**Understanding the role of saliva on aroma release from wine by
using static and dynamic headspace conditions**

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Abstract

The aim of this work was to determine the role of saliva on wine aroma release by using static and dynamic headspace conditions. In the latter conditions, two different sampling points ($t = 0$ and $t = 10$ min) corresponding with oral (25.5 °C) and post-oral phases (36 °C) were monitored. Both methodologies were applied to reconstituted dearomatized white and red wines with different non-volatile wine matrix composition and a synthetic wine (without matrix effect). All the wines had the same ethanol concentration and were spiked with a mixture of forty five aroma compounds covering a wide range of physicochemical characteristics at typical wine concentrations. Two types of saliva (human and artificial) or control samples (water) were added to the wines. The adequacy of the two headspace methodologies for the purposes of the study (repeatability, linear ranges, determination coefficients, etc) was previously determined. After application of different chemometric analysis (ANOVA, LSD, PCA), results showed a significant effect of saliva on aroma release dependent on saliva type (differences between artificial and human) and on wine matrix using static headspace conditions. Red wines were more affected than white and synthetic wines by saliva, specifically human saliva, which provoked a reduction in aroma release for most of the assayed aroma compounds independent of their chemical structure. The application of dynamic headspace conditions using a saliva bioreactor at the two different sampling points ($t = 0$ and $t = 10$ min) showed a lesser but significant effect of saliva than matrix composition and a high influence of temperature (oral and post-oral phases) on aroma release.

Key words: saliva, wine, aroma release, static HS-SPME-GC/MS, dynamic HS-SPME-GC/MS

INTRODUCTION

Aroma is one of the most outstanding aspects related to food preferences and choices, especially in the case of wine, in which consumption is mainly triggered by a hedonic motivation. Therefore, aroma represents a relevant aspect in wine research and many interesting works have focused in the characterization of aroma impact compounds of different wine types¹⁻³. However, the retronasal aroma profile of a food during consumption might better represent the aroma fraction involved in the interaction with the olfactory receptors than the orthonasal aroma profile, therefore, it should be more closely related with aroma perception⁴.

In the case of the consumption of liquid foods, such as wine, retronasal aroma is produced by the breathing airflow after swallowing sweeping the aroma molecules retained in the oral or throat cavities travelling via the nasopharynx from the mouth or throat to the nose⁵⁻⁷. It has been shown that orthonasal (odor sense when smelling a food) and retronasal aroma perception can be different⁸⁻¹⁰. Different factors involved in the intra-oral release of aroma compounds during consumption (saliva, interaction with mucosa, temperature, breathing flows, in-mouth air cavity volumes, change, etc.) seem to be related to these differences^{7, 11-15 16}.

Saliva is a complex dilute aqueous solution in which its composition varies depending on the respective physiological status, types of food consumed, oral hygiene, etc¹⁷. Saliva contains numerous inorganic salts (sodium, calcium, potassium, chloride, phosphate and bicarbonate)¹⁸ and organic components such as enzymes (amylase, lipases, proteases, etc.)^{17, 19, 20} and proteins (mucins, proline rich proteins, histidine rich proteins, etc)^{21, 22}. Previous studies have shown that saliva might exert an important role on aroma release through different physicochemical (dilution of aroma due to the aqueous phase of saliva, changes in the pH of the food, hydration

of the food which favors aroma release, interaction with salts causing a salting out effect, interaction with proteins); chemical (degradation of odorants); biochemical (degradation of odorant or release from aroma precursors), or even physiological effects (impact on velum-tongue seal formation and swallowing performance), which form part of many previous works performed on this topic^{19, 20, 23-25}.

Nonetheless, many of the studies performed on the saliva effect on aroma release in simple and real food systems seem to be contradictory. Some studies have shown that saliva reduces aroma release: e.g. in pectin gels²⁶ bell peppers²⁵ or French beans²⁴; whilst others have shown an increase in volatiles released from model gels²⁷ or primary and multilayer oil/water emulsions²⁸. There are also others works showing the lack of effect of saliva on aroma release: e.g. in model cheeses²⁹ and from starch and water liquid systems³⁰.

Undoubtedly, the physicochemical characteristics of the volatile compounds are outstanding parameters in determining the degree of interaction with saliva components²². In addition, saliva might induce an array of processes with sometimes opposite effects on aroma release and perception. Therefore, the overall impact of saliva needs to be specifically studied for each food system and aroma composition. Moreover, in many of the above mentioned works, different types of saliva had been used (human, artificial saliva with different compositions), therefore, a comparison of the effect of saliva performed in such different conditions is not straightforward.

As stated in a recent review on wine aroma analysis, the number of studies regarding aroma release during wine consumption using *in vitro* or *in vivo* approaches is scarce³¹, and research on the role of different intra-oral factors (such as saliva) which might be involved in aroma release during wine drinking is still incipient. The effect of saliva has been mainly studied because of its involvement in wine astringency³²⁻³⁷. However, there are very few studies

focused on the role of saliva on wine aroma release^{38, 39}. Although the relatively short-intra-oral period of consumption of liquid foods, could indicate a limited effect of saliva on aroma release, the formation of intra-oral (and pharyngeal) aroma reservoir⁵ and the fact that natural swallowing of saliva is continuously performed, makes the idea that saliva might exert an important role in the perception of wine aroma during consumption perfectly viable, but also affecting the persistence of aroma perception during the post-oral phase of wine consumption. Very recently, using *in vivo* conditions, it was shown that enzymatic degradation of palm wine odorants due to saliva was not noticeable among pyrazines, pyrrolines and most alcohols but was quite pronounced among aldehydes, esters and thiols⁴⁰.

Likewise, in other food systems, the few studies concerning the effect of saliva on aroma release from wines are contradictory. In the work of Genovese and collaborators³⁸, saliva induced, in general, a decrease on aroma release for most of the wine volatiles, and this effect seemed to be more important in white than in red wines. On the contrary, Mitropoulou and co-workers³⁹, observed an enhancement on the release of hydrophobic compounds from model wines and a decrease in the release of the most hydrophilic compounds in the presence of saliva, although this effect was dependent on the concentration of tannins and polysaccharides. Both works were, however, performed in very different conditions; by using dynamic conditions in the work by Genovese et al.(2009)³⁸, and by using a static headspace approach in the work of Mitropoulou et al.(2011)³⁹. The dynamic conditions are advisable to achieve more realistic conditions to that accounting for during food consumption, however, the static conditions have been shown to be better suited for the study of interacting effects that otherwise might be underestimated with the first approach^{23, 41}.

Therefore, the aim of this work was to determine the role of saliva on wine aroma release by using both static and dynamic headspace conditions. In an attempt to follow a systematic study,

avoiding the influence of different factors other than those of interest in this work (saliva effect and wine type), both methodologies were applied to reconstituted wines (with different non-volatile wine matrix composition) and a synthetic wine (with no matrix effect) keeping the concentration of ethanol and aroma compounds the same. In addition, two types of saliva (human and artificial) and control samples (with water) were used to better understand the different mechanisms that saliva might induce on the release of aroma compounds from wine.

MATERIAL AND METHODS

Wine samples

Two commercial Spanish wines representative of different wine matrix compositions were selected for this study: a young Verdejo white wine (W-wine), and a young Tempranillo red wine (R-wine).

