, limonene; **α-Ion**, α-ionone; **γ-But**, γ-butyrolactone; **E-W.Lac**, E-whisky lactone; **Z-W.Lac**, Z-whisky lactone; **C_{13}-nrsp**, C_{13}-norisoprenoids; **%AR**, percentage of aroma release 60 s after spat out the wine; **TPC**, total protein concentration; **TSEA**, total salivary esterase activity; **α-amy**, α-amylase activity.

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related olfactory dysfunction has been associated to multiple factors at both, the olfactory and brain level. Some examples include nasal diseases, alterations in the mucosal enzymes, a reduction in the selectivity of olfactory receptor cells (Doty & Kamath, 2014), damage in the olfactory epithelium (Lester et al., 2020) or changes in the neurotransmitter system; and it has also been related to the high amount of medication taken by the elderly population (Doty & Kamath, 2014). Understanding the impact of biological factors on aroma perception, which might also influence food choices, could be an interesting approach for the food industry to make more personalised foods and beverages.

Most of these studies have only considered the orthonasal perception of odorants, which is based on the odorant compounds that enter the nose with the airflow when sniffing. Although very valuable, they do not represent aroma perception during food/beverage intake. In this case, odorants are introduced into the mouth and transferred from the food to the saliva phase. Once in the mouth, they interact with this fluid, before they can be released into the air phase of the oral cavity and migrate to the nasal cavity via the nasopharynx, where they interact with the olfactory receptors (retronasal aroma). In fact, saliva has been highlighted as one of the main factors contributing to aroma release during food intake by its different effects, such as dilution, chemical and physical interactions or enzymatic metabolism of odorants (Muñoz-González, Feron, & Canon, 2018; Piombino et al., 2014).

As far as the authors know, only two recent scientific papers have dealt with the effect of age or gender on retronasal aroma release using *in-nose* proton transfer reaction mass spectrometry approaches (PTR-MS) and using flavoured model solutions (Muñoz-González, Feron, & Canon, 2020) or mint flavoured chewing-gum (Pedrotti, Spaccasassi, Biasioli, & Fogliano, 2019). However, in the case of wine, with a compositionally complex aroma profile and whose consumption is mainly triggered by hedonic clues, the influence of age and gender on retronasal aroma perception has been scarcely investigated. Only in one recent study, wine retronasal aroma perception considering differences in age and gender has been evaluated by a sensory approach using Time-Intensity assays. In this work, senior individuals (>55 years old) rated higher retronasal intensity and for longer times with some specific red wine aroma descriptors (smoked and black pepper) than younger adults (18–35 years old) (Criado, Muñoz-González, & Pozo-Bayón, 2021). These differences were partially related to variations in salivary flow and composition (proteins) between age-groups, which could have affected the kinetics of aroma release in the mouth during wine tasting (Criado et al., 2021). This hypothesis requires new *in vivo* analytical studies, focused on determining if saliva composition modifies the oral release of the many different types of compounds belonging to the wine volatile profile.

Interestingly, saliva composition can also be affected by age (Bel'skaya, Sarf, & Kosenok, 2020), and gender (Nascimento, Cassiani, & Dantas, 2012). For instance, some salivary parameters such as total protein concentration, antioxidant capacity, electrolyte content or salivary flow have been shown to be affected by age and/or gender (de Almeida, Gregio, Machado, De Lima, & Azevedo, 2008; Nagler & Hershkovich, 2005; Sun et al., 2014; Xu, Laguna, & Sarkar, 2019).

Hence, the aim of this work was to determine the effect of age and gender on oral volatile release using two compositionally different commercial wines (aged red wine and white wine), considering the physiological differences in saliva composition of a group of 32 individuals. For this, volunteers were grouped according to their age and gender, young-female (YF), young-male (YM), senior-female (SF) and senior-male (SM). Then, the oral aroma release of twenty-four volatile compounds was monitored after rinsing the mouth with the wine by using the *in-mouth* Head Space Sorptive Extraction (HSSE) method. Additionally, the salivary flow and composition (pH, viscosity, minerals, total protein content, total esterase and α-amylase activities) were determined for the 32 individuals. Finally, the relationship between the release of wine volatiles in the oral cavity and saliva composition was investigated.

2. Materials and methods

2.1. Individuals

Thirty-two healthy individuals were selected for this study depending on their age (young or senior adults) and gender (female or male). The young individuals (YF, YM) included young adults with ages ranging between 18 and 35 years old (n = 16), while the senior individuals were composed of adults older than 55 years old (n = 16). The number of participants was selected considering the complexity and restrictions imposed by the *in vivo* analytical approach we followed in this study. For instance: the availability of volunteers for performing all the assays at specific days and times, the high number of volatiles monitored in each assay in the mouth (over 20 volatile compounds), the long duration of each chromatographic run (>60 min), the need to perform the chromatographic runs (192 runs in total) immediately after the extraction of the aroma in the mouth and the costs associated with this type of instrumental *in vivo* analysis.

The experimental protocol of the study was explained to the volunteers, who gave their written consent prior to participating. The Bioethical Committee of the Spanish Council of Research (CSIC) authorized this study.

2.2. Saliva

2.2.1. Saliva collection

Saliva collection was conducted in the morning between 9:30 and 11:00 a.m. on three different days, always immediately before the *in-mouth* HSSE assay. Volunteers were not allowed to eat or drink 1 h before saliva donation. Stimulate saliva was collected during 5 min in sterile plastic tubes (VWR, Pennsylvania, EEUU) by chewing a little piece of Parafilm™, as previously described (Pérez-Jiménez, Muñoz-González, & Pozo-Bayón, 2020). The saliva collected three times from the same volunteer was mixed together. The final collected volume depended on the panellist, but it was enough to perform all the analytical and bioanalytical analyses.

