Individual differences and effect of phenolic compounds in the immediate and prolongued in-mouth aroma release and retronasal aroma intesity during wine tasting

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A B S T R A C T

To explore the role of phenolic compounds in oral aroma release during wine tasting, four rosé wines supplemented with three types of commercial phenolic extracts and a control wine were evaluated. Wines were aromatized with a mixture of six target aroma compounds. –*In vivo* oral aroma release was monitored in six volunteers at two different times after wine rinsing, just after spitting of the wine (immediate release), and four minutes later (prolonged release). To check the sensory meaning of these changes, descriptive analysis using a trained panel (n = 10) was also performed. Results showed a strong individual effect on total oral aroma release at the two sampling points. After the oral exposure to wines with phenolic extracts, a lower release of most target aroma compounds was also determined. Lower intensity scores for some aroma attributes in wines with phenolic extracts were found, showing a good agreement between the two scientific approaches.

1. Introduction

Phenolic compounds cover the largest fraction of the non-volatile components of wines. Flavonoids (flavonols, anthocyanins and flavan 3-ols) and non-flavonoids (phenolic acids and stilbenes) are the two major classes of wine phenolics. Among them, flavan-3-ols monomers and their oligomers and polymers, which are called proanthocyanidins or condensed tannins, are the most abundant in wine (Lesschaeve & Noble, 2005). They come from the skins, seeds and stems of the grapes. Different winemaking practices, such as different oenological supplies such as chips, staves, and commercial enological tannins are widely used, and therefore can largely modify wine phenolic composition (Chira & Teissedre, 2013). These compounds have a large contribution to wine sensory characteristics, such as color, astringency and bitterness.

Phenolic compounds can also affect wine aroma since they can interact with different types of aroma molecules, changing their volatility and modifying aroma release (Pozo-Bayón & Reineccius, 2009). There are many analytical studies focused on determining aroma-phenolic interactions at the molecular level. In a pioneer study using exponential dilution analysis and NMR, Dufour and Bayonove, (Dufour & Bayonove, 1999) confirmed the existence of weak interactions between catechin and aroma compounds in model wines, hydrophobicity being the main driving force in explaining this. Some other works (Jung & Ebeler, 2003) have shown that some phenolic acids, such as gallic acid, might reduce aroma volatility. In this case, interactions were due to π-π stacking of the galloyl ring of the phenolic compound with the aromatic ring of the odorant molecule. These studies have sometimes shown different results. Using natural tannin extract from grape skin and model wines, Mitropoulou and co-workers (Mitropoulou, Hatzidimitriou, & Paraskevopoulou, 2011) showed a reduction in the volatility of diethyl succinate, 2-phenylethanol and octanoic acid at lower tannin dose (up to 1 g/L). But, at higher concentration (5 g/L), the volatility of hydrophobic compounds such ethyl esters, isobutanol and linalool was markedly decreased. On the contrary, an increase in the volatility of isoamyl acetate and other hydrophilic compounds such as 2-methly-1-butanol, diethyl succinate and 2-phenylethanol was observed. Nonetheless, Lorrain and collaborators, (Lorrain et al., 2013) showed a reduction of the headspace concentration of ethyl octanoate in the presence of catechin, while this phenolic compound did not affect the volatility of other small aliphatic chain esters (ethyl butyrate, isoamyl acetate). It has been also shown that the addition of catechin in synthetic wines decrease the volatility of ethyl hexanoate by 10–20%, whereas the volatility of isoamyl acetate remains unaffected (Jung & Ebeler, 2003). In more complex systems, in which other wine matrix

components were also considered, catechin did not show a significant effect on aroma volatility compared to other wine matrix components, such as ethanol and glucose (Robinson et al., 2009). However, Villamor and co-workers (Villamor, Evans, Mattinson, & Ross, 2013), showed a "salting out" effect of tannins on certain aroma compounds, such as 2-methoxyphenol, 2-phenylethanol, β-damascenone and 1-octen-3-one, which was more enhanced at lower ethanol (8–10% v/v) and fructose (200 mg/L) concentration.

All the above mentioned works were conducted using - *in vitro* approaches (static and dynamic headspace conditions), which although very valuable in determining the chemical nature of aroma-phenolic interactions, do not represent the retronasal delivery of odorants during wine tasting. In fact, when the wine is introduced into the oral cavity, it is submitted to oral processing. Once in the mouth, wine components (aromatic and nonaromatic compounds) and oral fluids and structures (e.g. saliva, oral mucosa) act together, determining aroma release patterns (Pozo-Bayón, Muñoz-González, & Esteban-Fernández, 2016). However, the chemical and biochemical changes of these odorant compounds during wine oral processing remain scarcely investigated.

During wine oral processing, aroma compounds might interact with polyphenol-saliva proteins complexes (Mitropoulou et al., 2011) modifying aroma release, which might also depend on saliva composition (Muñoz-González, Feron, Brulé, & Canon, 2018). The formation of liquid coatings on oral and throat surfaces containing food matrix components making them able to act as aroma reservoirs has also been proven (Buettner & Schieberle, 2000; Buettner, Beer, Hannig, & Settles, 2001). In fact, the delayed aroma release from the oral/throat coatings could be behind the long lasting aroma perception (aroma persistence) (Buffo, Rapp, Krick, & Reineccius, 2005; Linforth & Taylor, 2000), closely related with wine quality.

In a recent work, Esteban-Fernandez and co-workers (Esteban-Fernández, Muñoz-González, Jiménez-Girón, Pérez-Jiménez, & Pozo-Bayón, 2018) using commercial wines with different chemical composition, showed differences in total oral aroma release depending on wine non-volatile matrix composition. For instance, a lower oral release of ethyl hexanoate in wines with higher content of flavan-3-ols was shown, while some phenolic acids produced a "salting out" effect. However, it is uncertain if differences in saliva composition among individuals might affect these results, or if the observed changes in oral aroma release might impact wine aroma perception.

Therefore, the fact that wine polyphenols might modulate the retention of aroma compounds in the oral and pharyngeal mucosa, needs to be further explored. Recent, scientific evidence underlines the idea that saliva can increase the stickiness of polyphenols to the oral surface prolonging their retention in the oral cavity (Ginsburg, Koren, Shalish, Kanner, & Kohen, 2012). This could be explained by the interaction of these compounds with proteins that form part of the mucosal pellicle, the bacterial-free adsorbed film of saliva proteins covering all oral surfaces. Ginsburg and co-workers (Ginsburg et al., 2012) showed that polyphenols in beverages can be retained in the oral cavity for long periods despite a constant saliva flow. More recently, Ployon et al. (2018) using a cell-based model showed the structural alteration of the mucosal pellicle by two types of tannins using microscopic techniques.