Reconstituted wines

Deodorization procedure

Wines were deodorized with Amberlite XAD-2 (Supelco, Bellefonte, PA, USA). Two 25 cm-length glass columns (Pobel, Madrid, Spain), one for each wine type, filled with 100 g of Amberlite XAD-2 were prepared by sequentially conditioning with 250 mL of dichloromethane, then methanol and finally 375 mL of a 12 % (v:v) hydroethanolic solution. After this, wine samples were filtered through glass wool and loaded into the column by slowly passing 750 mL of each wine.

Deodorized wines (750 mL of each) were transferred to 250 mL vials and were completely dried in a lyophiliser (Labconco, Kansas City, MO, USA). Five samples per wine type were prepared using this procedure. To replace the oxygen from the samples, all the dry samples were exposed to a Nitrogen atmosphere and stored at 4 °C until sample preparation. This procedure practically ensured the complete elimination of the original aroma compounds in the wines as was confirmed by HS-SPME-GC/MS analysis.

Wine reconstitution

Deodorized wines were reconstituted with a hydroalcoholic solution and spiked with a volatile mixture to a final ethanol concentration of 12 %. This aroma mixture composed of 45 aroma compounds (manufacturers: Aldrich, Fluka, Merck, Firmenich, Lancaster and Scharlau) representative of a typical wine aroma profile to produce the final concentration of each aroma compound shown in **Table 1**. This table also shows some of the typical gas chromatography and physicochemical properties of these compounds.

As well as the two types of reconstituted wine matrix, a synthetic wine (S-wine) representing a sample with ‘no matrix effect’ was prepared by mixing an hydroalcoholic solution with 4 g/L tartaric acid (Panreac, Barcelona, Spain) and adjusting the pH to 3.5 with NaOH (Panreac).

The influence of ethanol on the volatility of aroma compounds was not considered in this study, since it has been extensively demonstrated⁴²⁻⁴⁵. Therefore, ethanol was kept at the same concentration in all reconstituted and synthetic wines.

Human saliva

Stimulated human saliva was collected from 20 volunteers as described before⁴⁶. Participants could not consume food and water one hour before sampling. To stimulate production, volunteers chewed a little piece of Parafilm™ and spat out as much saliva in a bottle as they could. Sodium azide (NaN₃, Sigma-Aldrich, Saint Quentin Fallavier, France) was added at a final concentration of 0.02% to avoid bacteria and fungi contamination and development. To obtain most representative salivary composition, the different saliva samples were pooled, mixed and centrifuged at 15000 g for 15 min. After that, the salivary pool was filtered through a 0.22 µm Sartorius device under vacuum at 4 °C, to remove saliva bacteria. Finally, saliva was sampled into pots of 20 mL and stored at -80 °C until use.

Artificial saliva

Artificial saliva was prepared as previously described²⁴ by dissolving in 1 L of water (purified by a Milli-Q system) 5.028 g NaHCO₃, 1.369 g K₂HPO₄ x 3 H₂O, 0.877 g NaCl, 0.477 KCl, 0.441 g CaCl₂ x 2 H₂O and 2.16 g mucin (type 1-S from bovine submaxillary glands) from Sigma, (Milan, Italy). The artificial saliva was stored at 4 °C until use.

Static Headspace-SPME sampling procedure

In the human mouth, the average ratio liquid food/saliva had previously been shown to be 5/1 w/v³⁸. Therefore, blends containing the reconstituted white and red wines (W-wine, R-wine) or the synthetic wine (S-wine) were prepared by adding ten mL of the wines spiked with the volatile mixture in a 20 mL vial (Agilent Technologies, Palo Alto, CA, USA). After that, 2 mL of water, human or artificial saliva were added. The headspace vials were immediately closed with a screw cap and polytetrafluoroethylene (PTFE)/silicone septum (Supelco, Bellefonte, PA, USA) and were placed in the incubator of an automatic headspace sampling device (GERSTEL MPS 2, Gerstel Inc., Mülheim an der Ruhr, Germany) at 11 °C. The wine:saliva mixture was

previously pre-incubated for 12 min at 36 °C. In the control wine, the extraction was performed in the headspace of each vial at different incubation times (5, 15, 30 and 45 minutes) to follow the kinetic of aroma release and to determine the equilibrium time, using a DVB/CAR/PDMS (Divinylbenzene/Carboxen/Polydimethylsiloxane 50/30 µm thickness -2 cm length-) coated SPME fiber (Supelco, Bellefonte, PA). After the incubation time the fiber was exposed to the headspace above the sample for 2 min, and the vial was maintained at 36 °C. Desorption was performed in the injector of the GC system (Agilent 6890N) in splitless mode for 1.5 min at 270 °C. After each injection the fiber was cleaned for 30 min to avoid any memory effect. Each analysis was performed in triplicate (one injection per sample vial). Linearity and reproducibility of the procedure were previously determined by using a synthetic wine spiked with different amounts of the aroma solution (75, 150 and 300 µL) covering as closely as possible the wine aroma concentration expected in wines⁴⁷.

The results of this study are shown in supplementary Table 1 in the Supporting information. Herein, satisfactory values for the regression coefficients for most of the aroma compounds were obtained, which ranged from 0.910 to 1.000 and the regression RSDs were also acceptable, with values lower than 20% (except for γ -butyrolactone and ethyl dodecanoate). These results confirmed the lack of interactions between individual volatile aroma compounds in the mixture at the concentrations used⁴⁸, confirming the adequacy of the technique to perform this study.

Dynamic Headspace-SPME sampling procedure

A saliva bioreactor cell was used for these assays⁴⁹. This device was specifically designed to evaluate the particular role of saliva during liquid and semi-solid food consumption. It was composed of a water-jacketed glass flask (100 ml), which allowed a temperature control of the

sample set at 36 °C. This device has five orifices. The first permits clean air to enter the flask to purge the sample (100 mL/min), therefore, reproducing the dynamic conditions of the breathing phenomena. A second orifice is the purge gas outlet, which is connected through a heated transfer line to a flowmeter. In the third orifice the SPME fiber is inserted and the fourth opening is where the sample is introduced. Finally, in order to mix the sample as what might occur in the mouth, a fifth orifice allowed the introduction of a stir bar with digital speed control. An agitation rate of 150 rpm was employed. This last orifice was firmly sealed around the stir bar shaft with a septum to avoid leaks from the flask. During the experiment setup, the sample was added to the apparatus using a glass funnel.

Following the above mentioned 5/1 average ratio liquid food/saliva in the human mouth, 10 ml of water, human saliva or artificial saliva were transferred into the sample flask (100 ml) which was kept at 36 °C, and then 50 mL of wine were then added. The headspace was continuously flushed with purified Nitrogen gas (100 mL/min). Even if the experimental conditions were not directly comparable with conditions in the mouth, two sampling points were assayed to analyze the aroma release resulting from the incubation of control, red and white wines in contact with water, human saliva or artificial saliva (**Figure 1**). The first one, corresponding to an initial sampling time ($t = 0$ min), in which the saliva/wine mixture temperature raised from 25.5 °C to 32.3 °C that might correspond with the introduction of the sample in the mouth (oral-phase). The second sampling point ($t = 10$ min at 36 °C) was more related to the post-oral phase in which aroma from the remaining wine sample could be released within the oral cavity at physiological temperature. In both cases, extraction was performed for 2 minutes. Two or three replicates for each sample type were analyzed depending on the experiment.

It has been shown that inter-fiber repeatability is lower than the intra-fiber accuracy⁵⁰⁻⁵². Therefore, a preliminary inter-fiber repeatability study was performed in order to select the

most similar fibers to complete the study. For this study nine SPME fibers were used to recover the 45 aroma compounds of the aroma mixture added to synthetic wines, and the two SPME fibers exhibiting the lowest variation (less than 10 % RSDs for the extraction of the same aroma compound) were selected and used for the complete set of experiments.