2.2.2. Saliva analysis

Salivary flow was estimated by weighing the plastic tubes before and after saliva collection and it was expressed as mg/mL. Immediately after collection, salivary pH was measured using a CP-505 pH meter (Elmetron, Zabrze, Poland). Subsequently, saliva samples were centrifuged (15000g for 15 min at 4 °C) and supernatants from each individual were pooled separately. From here, 1.5 mL aliquots were prepared in Eppendorf tubes (Eppendorf Ibérica S.L.U. Madrid, Spain) and stored at −80 °C until analysis. The viscosity at a shear rate of 50 s^{-1}, minerals (Zn, Na, K, Ca, Mg), total protein concentration (TPC), total salivary esterase activity (TSEA) and α-amylase activity were determined in each saliva supernatant from the 32 individuals following the analytical methods already described (Criado et al., 2019; Pérez-Jiménez et al., 2020). The selection of these parameters was based in the correlations found between salivary parameters and aroma perception in previous sensory studies (Criado et al., 2019; C. Muñoz-González, Brule, Feron, & Canon, 2019; Perez-Jiménez, Chaya, & Pozo-Bayón, 2019; Pérez-Jiménez, Rocha-Alcubilla, & Pozo-Bayón, 2019).

2.3. Wines

Two wines (red and white) from the Marqués de Murrieta winery (La Rioja, Spain) were employed in this study. The white wine ("Pazo de Barrantes", vintage 2017) had a 13.3% ethanol content (*v/v*) and was made from the Albariño white grape variety, while the aged red wine ("Marqués de Murrieta", vintage 2014) presented an ethanol content of 14% (*v/v*) and was made from Tempranillo (84%), Graciano (9%),

Mazuelo (5%) and Garnacha (2%) red grape varieties. The both volatile and non-volatile (total polyphenols, total procyanidins, neutral polysaccharides, free amino acids and free amino acids plus peptides, colour intensity) analytical characterization of the wines was carried out as previously described (Perez-Jimenez & Pozo-Bayon, 2019), and is shown in Table S1.

2.4. In-mouth HSSE method

For the in-mouth volatile analysis, the previously published *in-mouth* HSSE methodology (Perez-Jimenez & Pozo-Bayon, 2019) was employed with little modifications. Briefly, this procedure consisted of rinsing the mouth with 15 mL of wine during 30 s and an in-mouth aroma extraction (during 30 s) using a PDMS (20 mm length × 0.5 mm film thickness) twister (Gerstel, Germany) one min after spitting out the wine. For this, the twister was placed in a perforated glass holder device, which allowed the monitoring of the oral headspace of the volunteers. Before wine rinsing, controls of the oral cavity after water rinsing were performed to ensure the absence of any of the wine volatile compounds that were monitored in this study. After the in-mouth extraction, the twister was briefly dried with a tissue and placed in the thermodesorption unit (TDU) (Gerstel) of the gas chromatograph-mass spectrometer (GC–MS). The same procedure was repeated three times per wine by each volunteer (n = 32). In total 192 chromatograms (32 individuals × two wines × three repetitions) were obtained. Between assays, the participants cleaned their mouth with a pectin water solution (1 g/L) and waited for 15 min.

All the oral aroma samplings were performed 60 s after spitting out the wine. This sampling time was selected to reduce intra-individual variability by ensuring the equilibration of volatile compounds between the liquid phase (residues of wine in the oral cavity) and the gaseous phase, as has already been shown (Esteban-Fernández, Rocha-Alcubilla, Muñoz-González, Moreno-Arribas, & Pozo-Bayón, 2016). In addition to this, the volatile released 60 s after wine intake could be more related to the wine aroma persistence (Perez-Jiménez et al., 2019).

2.5. Thermodesorption and GC–MS analysis

For the thermodesorption and GC–MS analysis of the twisters, the analytical conditions previously described (Perez-Jimenez & Pozo-Bayon, 2019) were selected. Briefly, the twisters were desorbed in the TDU in splitless mode coupled to a Cooled Injection System (CIS-4) (Gerstel) in order to trap the analytes by cryofocusing before they reached the column. The samples were analysed in a 6890 N GC coupled to a 5973 MS (Agilent). The analytes were separated in a DB-WAX column (30 m × 0.25 mm and 0.50 μm film thickness) provided by J&W Scientific (Folsom, CA). The temperature program of the oven was configured following the same conditions as previously described (Perez-Jimenez & Pozo-Bayon, 2019). The acquisition was configured in both scan and SIM modes (mas range from 35 to 350 *m/z*). All the volatile compounds were identified by comparison of mass spectra and retention times with those from reference standards. The amount of each volatile in the oral cavity (oral aroma release) was expressed as absolute peak areas (APAs).

2.6. Statistical analysis

All the analyses were performed using the XLSTAT software (v.2020.3.1) (Addinsoft, Paris, France), and a significance level of 0.05 was considered. Firstly, one-way ANOVA per each wine type (red and white) was carried out to check for significant differences in oral aroma release among the four groups of subjects (YF, YM, SF, SM). Secondly, a stepwise linear discriminant analysis (SLDA) was used to corroborate these differences among the four age-gender groups. The Wilk's lambda method was chosen as criteria for the selection of variables. One-way ANOVA was used to check for differences in saliva composition among age-gender groups. Finally, principal component analysis (PCA) to study the relationship between oral aroma release and saliva composition depending on the age-gender groups was performed per each wine type. In this case, aroma release and saliva compositional data were included in the same dataset.

3. Results

3.1. Differences in oral aroma release among age-gender groups

A total of 24 and 23 volatile compounds were identified in the oral cavity after white and red wine intake (Table S2), respectively. In general, the same chemical families of odorants were identified in both wines, although there were some differences between wines in some aroma compounds. For instance, more number of esters (ethyl butanoate and hexyl acetate) and alcohols (butanol and E-3-hexenol) were identified in the white wine, while more terpenes (α-pinene and β-pinene) and lactones (E- and Z- whisky lactones) were identified in the red wine. In addition to this, the amount of volatile compounds determined in both wines was quite different. For instance, higher peak areas of esters were determined in white wine, while higher peak areas of terpenes, furanic compounds or C_{13} norisoprenoids were determined in the red wine. These results showed the differences in oral volatile release depending on the wine type.