With all of these antecedents in mind, the aim of this work was to explore the role of phenolic compounds on oral aroma release during wine tasting, considering the individual effect and establishing whether this effect might also have consequences on aroma perception. For this, the same rosé wine was supplemented with three types of commercial phenolic extracts. Two of them composed of rich monomers (70% flavan-3-ol monomers, 28% procyanidins) and oligomers (21% flavan-3-ols monomers, 78% procyanidins) purified fractions obtained from a grape seed extract, and the third one, a red wine extract mainly composed of anthocyanins. All the wines were aromatized with a target mixture of six wine typical aroma compounds with different physicochemical properties. Oral aroma release was monitored in six volunteers by means of intra-oral SPME at two different times after wine rinsing, just after spiting off the wines (immediate aroma release) and four minutes later (prolonged aroma release). In addition, the sensory meaning of this effect was assessed using a trained panel (n = 10) in the recognition and retronasal evaluation of the aromatic descriptors associated to the odorant molecules used to aromatize the wines.

2. Material and methods

2.1. Wine samples

A low aromatic rosé wine from the Grenache grape variety (PDO Navarra 2014) with an ethanol concentration of 13% (v/v) and 252 mg gallic acid/L of total polyphenol content (measured by the Folin–Ciocalteau assay), was selected for this study. From this wine, three different wines were prepared by adding three types of phenolic extracts with different origin and phenolic composition. GSME-W and GSEO-W wines were made by adding the rich monomers (70% flavan-3-ol monomers, 28% procyanidins) and oligomers (21% flavan-3-ols monomers, 78% procyanidins) purified fractions respectively obtained from a commercial grape seed extract (Vitaflavan®). Both extract fractions were provided by Les Dèrives Resiniques & Terpéniques, S.A. (France). Their single phenolic composition was previously provided (Cueva et al., 2013; Sánchez-Patán et al., 2012). The third wine (RWE-W), was prepared with a red wine phenolic extract (Provinols™, Safic-Alcan Especialidades, S.A.U., Barcelona) mainly composed of anthocyanins (Sánchez-Patán et al., 2012). The specific phenolic composition of the three extracts is shown in Table 1S. The three types of extracts were added to the wines at the same concentration (150 mg/L). This concentration fitted in the lowest dose recommended by manufacturers in the case of using oenological tannin during winemaking. Previous assays confirmed their complete solubilisation in the wines and the absence of modification in wine astringency. In addition, the original rosé Grenache wine without polyphenols extracts was included in this study as a control wine (C-W).

All four wines had a similar pH ranging from 3.22 to 3.26 and a total polyphenol content measured by the Folin-Ciocalteu assay ranging from the minimum concentration determined in the control wine (252 ± 84 mg gallic acid/L), followed by the RWE-W wine (618 ± 99 mg gallic acid/L) and the highest concentration determined in the two wines supplemented with the procyanidins extracts, GSEM-W (731 ± 64 mg gallic acid/L) and GSEO-W (716 ± 61 mg gallic acid/L).

2.2. Wine aromatization

To reinforce the aroma profile of the wines, all of them were spiked with a mixture of six food-grade aroma compounds (Sigma–Aldrich, Steinheim, Germany) representative of the wine volatile profile: ethyl hexanoate (123-66-0), β-ionone (8013-90-9), linalool (78-70-0), guaiacol (90-05-1), β-phenylethanol (60-12-8) and isoamyl acetate (123-92-2). All of them are characterized by different physicochemical properties (Table 1). Before aromatization, six independent aroma stock solutions were prepared in food-grade ethanol (Panreac Química S.A., Barcelona, Spain). From here, each aroma compound was added to the wines to obtain a final concentration of 2 mg/L.

2.3. Volunteers

Six volunteers (four female and two male) between 22 and 42 years old, previously trained in the intra-oral SPME procedure participated in this analytical study. In addition, four more volunteers (all female) (21–34 years old), participated in the sensory analysis. They had no known illnesses and they had self-reported normal olfactory and gustatory functions. The sampling procedures were explained in detail to the subjects who also provided written consent to participate. This study was also approved by the Bioethical Committee of the Spanish

Table 1
Physicochemical properties of the aroma compounds employed in this study.

Compound	CAS number	MW (g mol^{-1})	BP (°C)	log P[a]	Descriptor[b]
Isoamyl acetate	123-92-2	130	134	2.26	Banana
Ethyl hexanoate	123-66-0	144	167	2.83	Apple
Linalool	78-70-6	152	204	3.38	Muscat
Guaiacol	90-05-1	124	211	1.34	Chemical
β-Phenylethanol	60-12-8	122	224	1.57	Honey
β-Ionone	8013-90-9	192	262	4.42	Violet

[a] log P = log of the octanol/water partition coefficient estimated from molecular modeling software *EPI* Suit (U.S EPA 2000–2007).
[b] Descriptors selected by consensus during the training of the panel used in this study.

National Council of Research (CSIC).

2.4. Saliva analysis

Flow, pH, and total proteins were determined in the stimulated saliva provided by the six volunteers who participated in the intra-oral SPME assay. To do so, volunteers could not consume food and water 1 h prior to sampling. To stimulate saliva production, participants chewed a little piece of Parafilm™ and then spat out as much saliva as they could during the 5 min collection period. Saliva samples were collected in previously weighed sterile tubes, and, then immediately weighed after collection. Salivary flow rate was calculated as mL/min (1 mg = 1 mL) (Öztürk et al., 2012). The pH determination was directly measured in the fresh saliva with a pH meter (Mettler Toledo, Barcelona, Spain). Subsequently, saliva samples were centrifuged at 15,000*g* for 15 min at 4 °C (Munoz-Gonzalez et al., 2014) and supernatants were frozen (−80 °C) until their analysis. Total protein content was determined using the Pierce BCA ™ Protein Assay Kit (Pierce Thermo Scientific, Illinois, USA).