In addition, because the dynamic HS-SPME sampling approach is based in a non-equilibrium situation, a linearity study was carried out in order to seek the relationship between the adsorbed amount of volatiles on the fiber and their initial concentration in the sample. To do so, a synthetic wine spiked with four different amounts of the aroma mixture, was submitted to the dynamic HS extraction conditions as explained above. These results are shown in the supplementary table 1 in the supporting information. As it can be seen there is good linearity, high coefficients of determination (R^2) (better than 0.9 in the assayed concentration range, except for γ -butyrolactone) and adequate regression RSDs for most of the assayed compounds independent of the time at which sampling was performed (0 and 10 minutes). The lack of fit test also showed the adequacy of the propose regression models (p values > 0.01 for most of the aroma compounds) (data not shown). Therefore, the adsorbed amount of aroma compounds in the SPME was linearly proportional to their initial concentration in the sample matrix, highlighting the adequacy of the technique for quantification purposes, which is in agreement with other theoretical and experimental studies performed in simpler aroma systems⁵³.

GC/MS analysis

The identification of volatile compounds was carried out with a Gas Chromatograph Agilent 6890N coupled to a quadrupole Mass Detector Agilent 5973. After desorption of the SPME fiber (270 °C, splitless), volatile compounds were separated on a DB-Wax polar capillary column (60 m $\times$ 0.25 mm i.d. $\times$ 0.50 μ m film thickness) from Agilent (J&W Scientific, Folsom,

USA). Helium was the carrier gas at a flow rate of 1 mL/min. The oven temperature was initially held at 40 °C for 5 min, then increased at 4 °C/min to 240 °C and held for 20 min.

For the MS system (Agilent 5973N), the temperatures of the transfer line, quadrupole and ion source were 250, 150 and 230 °C respectively. Electron impact mass spectra were recorded at 70 eV ionization voltages and the ionization current was 10 µA. The acquisitions were performed in Scan (from 35 to 350 amu) and SIM modes for some specific compounds as indicated in **Table 1**.

The identification of compounds was based on their retention indexes (RIs), comparison of retention times and mass spectra. RIs were calculated from the retention times of n-alkanes (C5–C30) on the same column. The mass spectra were compared with those from three databases: NIST 2.0, WILEY 138 and INRAMASS (internal database achieved using standard compounds).

To avoid possible wine matrix interaction phenomena⁴⁷ instead of using an internal standard compound, release data were referred to absolute peak area, once the precision of the data was proven.

Chemical wine matrix composition

Total acidity and pH, total polyphenols, neutral polysaccharides, residual sugar and nitrogen compounds (total nitrogen, free amino acids and peptides) were determined following previously described analytical procedures⁴⁷.

Saliva biochemical analysis

Protein concentration

The protein concentration was determined using Bradford protein assay Quick Start (Bio-Rad, France) with gamma-globulin as the standard for calibration.

Enzymatic activities

Lipolysis, proteolysis, lysozyme and amylase activities were measured as previously described^{17, 46}.

Statistical analysis

Aroma release data (absolute peak area) were submitted to two-way ANOVA to determine significant effects of the studied factors (saliva type and wine type). In addition, for each aroma compound and wine type (red, white and synthetic) differences between medium type (with human saliva, artificial saliva and water) were subsequently examined by least significant difference (LSD) test. The significance level was $P=0.05$ throughout the study. Principal component analysis (PCA) was also applied to examine the relationship between aroma release data and wine samples. The STATISTICA program for Windows version 7.1 was used for data processing (StatSoft, Inc., 2005, www.statsoft.com). Linear regression analysis to establish the calibration curves of each aroma compound and the lack of fit test to judge the adequacy of the models were performed by using the Statgraphics Centurion XV Version 15.2 (Manugistics, Rockville, MD, USA).

RESULTS & DISCUSSION

To understand the effect of saliva composition on the release of aroma compounds, two types of wines, a white and a red wine were previously deodorized, reconstituted to the same ethanol content and aromatized at the same concentration with the aroma mixture (**Table 1**). With this procedure, it was guaranteed that ethanol did not affect the partition of volatile compounds into

the headspace and that both wine matrices had the same concentration of aroma compounds. Therefore, the main differences between both wines were exclusively due to their matrix composition. **Table 2** shows the chemical composition of both reconstituted wines. The percentage of non-volatile residue and the pH values were very similar. The non-volatile residue was 2.17% (w/w) and 2.99 % (w/w) and the pH was 3.23 and 3.79 for the white and red wines respectively. Total acidity was slightly lower for the red wine (4.29 mg tartaric acid/L) compared to 5.66 mg tartaric acid/L in the case of white wine. The major differences were however, in the total polyphenol content, neutral polysaccharides, residual sugars and nitrogen containing compounds (amino acids and peptides) that were significantly higher in the red wine. These differences in matrix composition have been previously shown to affect the release of aroma compounds in static conditions⁴⁷. In addition to this, a synthetic wine with the same ethanol concentration and pH = 3.5 that could be considered as a wine with “no matrix” effect was also prepared.

For the saliva experiments, two types of saliva were used, artificial saliva with mucin prepared in agreement with the recipe previously described and human saliva collected from different volunteers and mixed together to form a single pool. The composition, regarding total protein content and enzymes (amylase, lipase, lysozyme and protease) was analyzed. The major enzymatic activity detected in the human saliva was lysozyme (698.06 U/mL) followed by proteolysis (16.77 U/mL) and amylase (8.01 U/mL) and in a lesser extent lipase (0.95 mU/mL). These values are in the same order of magnitude to those previously published^{17, 46, 54} except for proteolysis activity, which was higher in our study. In addition to the two types of saliva, control experiments were also performed by adding the same amount of water instead of saliva. With this control, we also eliminated the dilution effect exerted by saliva on volatile release, which has also been described^{28, 55}. In addition, this type of experiment could provide us

important information regarding whether saliva enzymes might have an impact on aroma release from wine as it has been previously shown in simple aroma/saliva mixtures^{19, 20, 56}.

Effect of saliva on aroma release using static headspace conditions

Although static headspace conditions do not mimic the dynamic conditions accounting for during drinking or eating, this technique has been largely used to study aroma interactions with food matrix components to determine their effect on aroma release^{23, 39, 47, 57}. Even so, different authors have shown that this is a reliable approach to investigate partitioning in more controlled and simple conditions, which allows us to envisage this subtle phenomena with importance on aroma release, that otherwise might be underestimated by using dynamic HS methods^{23, 41}.

In this work, the aroma release behavior of a mixture of forty five volatile compounds characteristic of the wine aroma profile and with very different physicochemical characteristics (**Table 1**) was evaluated in presence and absence of human and artificial saliva by using a previously validated static HS-SPME approach (see Table 1 in supporting information). Preliminary experiments were performed in order to determine the equilibration time (5, 15, 30, 45 minutes) for most volatiles of the aroma mixture. From the analysis of the kinetic profiles it was found that five minutes of incubation was enough for the equilibration of most of the aroma compounds of the mixture. Only ten of them (ethyl propanoate, isobutyl acetate, isobutanol, isoamyl acetate, 1-butanol, ethyl octanoate, furfuryl alcohol, α -terpineol, benzyl alcohol and decanoic acid) were not equilibrated after 5 minutes. Nonetheless, since the main objective of this work was to compare wine samples performed under identical experimental conditions, this should not be a constraint for the validity of the data and five minutes was adopted as the sampling time to perform the experiment, which are closer conditions to real physiological situations.