Furthermore, to study the effect of the age and the gender on the volatiles released 60 s after wine tasting, the individuals were grouped in four groups: young-female (YF), young-male (YM), senior female (SF) and senior male (SM). Then, to check if there were significant differences considering the four groups of individuals one-way ANOVA was performed with aroma release data from each wine type (red or white). These results are shown in Table S2.

As it can be seen in Table S2, results from one-way ANOVA showed significant differences among the four age-gender groups for most volatile compounds. Interestingly, a higher number of volatile compounds, mainly esters and alcohols, were affected by age-gender groups when tasting the white wine (22 compounds from 24 identified) than when tasting the red wine (16 compounds from 23 identified) (Table S2). This means that after tasting white wines, a higher percentage of aroma compounds (above 91%) were affected by the age-gender group, while this effect was minor (69%) in the case of red wines. The effect of wine matrix composition on oral aroma release using different–*in vivo* approaches has also been reported (Esteban-Fernández, Muñoz-González, Jiménez-Girón, Pérez-Jiménez, & Pozo-Bayón, 2018; Perez-Jimenez & Pozo-Bayon, 2019).

To ascertain the differences among the four age-gender groups based on the differences observed in oral aroma release, stepwise linear discriminant analysis was carried out (SLDA). For this analysis, only those volatile compounds which already showed significant differences among age-gender groups by ANOVA (Table S2) were employed. Two analyses, one for each wine type were also performed.

The SLDA model extracted 21 and 15 volatile compounds (variables) in the white and red wine, respectively, according to Wilks lambda criterion. The selected variables were employed to calculate the discriminant functions (data not shown) that were employed to classify the samples and to obtain the correct classification matrix, which is shown in Table 1. The percentage of correct classification (%CC) of the oral aroma release data in the previously defined four groups (YF, YM, SF, SM) for each wine type is also shown in Table 1.

As it can be observed (Table 1), the total correct classification percentage (%CC) of the samples assigned to the group that truly belonged was higher in the white wine (%CC = 100), than in the red one (%CC = 87.5). In the white wine, all the samples (volatile release data) from the four groups of subjects (YF, YM, SF, SM) were correctly classified. However, in the case of the red wine, although all the samples from the younger group (YF, YM) were correctly classified, in the case of the senior group, there were two samples, from both the SF and SM groups,

Table 1
Classification matrix of the samples in the different age-gender groups.

Wine	Observed groups	Predicted groups				N° of samples	%CC
White		YF	YM	SF	SM		
	YF	8	0	0	0	8	100%
	YM	0	8	0	0	8	100%
	SF	0	0	7	0	7	100%
	SM	0	0	0	9	9	100%
	Total	8	8	7	9	32	100%
Red							
	YF	8	0	0	0	8	100%
	YM	0	8	0	0	8	100%
	SF	1	0	5	1	7	71.43%
	SM	2	0	0	7	9	77.78%
	Total	11	8	5	8	32	87.50%

*YF: young-female; YM: young-male; SF: senior-female; SM: senior-male.

which were incorrectly classified in the group that truly belonged. This means more variability between males and females in the aroma release behaviour within the seniors when tasting red wines.

The graphically representation of these results is shown in Fig. 1. Here, the four age-gender groups remained quite separated, which means a good classification of the individuals depending on both their age and gender (Fig. 1a). In contrast, during red wine tasting (Fig. 1b), the classification by gender within seniors was less evident, although the classification by gender within the younger groups was still evident. This worse classification of the seniors than the young depending on their gender could be related to fewer differences among them in their oral aroma release during red wine tasting. In previous works, no differences in the *in-nose* release of odorants were reported depending on the gender when using PTR-ToF-MS (Pedrotti et al., 2019). However, in the above-mentioned work, only the gender and not the age of the volunteers was considered when comparing the individuals. Thus, the study of both combined factors (age and gender), could have affected these results compared to the above-mentioned study.

In order to compare the effect of the age-gender group on the oral release considering specific wine volatiles, oral aroma release data (absolute volatile peak area) were expressed as percentage of aroma release (%AR), considering 100% as the aroma release of the individual who presented the highest value from any of the four groups. Fig. 2 showed the box plot obtained with the %AR for each age-gender group and wine type. In this figure, only the compounds that showed significant differences among groups for each wine type (Table S2) are depicted.

As it can be observed (Fig. 2), oral aroma release was different for each age-gender group, but these differences were also dependent on the volatile chemical family and wine type. Despite this, some interesting trends related to differences by age-gender groups can be highlighted.

Remarkably, senior groups (SF, SM) showed a similar behaviour regarding the release of some specific types of volatile compounds, such as most esters (except phenethyl acetate), and the alcohols: pentanol, hexanol and phenylethanol, in the case of the white wine (Fig. 2a), and lactones, furanic compounds (except furfuryl alcohol), alcohols and esters in the case of the red wine (Fig. 2b). In fact, differences in oral release for most aroma compounds between senior males and female groups were lower than 10% (on average). In addition to this, the senior groups showed (on average) a higher release of terpenes (up to 25% more) than the younger groups, independent to the wine type assayed. This finding also agrees with results from a previous work (Muñoz-González, Feron, et al., 2020) in which the authors found a higher retronasal aroma release of ketones, aldehydes and terpenes as the age of