2.5. Intra-oral SPME procedure for oral aroma monitoring

Fifteen minutes before each experiment, the volunteers had to clean their mouths and rinse them with three different solutions: a bicarbonate solution, a pectin solution (1 g/L) and water, in order to have the most similar oral conditions when starting the assay. To monitor aroma release from the oral cavity after wine rinsing, the intra-oral SPME procedure previously described (Esteban-Fernández, Rocha-Alcubilla, Muñoz-González, Moreno-Arribas, & Pozo-Bayón, 2016) was used. For this, 15 mL of the aromatized wine were placed into the oral cavity, performing a soft rinsing, spitting out after 30 s. During rinsing, special care was taken to keep the lips closed, not to swallow and not to open the velum–tongue border prior to expectoration. Ten seconds after expectoration, a DVB/CAR/PDMS (divinylbenzene/carboxen/polydimethyl siloxane 50/30 µm film thickness, 2 cm length) coated SPME fiber (Supelco, Bellefonte, PA), with a home-made adaptor consisting of a plastic changeable tube inside a septum in which the SPME fiber was located, was placed into the oral cavity of the panelist. This device assured that, during the whole extraction time (2 min), the fiber did not touch the mouth surface and the aroma extraction was done in the free space of the mouth. This first oral aroma sampling (t = 0 min) was followed by a second in-mouth aroma extraction 4 min after wine expectoration (t = 4 min). Two minutes before the second sampling, the subject was instructed to breathe normally through the nose and to perform one swallowing event every 30 s (five times in total). An overview of the procedure is shown in Fig. 1. Each of the four wines was evaluated three times by each volunteer.

The two *in-mouth* samplings were performed with two different SPME fibers. The two SPME fibers were selected before starting the experiment considering their similarity in volatile recovery rates, considering that differences between them could not be higher than 5%. The SPME fiber with the oral aroma extract from the second oral sampling (t = 4 min) was immediately desorbed in the injector of the GC system (Agilent 6890N) (Agilent Technologies, California, USA) in splitless mode for 1.5 min at 250 °C. Volatile compounds were separated on a DB-Wax polar capillary column (60 m × 0.25 mm i.d. × 0.50 µm film thickness) from Agilent (J&W Scientific, Folsom, CA). Helium was the carrier gas at a flow rate of 1 mL/min. The oven temperature was initially held at 40 °C for 2 min, then increased at 8 °C/min to 240 °C and held for 15 min. The SPME fiber with the breath extract corresponding to the first sampling point (t = 0), was stored in the fridge (4 °C) in a sealed glass tube until the first GC run (corresponding to t = 4 min) finished. Preliminary experiments were performed in order to ensure that there were no significant losses of aroma during the storage of the fiber, which was not more than 1 h.

For the MS system (Agilent 5973N), the temperature of the transfer line, quadrupole and ion source were 270, 150 and 230 °C respectively. Electron impact mass spectra were recorded at 70 eV and the ionisation current was 10 µA. The acquisitions were performed in scan (from 35 to 350 amu) and SIM modes, looking for the specific ions of the six target compounds. The identification of the six compounds was based on the comparison of retention times and mass spectra with those provided in the NIST 2.0 database and using reference compounds injected in the same conditions than the samples. Since no internal standard was used during the intra-oral SPME extraction, absolute peak areas (APAs) were obtained to express aroma release. The use of APAs data to express aroma release was sufficient for this type of analysis, as the aim of the work was to compare the extent of intra-oral aroma release between wine samples and individuals. Fibers performance was periodically checked along the study by comparing the recoveries of the six aroma compounds in hydroalcoholic solution (12% v/v ethanol) using static HS-SPME conditions as previously described (Rodríguez-Bencomo et al., 2011).

2.6. Descriptive analysis

A total of ten judges were recruited from the staff members of CIAL on the basis of their interest and availability to perform this study. From them, six had also participated in the intra-oral SPME assay. All the sensory evaluations were conducted at 10:30 a.m. in an acclimatised room (21 °C), with controlled lighting and isolated from noise or smells that could interfere with the evaluations. Panelists were asked not to eat nor drink anything two hours before the tests. Wine samples (15 mL) were served in wineglasses according to standard UNE 87022-92. Samples were presented blind (labelled with three-digit random codes) and served in random order to minimize bias (Wakeling & MacFie, 1995). Aromatization of the samples was performed immediately before tasting the wines, as previously explained for the intra-oral aroma analysis. Sensory sessions of the four wines were carried out over two months (two 30 min sessions per week), and divided in training and wine evaluation.

Training in the recognition of the descriptors associated to each aroma compound was done individually. Once a consensus was reached on the terms, two references for each aroma descriptor were prepared at low and high concentrations. Panelists scored the attributes by means of unstructured 13-cm line scales that were anchored 2.3 cm from the ends of both extremes with the labels “low” and “high”, respectively. Judges were trained in the rating of both concentrations of each aroma and were asked to use the low and the high ends of the scales, respectively. The selected terms associated by the consensus of the panel to the chemical odorants were “banana” (isoamyl acetate), “apple” (ethyl hexanoate), “muscat” (linalool), “chemical” (guaicol), “honey” (β-phenylethanol), and “violet” (β-ionone). The high concentrations used in these training sessions were: 10 mg/L for isoamyl acetate, 5 mg/L for ethyl hexanoate, 2 mg/L for linalool, 2 mg/L for guaiacol, 100 mg/L for β-phenylethanol and 2 mg/L for β-Ionone. The minimum corresponded to the wines without aroma added.

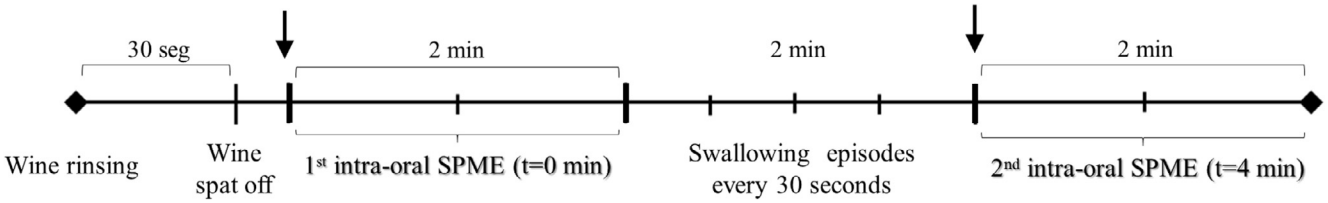

Fig. 1. Sampling procedure followed for oral aroma monitoring.

Table 2
Individual saliva composition.