Data corresponding to absolute peak areas of the aroma compounds determined by HS-SPME-GC/MS analysis in the three types of wines (white, red and synthetic) incubated with the two types of saliva (artificial and human) and water, were submitted to a two-factorial ANOVA to determine the magnitude of the effect of matrix composition and type of saliva on aroma release. Results of this analysis showed that both effects and the interactions (matrix composition $\times$ type of saliva) significantly affected the majority of aroma compounds. From a total of forty five aroma compounds, thirty seven were affected by the type of saliva and thirty three by matrix composition (data not shown). This showed the similar importance of both factors on aroma release in static headspace conditions.

To gain insight on the impact of saliva on aroma release depending on wine matrix composition, a LSD test was also carried out for each type of wine and for each aroma compound. **Table 3** shows these results taking into consideration the different aroma chemical families assayed. As it can be seen, in general, the addition of saliva (artificial or human) provoked a significant decrease (or higher retention) on the aroma release for most of the aroma compounds assayed. However, the extent of this effect was dependent on the type of wine, but also on the type of aroma chemical class. In this sense, it is important to highlight that human saliva exerted a high impact on the aroma release from red wines and practically all the aroma compounds assayed were less released when human saliva was added to the wine. However, in the case of white wines this effect was more dependent on the type of aroma compound. For example, the addition of human or artificial saliva did not affect the release of any of the alcohols of the aroma mixture. As it can be seen in the table, the effect of saliva seemed to be much lower in the case of synthetic wines.

To better understand the way in which both factors (type of saliva and wine matrix) affected the aroma release behavior, a PCA was also performed taking into consideration all the aroma

release data. Two principal components, PC1 and PC2 explaining 68.8 % of data variations were obtained (**Figure 2a**). As it can be seen in the graph, PC1 was mainly involved in the separation of the samples depending on the type of medium (with human saliva, artificial saliva or water). In agreement with previous results, the clearest separation (or differences) among wine samples were obtained for red wines. As it can be seen, red wines with human saliva showed positive values for PC1 while red wines with water showed high and negative values for this component. Red wines with artificial saliva showed an intermediate behavior and were placed between the other two types of wine samples (with human saliva and water). PC1 was negatively correlated with many volatile compounds (twenty five volatile compounds showed loadings lower than 0.8 and fifteen of them lower than 0.9). Among them, the variable projection (**Figure 2 b**) showed that some aroma compounds such as limonene (11), hexyl acetate (14), *cis*-3-hexen-1-ol (17), linalool (20) or 5-methylfurfural (21) among others, were strongly correlated with PC1. On the contrary, PC2 separated wines in function of wine type. Red wines exhibited negative values for this component, whilst white and synthetic wines appeared on the half top of the graph showing positive values for PC2.

These results underlined an effect of saliva on aroma release dependent on wine matrix composition. Even more interestingly, red wines seemed to be more affected than white and synthetic wines. The most outstanding effect provoked by human saliva was a reduction on the aroma release of most of the aroma compounds independently of their chemical structure. This global effect could be the result of the combination of single effects that could be differently affecting the volatile compounds employed in this study.

For instance, it is already known that wine polyphenols, which are more abundant and structurally different in red than in white wines, might interact with aroma compounds through different mechanisms depending on polyphenol structure decreasing the amount of aroma

release^{47, 58-60}. In addition to this effect, wine polyphenols (such as procyanidins) might form insoluble complexes with saliva proteins with colloidal structures³⁹ modifying the viscosity of the sample, and therefore, affecting aroma release. To check this hypothesis, the viscosity values of white and red wines with the two types of saliva and water were also determined. **Table 4** shows that the viscosity values determined in all the wines were very similar ranging from 6.9 mPa*s for the white wine with water to 7.4 mPa*s for the white wine with artificial saliva. Therefore, there were not any substantial differences between red or white wines. Although an increase in viscosity induced by saliva has been proposed in order to explain the lower aroma release observe in oil/water emulsions^{12, 28}, the low volume of saliva compared to the wine (1:5) employed in this study, might not be enough to provoke a clear effect, at least in static headspace conditions as used here. Therefore, this factor did not seem a determinant parameter responsible for the higher retention of aroma compounds determined in red wines and specifically in those with human saliva.

The buffering capacity of saliva might be another important factor to explain aroma release, since this property might induce changes in the pH of the food matrix^{12, 22}. In fact, this factor has been pointed out, since it might influence the overall perception of aroma compounds during the *in vivo* consumption of palm-wine⁶¹. To check this hypothesis, the pH values of the human and artificial saliva and the pH values of the wine/saliva mixtures were determined and they are also shown in **Table 4**. The original pH value for the artificial saliva was a little bit higher (8.4) than the pH of the human saliva (8.2). As expected and for both white and red wines, the addition of water practically did not change the pH while it increased with the addition of saliva. Artificial saliva seemed to induce higher changes in pH than human saliva and this could be due to its higher original pH compared to the human saliva. Therefore,

differences induced by changes in pH did not seem relevant to explain the differences in the behavior of the aroma compounds in both wines whatever the matrix and the type of medium.

To explain the retention effect induced by saliva, mainly in red wines, we have to propose additional hypothesis. Previous works using static headspace conditions but with other food matrices have shown the ability of the saliva protein mucin to bind aroma compounds via hydrophobic interactions leading to a reduction in aroma release into the headspace²³.

Moreover, this hypothesis has already been proposed to explain the lower release of a wide range of volatile compounds (e.g. esters, acetates, alcohols) from red and white wines³⁸.

However, in the present study, red wine with human saliva released lower amounts of aroma than the same wine with artificial saliva. The final amount of mucin in the wine/artificial saliva vial was 4.32 mg, while the amount of total protein (including mucin) in the wines samples added with human saliva was lower; 0.98 mg. Therefore, wines spiked with human saliva should have a minor interaction effect with mucin (and therefore higher aroma release) than wines spiked with artificial saliva, which does not explain our results. However, it is important to bear in mind, that human saliva contains other proteins different to mucin, for instance, proline-rich proteins (PRPs), histidine rich proteins (histatins or HRPs), lactoferrine, and enzymes (α -amylase, lipase, etc)²¹, which could be also involved in specific interactions with aroma compounds explaining the lower aroma release of wines with human saliva. In particular, PRPs, which represent up to 70 % of proteins originated from the parotid gland, are known to interact with tannins leading to the formation of some aggregates⁶². Moreover, it has been shown that depending on the protein and tannin concentrations, dense aggregates might coexist with non-aggregated proteins, the latter also showing a significant number of bound tannin molecules⁶². We could hypothesize that the formation of this second type of aggregates might interact with aroma molecules without substantially changing the viscosity of the

solution, as it was observed in the present study in the case of red wines added with human saliva.