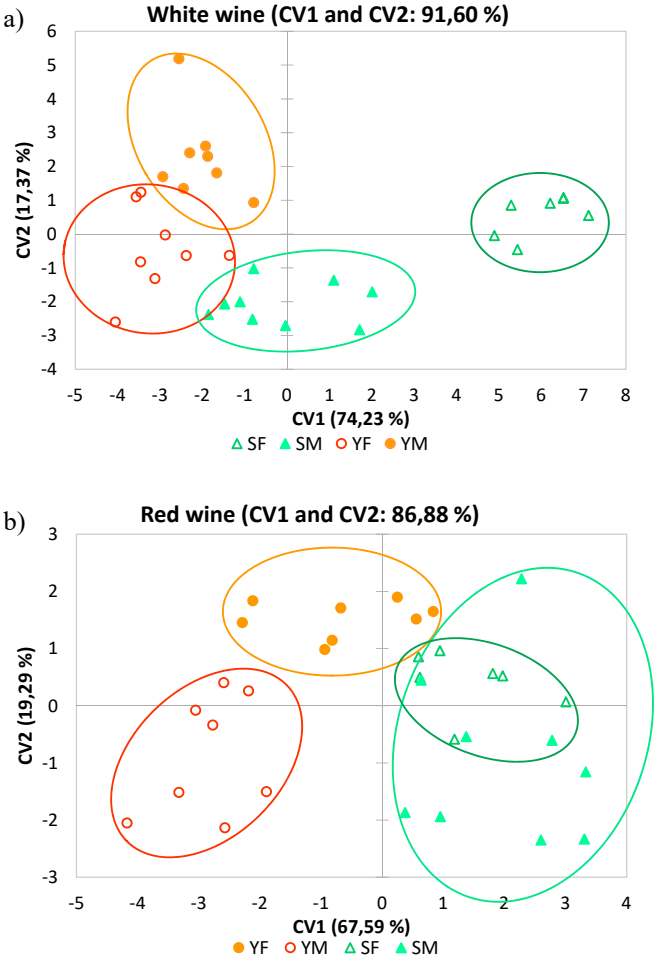

Fig. 1. Projection of oral aroma release data from the white (1a) and red wine (1b) assays in the plane defined by the first and second canonical variables (CV1 and CV2), obtained from the SLDA for the age-gender groups. (Each point represents a subject, ellipses are not related to statistical significance and only highlight the grouping of subjects). YF: young-female; YM: young-male; SF: senior-female; SM: senior-male. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

the participants increased. Additionally, the higher release of terpenes can be associated to the hydrolysis of wine glycosidic precursors by salivary enzymes and oral microbiota bacteria (Muñoz-González, Cueva, Pozo-Bayon, & Moreno-Arribas, 2015; Parker, Barker, et al., 2019; Parker, Onetto, et al., 2019).

On the other hand, the younger groups (YF, YM) showed greater differences in oral aroma release depending on gender. Most aroma compounds showed significant differences. A higher aroma release (between 10 and 29%) was found in YM compared to YF. Only E-3-hexenol, after the exposure to the white wine, and α-pinene, 5-methyl furfural and α-ionone after the oral exposure to the red wine did not show significant gender differences (<5% on average between YF and YM).

Considering the four age-gender groups, YF showed the lowest release of most aroma compounds in both wines, except for the compounds ethyl butanoate, ethyl pentanoate, furfural, 5-methyl furfural and α-ionone in the white wine, and the esters ethyl octanoate, ethyl decanoate and phenethyl acetate in the red wine. Excluding these compounds, the aroma released by YF was always between 5 and 20% (on average depending on the odorant type) lower than the aroma released from the other age-gender groups. On the contrary, from the four age-gender groups, the YM group showed the highest significant ($p < 0.05$) release of esters (except phenethyl acetate), alcohols (except E-