Individual	Flow (mL/min)	pH	Proteins (μg/mL)
#1	$2.24^{ab} \pm 1.18$	$7.27^{ab} \pm 0.12$	$786.15^{c} \pm 86.88$
#2	$1.06^{ab} \pm 0.73$	$7.23^{ab} \pm 0.18$	$729.58^{cd} \pm 22.16$
#3	$2.44^{a} \pm 1.23$	$7.62^{a} \pm 0.15$	$837.27^{c} \pm 27.26$
#4	$0.36^{b} \pm 0.21$	$6.56^{c} \pm 0.23$	$2141.76^{a} \pm 80.17$
#5	$1.00^{ab} \pm 0.69$	$7.09^{b} \pm 0.28$	$1151.09^{b} \pm 80.31$
#6	$0.81^{ab} \pm 0.45$	$6.46^{c} \pm 0.21$	$520.30^{d} \pm 126.20$

Differents letters within the same column denote statistical differences ($p < 0.05$).

Only when the entire panel was able to discriminate all the attributes and properly rate their intensity, wine evaluation was performed.

For the wine evaluation, 15 mL of each wine were served in wineglasses wrapped with aluminum paper and covered with plastic Petri dishes to prevent volatile loss. The judges had to take and keep the wine into the oral cavity doing soft rinses during 30 s and then expectorate, following a similar procedure to that described for the intra-oral SPME assay. During the training, judges were asked to retronasaly recognize the six aroma descriptors in all the wine matrices (supplemented with the different phenolic extracts) and to rate the aroma intensity on an unstructured 13 cm line scale. In order to remove the possible mouth wine polyphenols and to avoid a carry over effect, after each wine sample, panelists rinsed their mouth with a pectin and water solution (1 g/L) and then with tap water. For this, two wine evaluation sessions were performed. In each of them, judges rated the retronasal aroma intensity in the control wine and in the wines supplemented with the phenolic extract (GSEM-W, GSEO-W, RWE-W). Between sample tasting, panelists rested at least 10 min and vigorously rinsed their mouths with the same pectin and water solution previously described. Panelists were not informed about the nature of the samples and blind evaluated the wines.

2.7. Statistical analysis

Different statistical analyses were done on the individual and mean data.

Firstly, two way ANOVA was used in each sampling point (t = 0 min and t = 4 min) to determine significant differences in oral release of the six target aroma compounds considering individuals and wine type as factors. Secondly, Principal Component Analysis (PCA) was used to examine the relationship among wine type, oral aroma release data and individuals in both sampling points (t = 0 min and t = 4 min). Thirdly, one-way ANOVA was employed to check differences in oral aroma release for the same individual considering the different types of wines as a factor, and the tested aroma compounds as variables. Subsequently, least significant difference (LSD) tests were used for mean comparisons when appropriate.

Finally, for the sensory data, one-way ANOVA was also applied to check significant differences among the control and wines supplemented with the different phenolic extracts.

All the tests considered a significance level of 0.05 and XLSTAT v 19.01 software was used for data analysis.

3. Results and discussion

3.1. Oral aroma release depending on individual and wine type

To check if the addition of phenolic extracts might induce differences in oral aroma release, two *in-mouth* aroma samplings were performed just after spiting off the wines and four minutes later, using 6 volunteers and following the procedure depicted in Fig. 1. The first oral aroma monitoring could be more related to the immediate aroma release from oral mucosa, while the second one could be linked to the delayed release, or aroma persistence (Buffo et al., 2005; Linforth & Taylor, 2000). Since saliva composition might also impact individual differences in aroma release behavior (Ployon, Morzel, & Canon, 2017), the chemical and biochemical analysis of the stimulated saliva of the six individuals was also characterized (Table 2). These results, confirmed the large inter-individual variability in saliva composition among the volunteers of this work, as has been shown in previous studies (Neyraud, Palicki, Schwartz, Nicklaus, & Feron, 2012).

In order to check the effect of wine type and of individuals, aroma release data obtained from the first and second *in-mouth* monitoring were submitted to different statistical treatments as explained below.

Firstly, the effect of the studied factors (individual and wine type) on the oral release of the six target aroma compounds was tested by using a two-way ANOVA. Since a significant effect ($p < 0.05$) of both factors (and their interactions) on the oral aroma was found (data not shown), a PCA was applied in order to gain an understanding of the relationship between the factors and studied variables (aroma compounds).

3.1.1. Principal Component Analysis (PCA)

Aroma release data of individuals and wine type were submitted to PCA. The individual chemical and biochemical saliva composition (pH, saliva flow, total protein content) was also considered in this analysis as a supplementary variable. In addition, since the amount of aroma release in the first sampling time (t = 0 min) was much higher than in the second one (t = 4 min), one PCA per sampling time was performed. The graphic representation of these results is shown in Fig. 2. Here, the average values of three repetitions from the same wine and individual were considered. In Fig. 2a, the PCA with data from the first oral aroma monitoring showed that PC1 and PC2 explained more than 82% of data variation. PC1 explained 47.4% of data variation and it was positively and highly correlated with linalool (0.98), β-ionone (0.97) and β-phenylethanol (0.86). Total protein content was also highly and positively correlated (0.7) with PC1, while saliva flow (−0.65) was negatively correlated. As it can be seen in the figure, PC1 separated individual #4 from the rest. This individual showed higher oral release of the above mentioned aroma compounds as compared to the rest. This individual had the highest concentration of saliva proteins (2700 μg/mL) and the lowest saliva flow (0.36 mL/min) (Table 2). In addition, PC2, explained over 35% of data variation, and was positively and highly correlated to the two esters, isoamyl acetate (0.92) and ethyl hexanoate (0.93). Both compounds seemed to have a similar behavior regarding their oral release confirming results from previous works (Esteban-Fernández et al., 2018). As it can be seen in the figure, PC2 allowed us to separate between individual #2, with higher release of these two esters, from individual #4, who released lower amounts of these two esters. In