Moreover, besides tannins, other wine matrix components might be also involved in the formation of these types of aggregates. Mitropoulou et al. (2011)³⁹ have suggested, at least in reconstituted model wines, the possible formation of saliva protein-polyphenol-carbohydrate complexes able to encapsulate hydrophobic aroma molecules. In this sense, in addition to the higher concentration of polyphenols determined in the red wine employed in this study, the polysaccharide content was also higher (2502 mg mannose/L) compared to the white wines (1667 mg mannose/L) (see **Table 2**). The formation of these type of complexes involving saliva proteins and specific wine polyphenols (tannins) and polysaccharides, both at higher concentrations in red than in white wines, might explain why red wines, and specifically those with human saliva retained more aroma molecules. Moreover, the fact that the very high hydrophobic aroma compounds ($\log P > 2$) of the aroma mixture showed higher retention (lower aroma release) in red wines with saliva than in the white wines, might be in agreement with this hypothesis. The formation of these structures (protein-polyphenol-carbohydrate complexes) might, however, represent a reservoir of aroma molecules ready to be released by the exhalation breath during the *in vivo* red wine consumption, as it has been recently proposed⁶³.

Finally, the salivary metabolic activity might have also affected the release of certain aroma compounds. In this regard, a reduction of aldehydes to the corresponding alcohols and/or partial hydrolysis of certain aroma compounds such as esters might be expected^{19,20}. In the case of the aldehydes employed in the aroma mixture (furfural and 5-methyl furfural), the release of these compounds was lower in red wines with human saliva, which could be in agreement with a possible transformation by an NADP-linked aldehyde reductase¹⁹. However, the increase of

the corresponding alcohol (furfuryl alcohol in the case of furfural) was not significant in these samples. In addition, the involvement of aldehydes in the formation of condensation products such as Schiff bases (e.g. with salivary proteins) or other chemical reactions might be also possible¹⁹.

On the other hand, a reduction of esters in the HS of white but mainly red wines with saliva has been also shown in this work. However, this seems to be more related to the interaction of these compounds with the complex protein-polyphenol-carbohydrate than related to the esterase activity of saliva. Although the decrease in the release of some esters (ethyl butanoate, hexanoate, octanoate, etc) in wines with human saliva compared to control wines (without saliva) has been attributed to the esterase activity³⁸, the activity of these enzymes has only been proven in a very different environment (specifically, in an aqueous system at pH 5)²⁰ to that accounted for in wine (12 % ethanolic system at pH 3.5). Therefore, it seems difficult to obtain straightforward relationships between the decrease in ester release and saliva esterase activity.

Effect of saliva on aroma release using dynamic headspace conditions

In the present work, aroma release from different wine matrices in dynamic conditions was determined by using a bioreactor cell with controlled temperature and agitation conditions at two different sampling times (initial $t = 0$ min and final $t = 10$ min) (**Figure 1**). The initial sampling time ($t = 0$) might be related to the oral phase, in which the mixture of wine (generally cold) and saliva is at lower temperature (25.5 °C) than physiological temperature (36 °C). The final sampling time ($t = 10$) could be more representative of the post-oral phase, in which some volatiles could be released from the liquid sample remaining in the oral cavity after drinking⁵ at oral temperature (36 °C).

Aroma release data collected from $t = 0$ and $t = 10$ minutes are shown in **Tables 5** and **6** respectively. These data were submitted to two independent two way ANOVA (one for each sampling time), considering the global effect of saliva type (artificial, human, water) and type of wine matrix (red, white, synthetic). Results showed that in the oral-phase ($t = 0$) only nine aroma compounds were affected by saliva type while thirty of them were affected by wine matrix (data not shown). In addition, eleven compounds showed an effect of the interaction factor. In the case of the post-oral phase ($t = 10$ minutes) the application of the same statistical treatment also showed a higher influence of wine matrix composition (22 compounds significantly affected) compared to the saliva effect (7 compounds) and the interaction (5 compounds) (data not shown).

Compared to results from the previous static headspace analysis the influence of saliva on aroma release seemed to be lower. The minor effect of saliva addition by using the dynamic approach compared to the static headspace analysis could have been due to a displacement of the equilibrium, which might reduce the retention effect produced by proteins⁴¹ or by other non-volatile wine matrix molecules, such as the above mentioned protein-polyphenol-polysaccharides complexes. These findings are not surprising taking into consideration that several authors have already suggested that in spite that dynamic conditions might better simulate the consumption situation, static measurements are better suited for determining thermodynamic and kinetic parameters with good precision⁶⁴.

In terms of amount of aroma release, it is interesting to notice that higher release for most of the aroma compounds were found during the post-oral release step ($t = 10$ minutes) (**Table 6**) compared to the oral phase (**Table 5**). This could be due to the higher extraction temperature in the post-oral phase (36 °C) compared to the oral phase (25.5 °C). Previously, the effect of temperature (4, 23 and 60 °C) on volatile release from oil/water emulsions using an artificial

mouth system had been pointed out²⁸. These authors showed a similar effect between 4 and 23 °C (release less pronounced), compared to 60 °C. In the present work, using more realistic temperatures closer to what was expected during wine consumption (25.5 °C and 36 °C), most of the volatile compounds showed higher release when the temperature raised about 12 °C independent of the wine type. The increase in sampling temperature increases the partitioning of the volatiles into the gas phase following the vant'Hoff's law⁶⁵. In addition, in ethanol solutions (as wine) and using dynamic headspace conditions, Tsachaki et al. (2008)⁶⁶ showed that the evaporation of ethanol at the solution vapor interface might create a surface tension gradient, making new ethanol molecules move from the bulk phase to replenish the depleted surface areas, carrying along an appreciable volume of underlying liquid with aroma compounds. This phenomenon, called the Marangoni effect⁶⁷, might also explain the higher aroma release for most of the volatile compounds in the wines with a moderate increase in temperature.

To extract more conclusions on the role of saliva on aroma release using dynamic conditions, a LSD test for mean comparison was also performed for each type of wine (red, white and artificial) in the oral and post-oral phases. These results are also shown in **Tables 5** and **6**. Results show that during the oral-phase ($t = 0$), only three terpenes (α - and β -pinene and limonene) showed significant lower release in the three types of wines with saliva (human and artificial) (**Table 5**). The same compounds were significantly less released in white and red wines with saliva during the post-oral phase ($t = 10$) (**Table 6**). These compounds are characterized by high $\log P$ values, which seem to be in agreement with their involvement in the formation of hydrophobic interactions with wine polyphenols⁵⁸ or in their involvement in the formation of complexes with salivary proteins, polyphenols and polysaccharides.

Surprisingly and mainly during the oral-phase, a relatively high number of aroma compounds were highly released in the wines with saliva, which seem to contradict results from the previous experiment performed in static conditions. This could be due to the higher sensitivity of the dynamic HS conditions over the static HS, which might have improved the detection of some aroma compounds⁴¹. For instance, some lactones (*cis*- and *trans*- whiskylactones, γ -nonalactone), furanic compounds (furfural, 5-methylfurfural), volatile phenols (eugenol, ethylphenol), C13 norisoprenoids (β -damascenone, α -ionone, β -ionone), and terpene alcohols (linalool, terpinen-4-ol, α -terpineol, β -citronellol, nerol) were more highly released in red wines with human saliva (**Table 5**). Some of these compounds such as terpene alcohols, could have originated “de novo” from the corresponding grape glycosidic precursor that might have remained in the wine matrix after the desaramatisation step. The existence of saliva glycoside activity has been recently shown⁶⁸.