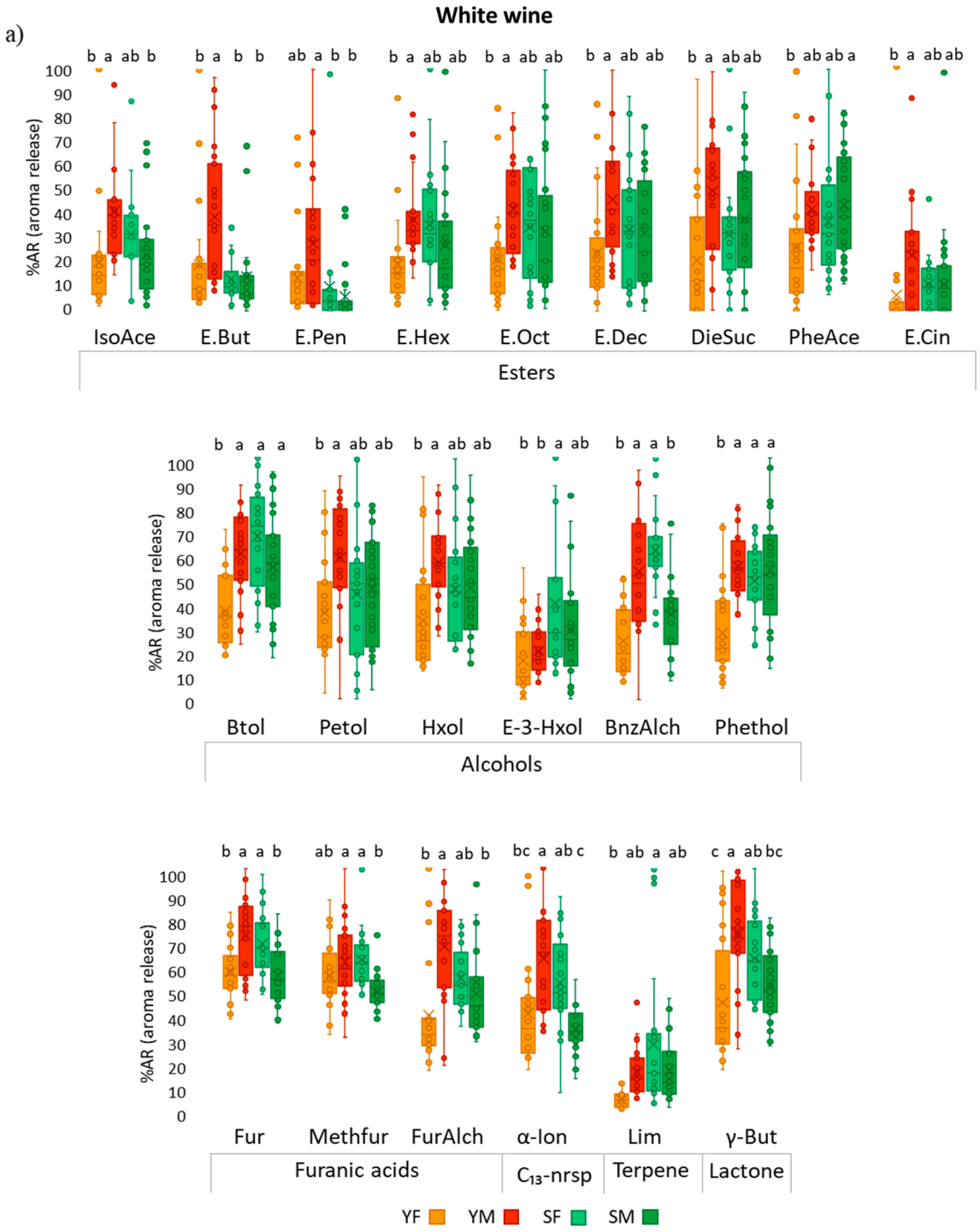

Fig. 2. Percentage of aroma release (%AR) 60 s after the oral rinsing with white (2a) and red wines (2b). Only volatile compounds that showed significant differences ($p < 0.05$) depending on the age-gender groups are shown. Letters above the boxes denote differences among groups from the Tukey test. YF: young-female; YM: young-male; SF: senior-female; SM: senior-male; IsoAce: isoamyl acetate; E.But: ethyl butanoate; E.Pen: ethyl pentanoate; E.Hex: ethyl hexanoate; E.Oct: ethyl octanoate; E.Dec: ethyl decanoate; DieSuc: diethyl succinate; PheAce: phenethyl acetate; E.Cin: ethyl cinnamate; Btol: butanol; Pntol: pentanol; Hxol: hexanol; E-3-Hxol: E-3-hexenol; BnzAlch: benzyl alcohol; Phethol: phenyl ethanol; Fur: furfural; MethFur: 5-methyl furfural; FurAlch: furfuryl alcohol; α-Pin: α-pinene; β-Pin: β-pinene; Lim: limonene; α-Ion: α-ionone; γ-But: γ-butyrolactone;; E-W.Lac: E-whisky lactone; Z-W.Lac: Z-whisky lactone; C_{13}-nrsp: C_{13}-norisoprenoids. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

3-hexenol and benzyl alcohol), lactones, furanic compounds and C_{13}-norisoprenoids in the white wine. YM group also showed the highest release of esters and alcohols (except phenethyl acetate and benzyl alcohol) in the red wine. In all the above-mentioned compounds, the YM group showed differences of up to 30% (mean value) in the %AR compared to the other age-gender groups. These results differed from those of a recent work, in which the authors did not find differences in aroma release from chewing gums between female and male young adults when using *–in nose* PTR-MS (Pedrotti et al., 2019). Differences in the type of aroma compound, matrix composition, techniques, and

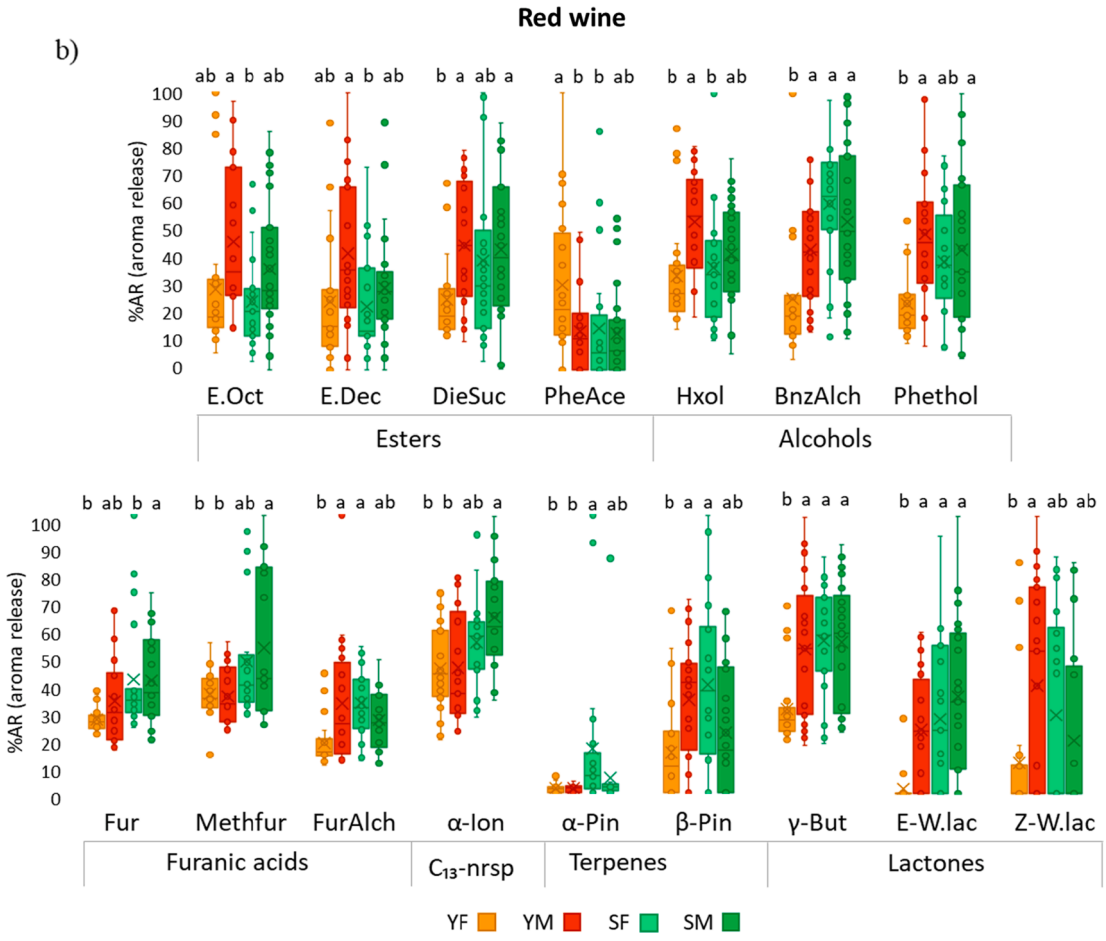

Fig. 2. (*continued*).

consumption procedures, could be the reason for this different result.