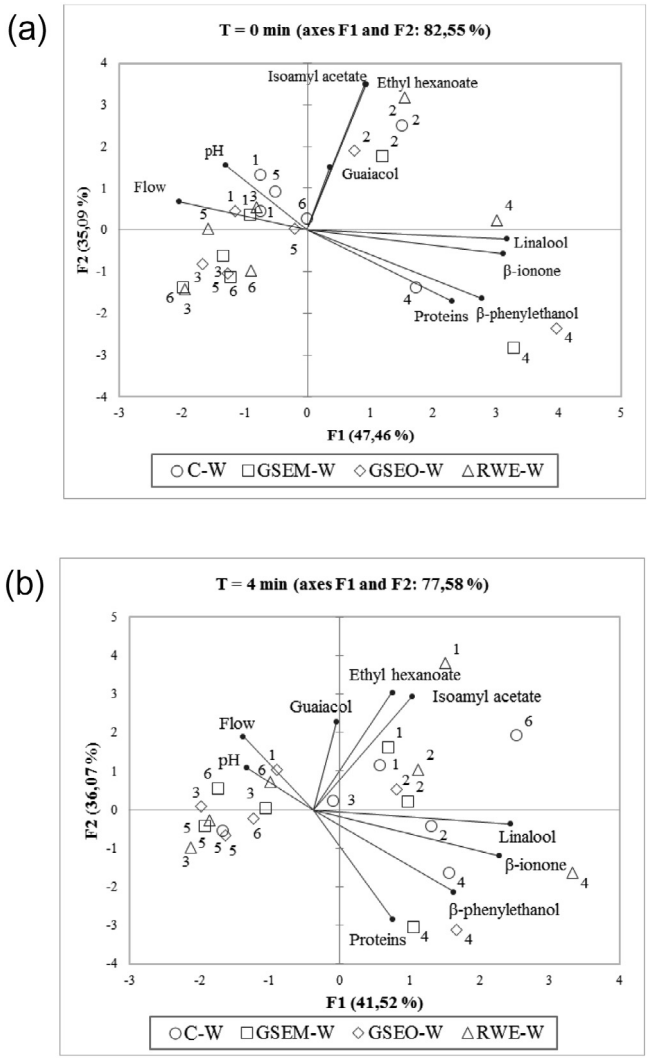

Fig. 2. PCA biplot of the intraoral aroma evaluation of the four types of wines (C-W, GSEM-W, GSEO-W, RWE-W) performed by the six volunteers considering all the aroma compounds and saliva composition obtained (a) immediately after spiting-off the wine (t = 0) and (b) four minutes later (t = 4 min). pH, saliva flow and proteins are supplementary (non-active) variables in the analysis.

addition individual #1, with a similar saliva total protein content than individual #2 showed a higher release of these esters compared to the rest of individuals (#3, #5 and #6) in all the assayed wines.

In the second sampling time (t = 4 min), (Fig. 2b), the PCA revealed a very similar picture compared to the first sampling point. PC1 and PC2 explained 41.5% and 36% of data variation respectively. Similarly to what happened in the first monitoring time, PC1 was positively and highly correlated with linalool (0.94) and β-ionone (0.89) and β-phenylethanol (0.67). Nonetheless, in this case, saliva compositional variables showed a minor correlation (< 0.6) with PC1. In this sampling point, PC2 was strongly correlated with the two esters, isoamyl acetate (0.8) and ethyl hexanoate (0.83) too, but differently to what happened in the first sampling point, also with guaicol (0.62). Total saliva protein content was negatively correlated (−0.76) with the second axis, meaning that, within this dataset, a higher protein concentration in saliva was associated to lower oral ester release. Also, saliva flow was moderately and positivity correlated with PC2 (0.54). Fig. 2b shows the graphic representation of these results. Similar to what happened in the first sampling point, four minutes after wine expectoration, individual #4 (located at the bottom right side of the graph), still released higher amounts of β-ionone, linalool and β-phenylethanol compared to the rest of the individuals, but lower amounts of the two esters and guaicol. On the contrary, individual #2, but also individual #1, which had a very similar protein content than individual #2, release higher amounts of the two esters and guaicol but lower linalool, β-ionone and β-phenylethanol. The rest of the individuals (#3, #5 and # 6) released lower amounts of all aroma compounds compared to individuals #1, #2 and #4. However, there was not a clear relationship between their saliva composition and their different aroma release behavior. The limited number of compositional saliva parameters that have been used in this study (only three) and others physiological aspects (oral cavity volume, breathing flows, etc) that were not considered in this study could explain this fact.

Thus, results from PCA showed on one hand, that aroma release data seem to be better grouped by individuals than by wine type. On the other hand, although total aroma release was lower in the second sampling point, the individuals' relative location was similar in both sampling points. Regarding individual differences, it is worth noticing the different aroma release behavior depending on compound type. Even the release behavior of specific odorant molecules is largely dependent on the individual. These differences were more evident for individuals #4 and #2, and also for individual #1, which behaved more similar to #2 in the second sampling point (t = 4 m). While individual #4 released higher amounts of some compounds (linalool, β-phenylethanol, β-ionone) that are quite different considering their physicochemical characteristics (Table 1), individual #2 (and #1 mainly in the second sampling point), released higher amounts of the two esters, but lower in the above mentioned odorant compounds. Individual differences in aroma release depending on compound type might be linked to differences in saliva composition. As previously shown (Ployon et al., 2017), saliva might affect the release of aroma compounds through different effects. Some of them have been related to the dilution of aroma compounds, making these compounds less available to be released during exhalation (Van Ruth & Roozen, 2000). Individual #4 exhibited the lowest saliva flow, and therefore, aroma compounds could be less affected by saliva dilution, therefore being more available for release compared to individuals with higher salivary flow. Interestingly, individual #4 also showed the highest saliva protein content. The retention of aroma compounds by saliva proteins through non-covalent interactions has been described (Friel & Taylor, 2001). Moreover, esters can interact with some types of saliva proteins like α-amylase and mucins, reducing their release to the headspace (Pagès-Hélary, Andriot, Guichard, & Canon, 2014). In this present work, the lowest salivary flow of individual #4, might explain the lower aroma dilution, and higher aroma release found for certain aroma compounds (linalool, β-ionone and β-phenylethanol). Nonetheless, the fact that this individual also had the highest protein content, might explain the higher retention of esters to the saliva proteins and, consequently, the lower oral release of these compounds. As far as the authors know, these results show for the first time the impact of saliva total protein content on oral ester release, using an *–in vivo* approach, confirming results from previous *–in vitro* experiments. However, a large set of individuals with differences in saliva composition will be necessary to confirm these results.

Besides, other saliva compositional factors, such as differences in salt content, might exert an effect on the conformational state of the saliva proteins affecting their aroma binding performance (Friel & Taylor, 2001). The effects of saliva enzymes (Muñoz-González et al., 2018; Ployon et al., 2017) and other individual physiological features, such as the volume of the oral cavity (Pagès-Hélary et al., 2014) might also contribute in explaining the individual differences in aroma release.

Although, as it was shown in the PCA, there was a better discrimination among oral release data by individuals than by wine composition, differences in aroma release depending on the wine type were also found within the same individual. Therefore, a careful

examination of the wine type effect in aroma release will be explained in the following section.