In order to better understand the impact of saliva on aroma release in the three types of wine matrices, aroma release data (peak area) taken at $t = 0$ and $t = 10$ minutes were independently submitted to PCA. **Figure 3a** shows the representation of the two first principal components obtained after the application of this test to aroma release data collected from the wines at $t=0$. Both PCs explained more than 65% of data variation. As it can be seen (**Figure 3**), similarly to what happens in static conditions, the main differences among wine samples were found in the case of red wines. PC1 clearly separated among red wines with water and artificial saliva from red wines with human saliva. The latter exhibited high and negative values for this component. The representation of the variables on the basis of the two first components (**Figure 3b**) shows how some variables such as *trans* and *cis*-hexenol (16, 17), linalool (20), 5-methylfurfural (21), terpinen-4-ol (22), nerol (29), β -phenylethyl acetate (30), β -damascenone (32) and β -ionone (38) among others, were strongly and negatively correlated with PC1. Most of these

compounds match with those previously shown in the LSD test (**Table 5**), as significantly more released in red wines with saliva, which is the same conclusion obtained by PCA. In **figure 3a**, PC2 also shows a separation of the samples depending on wine matrix composition. Red wines appeared on the top of the graph showing high values for this component, whilst white and mainly synthetic wines appeared in the low part of the graph with lower and even negative values for this component, especially in the water medium. As it can be seen (**Figure 3b**), the most correlated (negatively) variables were -in general, ethyl esters such as ethyl octanoate (18), ethyl decanoate (23) and ethyl dodecanoate (31) and some nonalcoholic terpenes such α -pinene (3), β -pinene (8) and limonene (11). It seems that these compounds (with high Log P value) might interact more with saliva protein and wine matrix than with synthetic wine. In the case of these three terpene compounds, this result was the same previously shown in the LSD test.

Similarly to the results found in the previous ANOVA and LSD test, results from the PCA performed with release data collected during the post-oral phase ($t = 10$ minutes) did not show a clear grouping of wine samples depending on the medium composition (with human saliva, artificial saliva or water) (**Figure 4a**). However, an influence on the wine matrix composition was indeed manifested. As it can be seen, PC1 separates between red on the positive side of the graph and white and synthetic wines on the other side (**Figure 4a**) showing differences on their aroma release behavior. Red wines exhibited higher values for this component than white wines. The projection of the variables on the plane defined by the first and second components (**Figure 4b**) shows that PC1 was highly correlated (negatively) with some aroma compounds such as, terpinen-4-ol (22), β -citronellol (28), α -ionone (33), β -phenylethyl alcohol (37), 4-ethylguaicol (40) and 4-ethyl phenol (44), among others. In addition, PC2 also allowed a separation between synthetic wines with positive values for this component and white and red

wines with negative values for it. In this case, PC2 was strongly and negatively correlated with some alcohols such as 1-butanol (10) or isoamyl alcohols (12) but positively with some non-alcoholic terpenes such as α - and β - pinene (3, 8) and limonene (11).

In conclusion, the main finding of this work is that saliva has an important effect on aroma release from wine and this effect was different depending on wine matrix composition. In addition, we found differences depending on using human or artificial saliva, therefore proving that other proteins than mucins seem to have an important role on aroma release. Moreover, it has been shown that the effect of saliva on wine aroma release is more evident when using static than dynamic headspace conditions. In general, human saliva produces lower release for most of the wine volatile compounds, and this effect was more important in red than white wines. The interaction of aroma compounds with other proteins different to mucin and/or the formation of complexes involving saliva glycoproteins-wine polyphenols-wine polysaccharides and aroma compounds, preferentially for those aroma compounds with high log *P* values (hydrophobic), seem to be responsible for the observed effect. In addition, large differences in the amount of aroma released depending on sampling temperature during the oral and post-oral phases invite us to think about the importance of this second step of wine consumption as a mechanism in releasing aroma compounds from oral or throat wine reservoirs influencing long lasting perception of aroma after swallowing. Finally, in spite of the minor impact of saliva observed in dynamic conditions, it is important to bear in mind that *in vivo* consumption conditions, could represent a more dynamic process to that used in the present work, in which saliva is continuously produced and replenished (incorporating more proteins to interact with aroma compounds, or enzymes) and also “fresh” sample is continuously being provided. Therefore, the extent of its effect could be higher than that determined with the experimental *in vitro* dynamic headspace conditions used in this study. On overall, this work will contribute to

gain insight on the role of oral physiology on wine aroma perception, which should be taken into consideration in the production of high quality wines for targeted groups of consumers.

ASSOCIATED CONTENT

*** Supporting Information**

Table with the Linear ranges and regression parameters calculated for the aroma compounds by using static and dynamic (t = 0 and t = 10 minutes) HS-SPME-GC/MS approaches. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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Notes

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FIGURE CAPTIONS

Figure 1. Representation of the sampling procedure employed during the dynamic HS-SPME-GC/MS analysis.

Figure 2. Graphic representation of the wine samples (2a) and of the variables (2b) obtained using PCA with the aroma release data from the static HS-SPME-GC/MS. Numbers of the variables in Figure 2b correspond to the compounds listed in Table 1. RW, SW and WW mean red, synthetic and white wines respectively.

Figure 3. Graphic representation of the wine samples (2a) and of the variables (2b) obtained using PCA with the aroma release data from the dynamic HS-SPME-GC/MS analysis at $t = 0$ (oral phase). Numbers of the variables in Figure 3b correspond to the compounds listed in Table 1.

Figure 4. PCA of the Graphic representation of the wine samples (2a) and of the variables (2b) obtained using PCA with the aroma release data from the dynamic HS-SPME-GC/MS analysis at $t = 10$ (post oral phase). Numbers of the variables in Figure 4b correspond to the compounds listed in Table 1.

836 **Table 1.** Chromatographic and physicochemical characteristics of the volatile compounds employed in this study.

Nº	Compound	RI exp ^a	RI lit ^b	Ion Q ^c (m/z)	MW ^d (g/mol)	LogP ^e	BP ^f (°C)	DESCRIPTOR ^g	CAS number	Concentration ^h (mg/ L)
1	Ethyl Propanoate	< 1000	950	57	102	1.2	99.1	fruit	105-37-3	0.61
2	Isobutyl acetate	1018	1018	56	116	1.8	116.5	fruit, apple, banana	110-19-0	0.33
3	α -pinene	1030	1035	93	136	4.8	156.0	pine, turpentine	80-56-8	0.20
4	Ethyl butanoate	1043	1040	71	116	1.9	121.5	apple	105-54-4	0.54
5	Ethyl 2-methylbutanoate	1060	1056	57	130	2.3	133.0	apple	7452-79-1	0.29
6	Butyl acetate	1079	1079	43	116	1.8	126.1	pear	123-86-4	0.35
7	Isobutanol	1100	1103	74	74	0.8	108.0	wine, solvent, bitter	78-83-1	1.38
8	β -pinene	1120	1118	93	136	4.4	164.0	pine, resin, turpentine	127-91-3	0.25
9	Isoamyl acetate	1131	1117	70	130	2.3	142.5	banana	123-92-2	0.69
10	1-butanol	1154	1145	56	74	0.8	117.0	medicine, fruit	71-36-3	0.93
11	Limonene	1217	1208	68	136	4.8	176.0	lemon, orange, citrus	5989-27-5	0.23
12	Isoamylic alcohols	1217	1208	55	86	1.3	128.0	wine, onion	123-51-3	30.01
13	Ethyl hexanoate	1247	1231	88	136	2.8	167.0	apple peel, fruit	123-66-0	0.89
14	Hexyl acetate	1286	1276	56	144	2.8	171.5	fruit, herb	142-92-7	0.92
15	1-Hexanol	1364	1362	56	102	2.0	156.0	resin, flower, green	111-27-3	0.91
16	<i>trans</i> -3-Hexen-1-ol	1376	1386	67	100	1.6	156.5	mossss, fresh	928-97-2	0.31
17	<i>cis</i> -3-Hexen-1-ol	1399	1398	67	100	1.6	156.5	grass	928-96-1	0.33
18	Ethyl octanoate	1453	1444	127	172	3.8	208.5	fruit, fat	106-32-1	0.79
19	Furfural	1487	1466	95	96	0.4	161.7	bread, almond, sweet	98-01-1	0.85
20	Linalool	1557	1544	93	154	3.0	198.0	flower, lavender	78-70-6	0.24
21	5-Methylfurfural	1603	1573	109+110	110	0.7	187.0	almond, caramel, burnt	620-02-0	0.54
22	Terpinen-4-ol	1633	1606	93	154	3.3	209.0	turpentine, nutmeg, must	2438-10-0	0.30
23	Ethyl decanoate	1658	1636	101	200	4.8	241.5	grape	110-38-3	0.38
24	Furfuryl alcohol	1677	1672	98	98	0.3	171.0	burnt	98-00-0	0.55