3.2. Relationship between oral aroma release and salivary composition in the age-gender groups

Since saliva has been described as one important factor involved in retronasal aroma release (Canon, Neiers, & Guichard, 2018), in a second step of the work, the differences in oral aroma release among age-gender groups were related to differences in salivary parameters such as pH, flow rate, rheological properties (viscosity at a shear rate of 50 s^{-1}), minerals (Zn, Na, K, Ca, Mg), total protein concentration (TPC), total salivary esterase activity (TSEA) and α-amylase activity. Firstly, salivary data were submitted to one-way ANOVA and Tukey test to check for significant differences ($p < 0.05$) among the four age-gender groups (YF, YM, SF, SM). Results are shown in Table 2.

As it can be observed (Table 2), pH values ranged between 7.32 and 7.43 among age-gender groups, which are within normal pH values (Humphrey & Williamson, 2001). A small, but significant difference in salivary pH was observed in YM (7.32) compared to SF women (7.45). The minor effect of age and gender in pH is in agreement with previous works that studied the effect of these two factors separately (Fenoli-Palomares et al., 2004). Therefore, the small differences found in salivary pH could be due to factors such as oral hygiene conditions (e.g. caries) (Farsi, 2008) or diet (Ccama Quispe, 2016), and should not be related to gender and age.

On the contrary, no significant differences ($p < 0.05$) were found in the salivary flow neither by gender nor by age group (Table 2). These results seem contradictory to the reduction in salivary flow with aging that had been previously reported by other authors (Criado et al., 2021). Nonetheless, several revisions on this topic did not find a consensus explaining that a decrease in salivary flow from the seniors might be caused by age itself (Affoo, Foley, Garrick, Siqueira, & Martin, 2015; de Almeida et al., 2008; Xu et al., 2019). In fact, the low flow rate in the elderly population could be related to individual factors others than age,

Table 2
Average values of salivary composition determined in the four age-gender groups (YF, YM, SF, SM) and results from ANOVA. Different letters denote significant differences ($p < 0.05$) from Tukey test.

Age-gender groups	pH	Flow	Viscosity	Na	K	Ca	Mg	Zn	TPC	TSEA	α-amylase
YF	7.43 ab	1.06 a	2.40 x10^{-3} a	146.48 a	3303.59 a	52.52 a	11.54 bc	1.32b	787.07 ab	0.68 a	55.72 ab
YM	7.32b	1.29 a	2.30 x10^{-3} a	102.41 a	2233.39 a	47.46 a	10.42c	1.55 ab	595.62b	0.49 a	29.76b
SF	7.45 a	1.28 a	2.50 x10^{-3} a	101.28 a	3017.69 a	50.01 a	14.36 ab	2.34 a	939.44 a	0.53 a	56.29 ab
SM	7.36 ab	1.22 a	2.30 x10^{-3} a	100.15 a	2909.91 a	53.05 a	15.32 a	2.29 a	1007.27 a	0.46 a	65.02 a
Pr > F	**0.0169**	0.1052	0.9456	0.3173	0.2611	0.1718	**0.0006**	**0.011**	**0.0028**	0.3182	**0.0456**

YF: young-female; YM: young-male; SF: senior-female; SM: senior-male; Flow: mL/min; Viscosity shear 50; Na, K, Ca, Mg, Zn in mg/L; TPC: total protein concentration in mg/L; TSEA: total salivary esterase activity in UI/min; α-amylase in U/mL.

such as the use of medication, alterations in the salivary glands or oral diseases (de Almeida et al., 2008; Xu et al., 2019), and additional factors related to the design of the study, such as the collection of the saliva samples (Affoo et al., 2015).

On the other hand, from all the minerals determined in the saliva, only Mg and Zn content was significantly higher ($p < 0.05$) in senior than in younger groups (Table 2). An increase in the mineral content of saliva in the elderly (70–86 years) and middle-aged people (30–69 years) compared to younger people (20–29 years) has also been shown by other authors (Nagler & Hershkovich, 2005).

Regarding the TPC, the senior groups (SF, SM) showed a higher TPC than the younger groups (YF, YM) (Table 2), which is in agreement with some previous works (Criado et al., 2021; Nagler & Hershkovich, 2005; Sun et al., 2014). An increase in salivary protein content in elderly people has been related to a lower salivary flow, which although has not been found in the present study, it is frequently observed with increasing age (Affoo et al., 2015), which might also provoke periodontal diseases (Koss, Castro, Salúm, & López, 2009). New studies with a higher number of individuals should be carried out to confirm this.

Additionally, as shown in Table 2, a higher α-amylase activity was also found in the senior groups (SM, SF), which agrees with previously reported results (Arhakis, Karagiannis, & Kalfas, 2013; Nagler & Hershkovich, 2005) and with the highest TPC found in this age group. Nonetheless, no significant differences were found in TSEA among the age-gender groups.

The relationship between oral aroma release and saliva composition in the four age-gender groups (YF, YM, SF, SM) was explored by applying principal component analysis (PCA), one per each wine type (red and white). For this, the oral aroma release data that showed significant differences depending on age-gender (Table S2) were used. Additionally, salivary parameters (pH, Mg, Zn, TPC, α-amylase) that also showed significant differences among the age-gender groups (Table 2) were included in the PCA as well. The two PCAs corresponding to the white and red wines are shown in Fig. 3a and 3b, respectively.