3.1.2. One-way ANOVA and LSD test

In order to evaluate differences in oral aroma release depending on wine composition, and specifically, the effect of phenolic extracts, a one-way ANOVA was applied to the aroma release data from each individual separately, only taking into account the effect of wine type on the three replicates of the same individual and aroma. Least significant difference (LSD) test was used for means comparison. This data treatment was independently carried out for the two sampling points (t = 0 and t = 4 min). These results are shown in Table 2S (Table2Sa and Sb). Here, a significant effect of the wine type in oral aroma release was observed for most individuals and aroma compounds for both sampling times. A careful examination of the LSD results showed that the effect of a wine on aroma release was not identical in each individual. This together with differences in aroma release depending on odorant type, made it difficult to extract straightforward conclusions regarding the impact of phenolic extracts. Nonetheless, results showed some interesting general patterns. For instance, a general decrease in aroma release after the oral exposure to wines with phenolic extracts (GSME-W, GSEO-W, RWE-W), was observed. This happened for all six aroma compounds in individuals #5 and #6, for five compounds in the case of individual #3, and for three compounds in the case of individuals #1 and #4. This situation was very similar in the second monitoring time. In order to further understand the effect of phenolic extracts on oral aroma release, Fig. 3 shows the comparison between oral aroma release in the control wine (considering this as 100% of aroma release) and in the wines with phenolic extracts, in the first and second sampling points (t = 0 and t = 4 min). Values lower than 100%, show lower aroma release compared to the control, which could be attributable to the retention effect exerted by phenolic compounds, while values higher than 100% mean higher release in the wines with phenolic extracts which could be due to a "salting out" effect provoked by these extracts. Interestingly, Fig. 3a shows very similar results for the two esters (isoamyl acetate and ethyl hexanoate). These compounds showed very comparable trends. Except for individual #4, a general decrease in oral release for both compounds was observed for most individuals in the wines with any of the phenolic extracts assayed. These results showed a good accord in the observed effect when considering the same type of chemical odorant (esters). On the other hand, these results are also in agreement with those recently reported by Esteban-Fernandez and coworkers (Esteban-Fernández et al., 2018) which also showed a negative correlation between the amount of flavonoids in commercial wines and the oral aroma release of these two esters. The hydrophobic interactions among these odorants and saliva proteins-polyphenol complexes might be the reason.

However, polyphenol extracts did not significantly affect the oral release of linalool. Only individual #6 shows a significant reduction in aroma release. On the contrary, the oral release of guaicol was significantly reduced in most individuals after the oral exposure to wines with phenolic extracts. This effect was also more evident for the wines spiked with the grape seed extracts (GSEM-W and GSEO-W). In fact, around 40% lower guaicol release was observed for individuals #1 and #6 after the oral exposure to GSEM-W wine.

It is also noteworthy that while ethyl hexanoate and isoamyl acetate can be considered as hydrophobic compounds (log P values = 2.26 and 2.83), which might explain their higher interaction with saliva proteins-polyphenols complexes, guiacol, the most polar compound from those assayed (log P = 1.34), also showed lower oral release in wines with phenolic extracts. On the basis of its low hydrophobicity, this quite intriguing behavior has however, already been pointed out in previous studies (Esteban-Fernández et al., 2016). In the above mentioned work, a very high adsorption of this compound to oral mucosa after the oral exposure to wine was observed. Authors hypothesized that wine polyphenols anchored to the mucosa pellicle on the surface of the oral cavity, might favor the oral binding of certain aromatic polar compounds, such as guaicol, through interactions between the galloyl ring of the phenolic compound and the aromatic ring of the odorant molecule. This is a type of π-π staking interaction with stability provided by hydrogen bonding, and it was previously used to explain the interaction between some odorants and wine polyphenols using static headspace methods (Aronson & Ebeler, 2004; Jung & Ebeler, 2003; Rodríguez-Bencomo et al., 2011). Results from the present work confirm these results in physiological conditions closer to wine consumption.

Contrarily to what happened for the above four mentioned compounds, β-phenylethanol did not show this trend. The oral exposure to wines with phenolic extracts did not show a significant effect on the oral release of this compound for most individuals. Only, individual #4 and #5 showed a higher release of this compound after the oral exposure to wines with grape seed extracts (GSEM-O, GSEO-W). For these two individuals β-phenylethanol was around 40–50% higher released after the oral exposure to these wines. This "salting out" effect might be due to changes in the ion strength of the medium as consequence of adding an apolar extract (grape seed tannins), which might reduce the solubility of polar aromatic compounds such as β-phenylethanol, favoring their release. However, the addition of a more polar extract (red wine extract rich in anthocyanins) did not affect the release of this odorant molecule. A gradual increase of β-phenylethanol release above the wine headspace has also been shown during the addition of grape skin tannin (from 0.5 to 10 g/L) to model wines (Mitropoulou et al., 2011). In addition, an increase in the headspace concentration of β-phenyl ethanol was also found in model wines with low ethanol (8–10%) and fructose concentration (200 mg/L) in wines spiked with grape seed tannins. The higher tannin-self aggregation at lower ethanol level, led to a decrease in the potential binding sites of odorants to these compounds which might explain this effect (Villamor et al., 2013). Since the wine used in this study had higher ethanol content (13% v/v), the first hypothesis seems the most likely mechanism in explaining these results.

Finally, for the majority of individuals, the oral release of β-ionone, the most hydrophobic compound from those studied in the present work, was also significantly affected by the wine type. Only individual #1 was not affected. Nonetheless, the effect of phenolic extracts did not follow a clear trend for this compound. Similar to what happened for the rest of the hydrophobic compounds, a general decrease in oral aroma release was observed in wines with phenolic extracts. This effect was statistically significant after the oral exposure to wines with grape seed extract (GSEM-O, GSEO-W) but also in the case of the wine supplemented with the red wine extract (RWE-W). Nonetheless, for some individuals (#4 and #5), the effect of phenolic extracts was the opposite, and higher β-ionone release was observed after the oral exposure to GSEO-W wine.

Four minutes after spitting-off the wines, the effect of phenolic extracts was also significant for many aroma compounds, and especially for the two esters, isoamyl acetate and ethyl hexanoate (Fig. 3b). Similarly to what happened in the first sampling point, a lower oral release of these two esters was determined after the oral exposure to wines with phenolic extracts for most individuals. However, a "salting out "effect was also observed for some other individuals (#1, #4 and #6) after the oral exposure to RWE-W wine. Interestingly, this enhancing aroma release effect was also observed for other aroma compounds, such as linalool, guaicol, and β-phenylethanol. β-Ionone was however, less released after the exposure to wines with phenolic extracts; this effect was less significant than in the first sampling point, which could be due to the lower amount of aroma remaining in the oral cavity during the second sampling, making it more difficult to find differences among wine types.