25	γ -butyrolactone	1674	1647	42	86	-0.6	204.0	caramel, sweet	96-48-0	1.97
26	Diethyl succinate	1693	1647	101	174	1.2	217.7	wine, fruit	123-25-1	0.69
27	α -Terpineol	1725	1688	59	154	3.0	217.5	oil, anise, mint	10482-56-1	0.20
28	β -Citronellol	1780	1768	69	156	3.9	224.0	rose	106-22-9	0.28
29	Nerol	1820	1792	69	154	3.6	225.0	sweet	106-25-2	0.23
30	β -phenylethyl acetate	1852	1829	104	164	2.3	232.6	rose, honey, tobacco	103-45-7	0.74
31	Ethyl dodecanoate	1860	1842	88	228	5.7	281.2	leaf	106-33-2	0.43
32	β -Damascenone	1860	1815	190	190	4.2	275.0	apple, rose, honey	23726-93-4	0.20
33	α -ionone	1894	1840	93	192	3.9	259.5	wood, violet	127-41-3	0.10
34	Hexanoic acid	1900	1829	60	116	2.1	203.0	sweat	142-62-1	0.83
35	Benzyl alcohol	1909	1897	79	108	1.1	205.3	sweet, flower	100-51-6	0.74
36	<i>trans</i> -whiskey lactone	1935	1977	99	156	2.0	260.6	flower, lactone	80041-01-6	0.69
37	β -phenylethyl alcohol	1948	1925	91	122	1.4	218.2	honey, spice, rose, lilac	60-12-8	3.28
38	β -ionone	1985	1912	177	192	3.8	262.9	raspberry, violet, flower,	79-77-6	0.10
39	<i>cis</i> -whiskey lactone	2010	1985	99	156	2.0	260.6	coconut	80041-00-5	0.69
40	4-ethylguaicol	2067	2031	137	152	2.4	248.39	spice, clove	2785-89-9	0.35
41	γ -Nonalactone	2081	2042	85*	156	2.1	243.0	coconut, peach	104-61-0	0.17
42	Octanoic acid	2107	2083	60	144	3.1	239.0	sweat, cheese	124-07-2	1.96
43	Eugenol	2205	2164	164*	164	2.3	253.2	clove, honey	97-53-0	0.21
44	4-Ethylphenol	2205	2170	107*	122	2.6	217.9	must	123-07-9	0.40
45	Decanoic acid	2328	2361	60	172	4.1	278.6	rancid, fat	334-48-5	0.78

837 ^a Experimental retention index calculated with an alkane mixture (C5–C30) on DB-WAX column.

838 ^b Linear retention index from literature (NIST Chemistry Webbook).

839 ^c Ion of quantification (* Compound determined in SIM mode).

840 ^d Molecular weight.

841 ^e Hydrophobic constant estimated using molecular modeling software EPI Suite (U.S. EPA 2000-2007).

842 ^f Boiling point estimated using molecular modeling software EPI Suite (U.S. EPA 2000-2007).

843 ^g From Flavornet (<http://www.flavornet.org>; accessed October 2009) database, from NIST web chemistry book (2005) (<http://www.webbook.nis.gov/chemistry>).

844 ^h Final concentration in the wine.

845

846 **Table 2.** Chemical composition of the red and white wines employed in this study.

	White Wine		Red Wine	
	Mean	SD	Mean	SD
Non-volatile residue (% w/w)	2.17	0.11	2.99	0.08
pH	3.23	0.01	3.79	0.01
Total acidity (mg tartaric acid/L)	5.66	0.1	4.29	0.2
Total polyphenols (mg gallic acid/L)	269.95	17.2	1647.98	292.8
Neutral polysaccharides (g mannose/L)	1.67	0.5	2.50	0.9
Residual sugars (g/L)	1.12	0.2	3.68	0.5
Total nitrogen (mg/L)	239.96	32.9	406.00	65.7
Amino acids + peptides (mg N/L)	49.54	2.2	133.51	10.9
Amino acids (mg N/L)	30.67	0.8	58.57	1.4
Peptides (mg N/L)*	18.87	-	79.94	-

847

848 Values are average of two determinations except for pH (average of three determinations).* This value is indirectly
849 determined as the difference between the analytical determination of amino acids plus peptides and free amino
850 acids, therefore SD (Standard deviation) values are not included in the table.

851 **Table 3.** Average aroma release values and results of LSD test in the wines determined by static HS-SPME-GC/MS.

	Synthetic wine			White wine			Red wine		
	Water	Artificial saliva	Human saliva	Water	Artificial saliva	Human saliva	Water	Artificial saliva	Human saliva
Terpenes									
α -pinene	495.6 b	398.4 a	412.7 a	466.3 b	392.7 a	381.6 a	560.3 c	442.8 a	517.5 b
β -pinene	584.8 b	483.3 a	488.4 a	536.5 b	461.1 a	432.7 a	648.0 c	533.0 a	574.1 b
Limonene	95.1 b	76.5 a	77.9 a	89.0 b	72.8 a	72.1 a	99.5 b	82.9 a	82.5 a
Linalool	2.3 a	2.1 a	2.2 a	2.2 a	2.1 a	2.1 a	2.4 b	2.4 ab	2.2 a
Terpinen-4-ol	1.8 a	1.6 a	1.6 a	1.7 a	1.6 a	1.7 a	1.8 b	1.7 ab	1.6 a
α -terpineol	1.2 b	1.0 a	1.0 ab	1.2 b	1.1 a	1.2 b	1.2 b	1.0 a	1.0 a
β -citronellol	2.3 b	2.0 a	2.0 a	2.1 a	2.0 a	2.0 a	2.3 b	2.1 ab	2.0 a
Nerol (cis-geraniol)	1.7 b	1.5 a	1.5 ab	1.6 b	1.5 a	1.4 a	1.7 b	1.6 ab	1.5 a
Esters									
Ethyl propanoate	19.1 b	17.9 a	19.0 b	17.9 a	17.6 a	17.2 a	20.1 a	18.9 a	18.4 a
Isobutyl acetate	11.8 b	11.0 a	11.6 b	10.8 a	10.9 a	10.5 a	12.2 a	11.4 a	11.2 a
Ethyl butanoate	39.1 ab	38.2 a	41.4 b	35.9 a	36.1 a	38.0 a	41.9 b	38.1 a	36.9 a
Ethyl 2-methylbutanoate	29.4 c	26.6 a	27.7 b	26.4 a	25.5 a	25.4 a	30.4 b	27.9 ab	27.5 a
Butyl acetate	33.6 a	31.7 a	27.0 a	31.6 a	30.8 a	31.0 a	36.5 b	34.1 a	32.8 a
Isoamyl acetate	78.9 c	71.3 a	74.5 b	72.9 a	71.2 a	70.1 a	82.7 b	74.5 a	73.5 a
Ethyl hexanoate	194.5 b	173.2 a	179.0 a	179.9 b	169.7 a	170.6 a	201.4 b	183.7 a	176.6 a
Hexyl acetate	116.9 b	104.7 a	106.8 a	107.7 b	102.3 a	102.7 a	121.3 b	111.1 a	106.1 a
Ethyl octanoate	101.0 b	83.8 a	85.8 a	88.5 b	82.1 a	82.3 a	95.0 b	87.5 a	82.5 a
Ethyl decanoate	122.2 b	96.5 a	102.0 a	104.1 b	93.4 a	93.0 a	102.8 b	106.5 b	93.8 a
Diethyl succinate	1.6 a	1.4 a	1.5 a	2.2 b	2.0 a	2.1 ab	2.9 b	3.4 c	1.7 a
Beta-phenylethyl acetate	13.4 b	12.2 a	12.5 ab	12.8 a	12.4 a	12.6 a	13.6 b	12.7 ab	12.1 a
Ethyl dodecanoate	294.3 b	216.3 a	212.4 a	215.8 b	187.4 a	182.4 a	218.5 b	243.5 c	163.3 a
Alcohols									
Isobutanol	0.1 a	0.1 a	0.1 a	0.1 a	0.1 a	0.1 a	0.3 b	0.3 b	0.2 a