As it can be seen in the PCA from the white wine (Fig. 3a), the two principal components explained 54.46% of data variation. PC1 explained 42.03% of the variability and was positively correlated to hexanol (factor loading = 0.95), γ-butyrolactone (0.89), ethyl decanoate (0.87), diethyl succinate (0.86), phenethyl acetate (0.85) and isoamyl acetate (0.84). PC1 separated YM group, which showed positive values of this PC, from the YF group, which showed negative values of PC1. While the senior groups (SF, SM) showed an intermediate behaviour with PC1 values close to zero. Therefore, and as it has been previously explained (Section 3.1), YM showed a higher release of the above-mentioned volatile compounds, contrarily to the YF group, which showed, in general, a lower release of these compounds. Additionally, and as previously shown (Section 3.1), both senior groups (SF; SM), showed a more similar aroma release behaviour after tasting the white wine. On the other hand, PC2 only explained 12.43% of the data variation, but it was positively correlated to ethyl pentanoate (0.79) and ethyl butanoate (0.60), and negatively and weakly (<−0.50) correlated to salivary parameters (α-amylase, Zn, TPC, Mg). PC2 mainly separated individuals from the young groups (YF, YM), which showed positive values for this PC, and thus a higher release of ethyl pentanoate and ethyl butanoate, particularly the YM group, from the individuals from the senior groups (SF, SM) which showed negative values of PC2 and thus, a lower release of ethyl butanoate and ethyl pentanoate and a relatively higher amount of α-amylase, Zn, TPC and Mg saliva parameters, which is in agreement with results shown in Table 2.

Interestingly, the PCA obtained with data from the red wine (Fig. 3b), showed quite a different picture compared to the white wine. In this case, the principal components (PC1 and PC2) explained 51.9 % of the total variability. PC1 explained 33.6% and was highly correlated to γ-butyrolactone (0.90), hexanol (0.80), furfuryl alcohol (0.79), benzyl alcohol (0.77) and diethyl succinate (0.76). PC1 separated the senior (SF, SM) and YM groups, which showed positive values of this

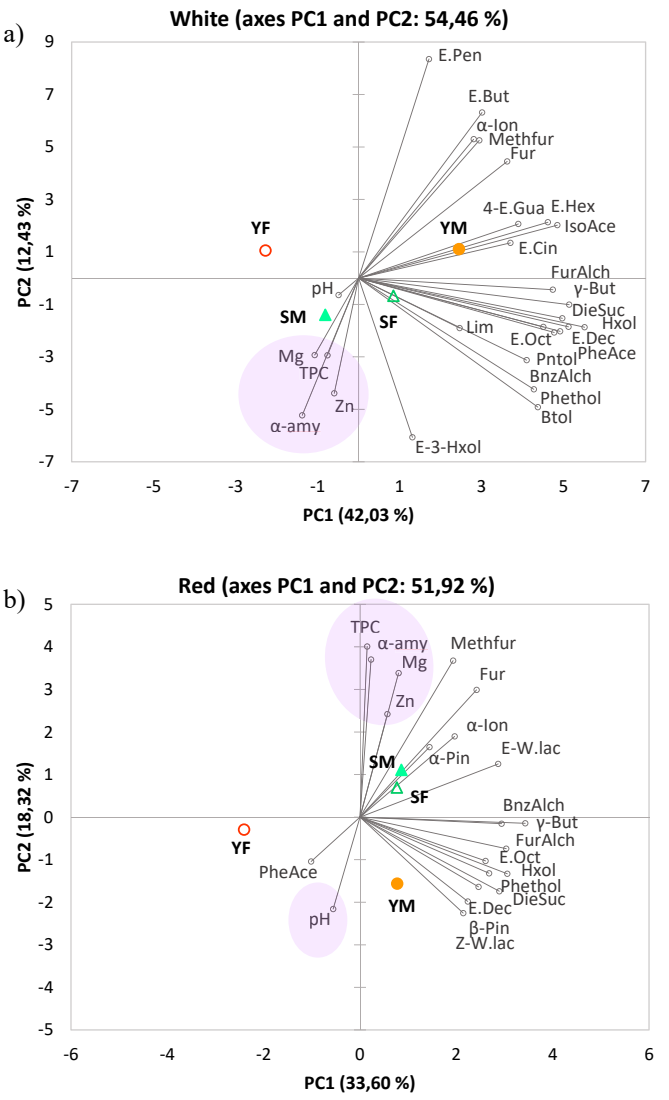

Fig. 3. Graphical projection of the two first components obtained after the application of PCA to oral aroma release data after white wine (a) and red wine tasting (b) and saliva composition (marked in purple) in the four age-gender groups. Only those variables that showed significant differences among groups ($p < 0.05$) have been included. For each age-gender group only the centroids are represented. YF: young-female; YM: young-male; SF: senior-female; SM: senior-male; IsoAce: isoamyl acetate; E.But: ethyl butanoate; E.Pen: ethyl pentanoate; E.Pen: ethyl pentanoate; E.Hex: ethyl hexanoate; E.Oct: ethyl octanoate; E.Dec: ethyl decanoate; DieSuc: diethyl succinate; PheAce: phenethyl acetate; E.Cin: ethyl cinnamate; Btol: butanol; Pntol: pentanol; Hxol: hexanol; E-3-Hxol: E-3-hexenol; BnzAlch: benzyl alcohol; Phethol: phenylethanol; Fur: furfural; MethFur: 5-methyl furfural; FurAlch: furfuryl alcohol; α-Pin: α-pinene; β-Pin: β-pinene; Lim: limonene; α-Ion: α-ionone; γ-But: γ-butyrolactone;; E-W.Lac: E-whisky lactone; Z-W.Lac: Z-whisky lactone; TPC: total protein concentration; α-amy: α-amylase activity. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