3.2. Effect of phenolic extracts on retronasal aroma perception

After establishing the effect of phenolic extracts on oral aroma

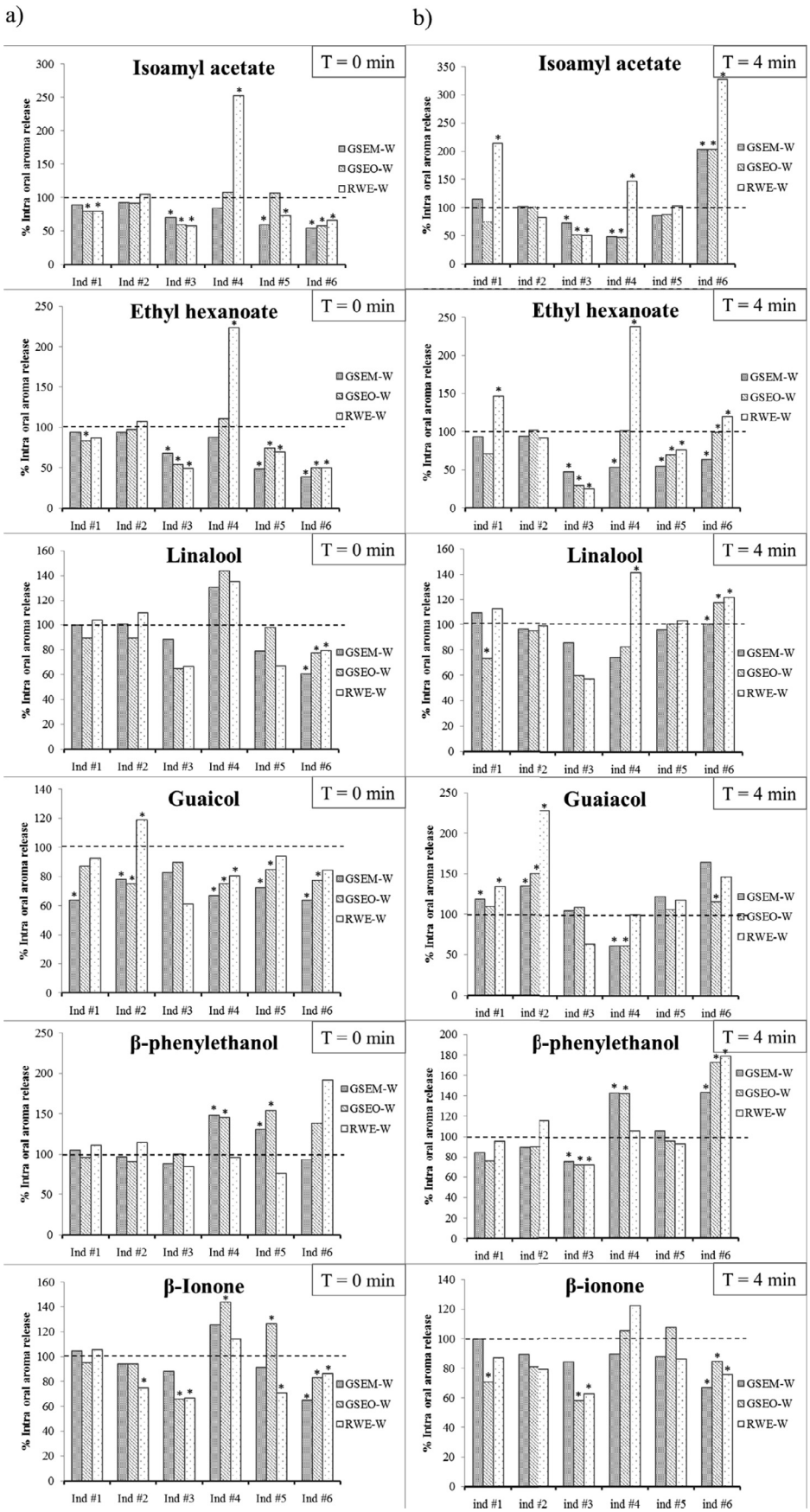

Fig. 3. Comparison between aroma release in the control wine (considering these values as 100% of aroma release) and in the wines with phenolic extracts, (a) in the first sampling point immediately after spiting off the wine and (b) four minutes later. Asterisks denote statistically significant differences among wine types from LSD test results ($p < 0.05$).

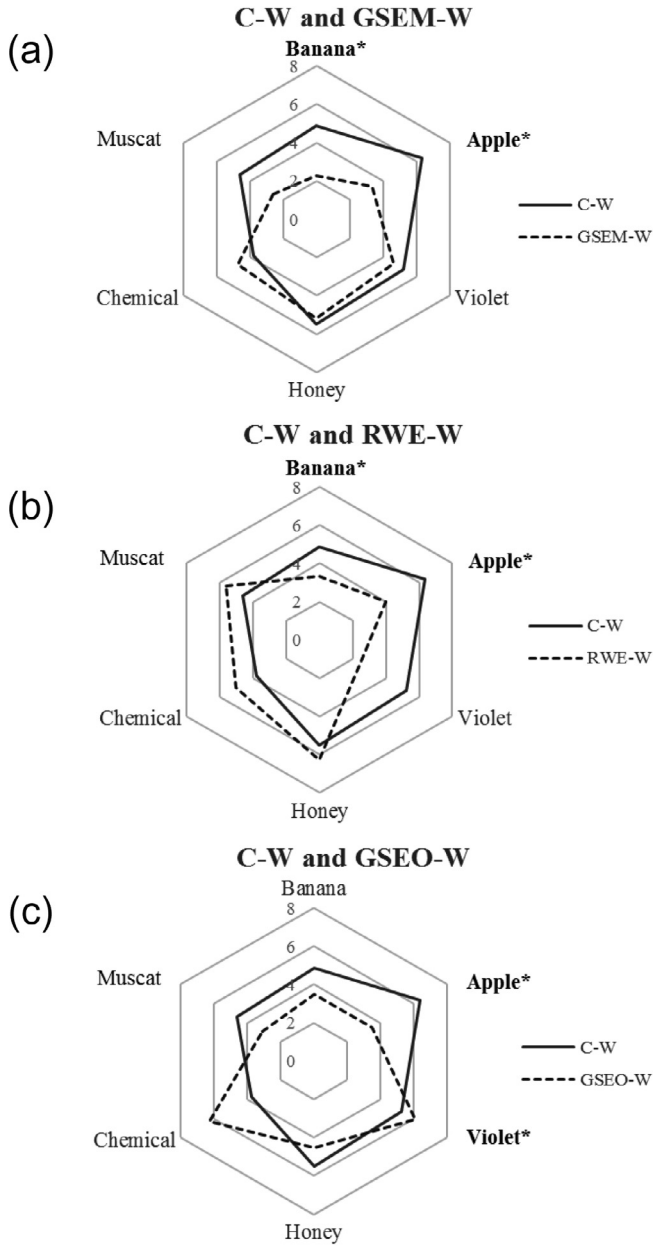

Fig. 4. Intensity scores of the aroma descriptors determined by the trained panel (n = 10) in the control wine (C-W) and in the wines supplemented with the phenolic extracts (GSEM-W, GSEO-W, RWE-W). Asterisks denote statistically significant differences from ANOVA results ($p < 0.05$).

release, it was interesting to determine whether this effect could also have a sensory meaning affecting retronasal aroma perception. Therefore, a descriptive sensory analysis comparing the intensity of aroma attributes associated to the chemical odorants used to aromatize the wines in the control wine (C-W), and in the three types of wines supplemented with the phenolic extracts (GSEM-W, GSEO-W, and RWE-W) was carried out. To do so, ten assessors participated in this study. Six of them also participated in the oral aroma release study.

To compare data obtained under the same conditions, the aroma intensity evaluation of the four wines was performed in the same session. A total of two sessions were finally performed. The scores obtained for each aroma attribute in the wines with phenolic extracts were compared to those obtained for the control wine and are shown in Fig. 4. Results confirmed that wines with phenolic extracts exhibited significantly lower intensity for the attributes "banana" and "apple" which were associated with the compounds isoamyl acetate and ethyl hexanoate. This effect was statistically significant ($p < 0.05$) for the two aroma attributes in GSEM-W and RWE-W wines, while only "apple" was statistically significant in GSEO-W wine. In the latter, the intensity of the attribute "violet" was rated significantly higher than the control, although in the other two types of wines this effect was not statistically significant. Lorrain and co-workers (Lorrain et al., 2013) also showed an agreement between the lower headspace concentration of certain esters induced by adding catechin (2 g/L) into a model wine and the higher odor threshold determined for these odorants (more than three times higher) compared to a control wine (without catechin). In addition, in these two wines supplemented with grape seed extract, the attribute "muscat" was also rated higher in the control than in the wines with phenolic extracts. None of the other aroma attributes were significantly affected by the phenolic extracts. In the case of the attribute "honey" associated to the compound "β-phenylethanol", it was scored slightly higher in the wines with phenolic extracts (RWE-W and GSE-O), following an opposite trend to the above mentioned aroma attributes. This compound was the only one exhibiting higher oral aroma release for certain individuals in wines with phenolic extracts by using the intraoral-SPME approach. However, the higher odor threshold value for this compound (10,000–14,000 μg/L) (Francis & Newton, 2005) compared to the other compounds tested in this work, could be the reason for the lack of differences in the perceived retronasal intensity in the wines with phenolic extract. In addition, the intensity of the attribute "chemical" was higher (but not statically significant) in the wines with phenolic extracts. In this case, "chemical" might have been interpreted by the panel as an unpleasant odor associated to wines with phenolic extracts, increasing the intensity score for this attribute, but without a relationship to a higher oral release of guaicol.

Therefore, by sensory analysis, a statistically significant lower intensity of some aroma attributes ("apple" and "banana") associated to some ester compounds (ethyl hexanoate, isoamyl acetate) was found. The attribute "muscat" also associated to linalool showed lower scores (but not statistically significant) in the wines with phenolic extracts. Even considering that perceptual interactions (masking, synergism, antagonism, etc.) among odorants can happen during wine tasting (Lytra, Tempere, Revel, & Barbe, 2012), these results matched pretty well with the lower oral release of the aroma compounds determined by intra-oral SPME in wines with phenolic extracts. Nonetheless, since these phenolic extracts also exerted an effect on oral aroma release a long time after the wine disappeared from the oral cavity, it would be interesting to perform new sensory studies, using dynamic temporal methods, with a higher number of individuals, in order to check the effect of these extracts on the long lasting aroma perception of these aroma attributes.

4. Conclusions

Results from this work have proven that phenolic compounds from different origin (grape seed and red wine extracts) can exert a significant effect on oral aroma release during wine tasting. Although this effect can be different in each individual, there was a general trend showing that wines supplemented with phenolic extracts produce lower oral release for most aroma compounds. This reduction was more pronounced in wines with grape seed extracts than in those supplemented with a red wine extract. In addition, the reduction in oral release was mainly observed for hydrophobic aroma compounds, which confirm that in a physiological environment, such is the mouth; hydrophobicity seems to be the main force governing polyphenol-aroma interaction involved in oral aroma release. Nonetheless, the large reduction in oral release determined for the polar compound guaicol in wines with phenolic extracts, suggest that other type of interactions, (π-π staking between galloyl rings of flavonoids and aromatic rings of this odorant) are also relevant at a physiological level. On the contrary, wines with phenolic extracts induced a higher oral release of some polar compounds, such as β-phenylethanol, which could be more

affected by the changes in the ionic strength of the wine. In addition, the effect of phenolic compounds on oral aroma release is practically the same just after wine expectoration and four minutes later, thus affecting the immediate and prolonged oral aroma release. Finally, it has been proven that changes in oral aroma release induced by phenolic extracts were also evident at a sensory level. In spite of the low number of individuals in the sensory panel (n = 10), a statistically significant lower intensity of some aroma attributes such as “apple” and “banana” associated to some esters (ethyl hexanoate, isoamyl acetate), was also found in wines supplemented with phenolic extracts, even at the very low dose like that used in this study, confirming the good agreement between the *-in vivo* analytical approach using intra-oral SPME and the sensory findings. Overall, from a technological point of view this study provides new insights for the development and/or improvement of polyphenol base oenological formulations to enhance wine aroma persistence.

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Conflict of interest

The authors declare no conflict of interest in publishing this work.

Ethical consent

This work has been approved by the Bioethical Committee from the Spanish National Council of Research (CSIC).

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodchem.2019.01.152.

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