component and thus, a higher release of the above-mentioned aroma compounds, from the YF group with negative values of PC1 and a lower release of these compounds, which is in agreement with the results shown before (Fig. 2). Considering PC2, this component explained 18.32% of the total data variability and was positively correlated to the salivary parameters, TPC (0.78), α-amylase (0.72) and Mg (0.65) and the release of 5-methyl furfural (0.71) and furfural (0.58). PC2 separated the senior groups (SF, SM), with positive values of this PC and a higher TPC, α-amylase, Mg content and higher release of furanic compounds, from

the young groups (YF, YM) with negative values of PC2 and thus, lower content of the above mentioned salivary parameters and furanic compounds release. Interestingly, in a recent work that used a time-intensity sensory methodology to rate the dynamics of retronasal aroma intensity immediately after wine tasting, it showed that senior individuals (>55 years old) perceived a greater aroma intensity of the smoked and black pepper wine aroma attributes, associated to very polar volatile compounds with aromatic rings (guaiacol, 4-vinylphenol) than younger individuals (18–25 years old) (Criado et al., 2021), which seems to be in agreement with the present findings. Additionally, and according to the results from the present work, the authors found a positive correlation between the retronasal intensity of both red wine attributes and the salivary total protein content of senior individuals (Criado et al., 2021; Muñoz-González, Canon, Feron, Guichard, & Pozo-Bayon, 2019; Muñoz-González et al., 2020). To explain the contribution of salivary proteins to the higher volatile release, a salting out effect of volatile compounds in the oral cavity, or a higher retention of some volatiles by salivary proteins from the mucosal pellicle have been proposed (Criado et al., 2021; Muñoz-González et al., 2019)

Hence, PCA (Fig. 3) confirmed the results of the previous ANOVA and SLDA, also showing differences in volatile release linked to age-gender groups, whose effect was different depending on the wine type. Additionally, PCAs showed large differences in the aroma release behaviour by age-gender groups. Likewise, senior groups (SF, SM) released higher amounts of furanic compounds, furfural and 5-methyl furfural with the red wine compared to the younger individuals. This is opposite to what was observed with the white wine, in which senior individuals released a lower amount of furanic compounds than the younger groups. Similarly, seniors (SF, SM) also released a higher amount of α-ionone than young did, with red wine compared to the white one. Besides this, seniors, and particularly the SM group, presented the highest salivary values of TPC, Mg and Zn and α-amylase. These results point out that the impact of saliva on aroma release during wine tasting could be modulated by the wine matrix.

Furthermore, PCAs from both wines (Fig. 3) showed that gender differences mainly occurred in the younger groups than in the senior groups. SM and SF groups showed a more similar volatile oral release behaviour. As previously explained, the YF group showed the lowest release of most of the volatile compounds compared to the other three age-gender groups in both wines. This did not seem to be related to saliva composition (Fig. 3, Table 2). Nonetheless, it is important to highlight some limitations of this study. For instance, the number of volunteers in each group might not be enough to find a more straightforward relationship between saliva and oral aroma release. Additionally, there are other salivary components that have not been considered in this study (individual protein composition), and/or other physiological factors (oral cavity volume, breathing flow, etc), which could also affect oral volatile release. Among them, the volume of the oral cavity has been pointed out (Carolina Muñoz-González et al., 2019), being generally smaller in female than male young adults (<55 years) (Nascimento et al., 2012; Pedrotti et al., 2019). Therefore, the lower aroma release of YF compared to the other age-gender groups deserves further investigations. On the other hand, whether gender and age might impact the liking and the emotional response to wines, as it has been previously shown by other authors (Mora, Urdaneta, & Chaya, 2018), and if this could also be related to differences in saliva composition, they will be investigated in future works using sensory tests with wine consumers.

4. Conclusions

This study shows for the first time the combined effect of age and gender on wine oral volatile release using an *in vivo* analytical approach and considering the wine type (red and white) and its relationship with salivary composition. Results confirmed significant differences in oral aroma release among age-gender groups (younger male, younger female, senior male, senior female), which also depended on the type of volatile compound and wine type. The senior group showed a similar oral volatile release behaviour between females and males. The higher release of furanic compounds and C_{13}-norisoprenoids in senior groups after red wine tasting was related to some differences in their saliva composition (higher TPC and α-amylase salivary content). On the contrary, the effect of gender on volatile release was larger in younger individuals. The YM group showed a higher release of esters and alcohols regardless of the type of wine (white and red). On the other hand, the YF group showed the lowest oral release in both wines from the four age-gender groups; but this did not seem to be explained by saliva composition. Additionally, as revealed in the SDLA, a higher variability between males and females in the oral aroma release behaviour within the seniors compared to the younger group was found when tasting red wines This study also confirmed the modulation exerted by wine matrix composition, which was more evident in red than white wine. In addition to that, the results from this work also showed the effect of saliva on aroma release during wine tasting. The differences on aroma release between age-gender groups that have been observed in this work, could have consequences on the sensory perception of the wine. Although changes in volatile release do not necessarily produce changes in aroma perception, these results could be interesting as a starting point in making more personalised wines adapted to target consumer groups, as it will be tested in future sensory studies.

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CRediT authorship contribution statement

María Pérez-Jiménez: Investigation, Methodology, Formal analysis, Data curation, Writing – original draft. **Carolina Muñoz-González:** Formal analysis, Data curation, Writing – review & editing. **Carolina Chaya:** . **Virginia Fernández-Ruiz:** Investigation, Methodology, Formal analysis. **María Dolores Álvarez:** Investigation, Methodology, Formal analysis. **Beatriz Herranz:** Investigation, Methodology, Formal analysis. **Maria Ángeles Pozo-Bayón:** Conceptualization, Funding acquisition, Supervision, Data curation, 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.

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The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

Ethical consent

The study was approved by the Bioethical Committee of the Spanish National Council of Research (CSIC) (June, 2017).

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.

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