1-butanol	1.0 a	1.0 a	1.0 a	1.0 a	1.0 a	1.0 a	1.2 c	1.1 b	1.0 a
Isoamyl alcohols	53.4 a	52.1 a	56.1 a	52.7 a	52.1 a	51.1 a	59.8 b	58.1 b	54.0 a
1-hexanol	8.4 a	8.1 a	8.2 a	8.1 a	8.1 a	8.0 a	9.2 b	8.6 a	8.2 a
<i>trans</i> -3-hexen-1-ol	1.0 a	0.9 a	1.0 a	1.0 a	0.9 a	1.0 a	1.1 b	1.0 ab	1.0 a
<i>cis</i> -3-hexen-1-ol	1.1 a	1.0 a	1.0 a	1.0 a	1.0 a	1.0 a	1.1 b	1.1 ab	1.0 a
Benzyl alcohol	0.4 a	0.3 a	0.4 a	0.4 a	0.3 a	0.4 a	0.4 a	0.4 a	0.4 a
β -phenylethyl alcohol	3.8 a	3.1 a	3.6 a	3.4 a	2.9 a	3.0 a	3.7 a	4.0 a	3.4 a
Lactones/Furanic									
Furfural	3.0 b	2.7 a	2.9 b	2.7 b	2.6 a	2.7 ab	3.0 b	2.8 b	2.7 a
5- methylfurfural	1.3 b	1.1 a	1.2 ab	1.2 a	1.1 a	1.2 a	1.3 b	1.2 b	1.2 a
γ -butyrolactone	0.2 a	0.2 a	0.2 a	0.3 b	0.2 a	0.2 ab	0.4 a	0.5 a	0.4 a
Furfuryl alcohol	0.2 a	0.2 a	0.2 a	0.2 a	0.2 a	0.2 a	0.2 a	0.2 a	0.2 a
<i>trans</i> -whiskey lactone	0.9 a	0.8 a	0.8 a	0.8 a	0.8 a	0.8 a	0.9 a	0.9 a	0.8 a
<i>cis</i> -whiskey lactone	0.7 a	0.6 a	0.6 a	0.6 a	0.6 a	0.6 a	0.7 b	0.6 ab	0.6 a
γ -nonalactone	0.5 a	0.4 a	0.4 a	0.4 a	0.3 a	0.4 a	0.4 b	0.4 b	0.3 a
Volatile phenols									
2-methoxy,4-ethylphenol	1.7 b	1.5 a	1.5 ab	1.6 a	1.5 a	1.6 a	1.7 b	1.6 b	1.5 a
Eugenol	0.3 b	0.3 a	0.3 ab	0.3 a	0.3 a	0.3 a	0.3 b	0.3 b	0.3 a
4-ethylphenol	1.3 a	1.1 a	1.2 a	1.2 a	1.1 a	1.1 a	1.3 a	1.3 a	1.2 a
C13-norisoprenoids									
β -damascenone	1.6 a	1.5 a	1.6 a	1.6 a	1.5 a	1.5 a	1.6 b	1.5 ab	1.4 a
α -ionone	2.5 a	2.3 a	2.3 a	2.4 b	2.2 a	2.3 a	2.4 b	2.3 b	2.2 a
β -ionone	4.4 b	4.0 a	3.9 a	4.2 b	3.9 a	4.0 ab	4.2 b	4.0 ab	3.7 a
Acids									
Hexanoic acid	1.0 b	0.8 a	1.0 b	0.9 a	0.8 a	0.9 a	1.1 b	0.8 a	0.9 a
Octanoic acid	2.7 b	2.2 a	2.7 ab	2.6 b	2.4 a	2.3 a	2.8 b	2.1 a	2.3 a
Decanoic acid	2.5 b	1.3 a	1.4 a	1.5 b	1.3 ab	1.2 a	1.4 b	1.1 a	1.4 b

852 All values (area: arbitrary unit) are divided by a factor of 10.000. Different letters for the same aroma compound in the same wine type (synthetic, white, red) denote statistical differences among saliva types after
853 applying LSD test.

854

855 **Table 4.** Viscosity and pH values determined for the saliva samples and wine/saliva mixtures
856 (*n* = 3).
857

	Viscosity (mPa x s)			pH		
	Water	Human saliva	Artificial saliva	Water	Human saliva	Artificial saliva
	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)
Salivas	-	7.0 (0.1)	7.9 (0.2)	-	8.2 (0.1)	8.4 (0.2)
Red wine	7.2 (0.1)	7.3 (0.1)	7.1 (0.1)	3.8 (0)	4.0 (0.1)	4.2 (0.1)
White wine	6.9 (0.0)	7.3 (0.3)	7.4 (0.5)	3.1 (0)	3.3 (0.1)	3.7 (0.1)

858 **Table 5.** Average aroma release values and results of LSD test in the wines determined by dynamic HS-SPME-GC/MS at t = 0 (oral-phase).

	Synthetic wine			White wine			Red wine		
	Water	Artificial saliva	Human saliva	Water	Artificial saliva	Human saliva	Water	Artificial saliva	Human saliva
Terpenes									
α -pinene	49.1 b	0.2 a	19.0 a	7.2 b	2.0 a	0.7 a	10.9 c	1.3 a	3.7 b
β -pinene	45.1 b	0.0 a	16.3 a	9.9 b	3.2 a	1.1 a	19.4 c	3.3 a	7.3 b
Limonene	14.8 b	0.9 a	7.1 a	4.6 b	3.4 b	1.6 a	3.9 c	1.4 a	2.3 b
Linalool	1.7 a	1.6 a	1.7 a	1.9 a	1.9 a	1.9 a	1.8 a	1.6 a	2.1 b
Terpinen-4-ol	1.2 a	1.1 a	1.1 a	1.3 a	1.3 a	1.3 a	1.3 ab	1.1 a	1.5 b
α -terpineol	1.0 a	1.1 a	0.9 a	0.7 a	0.8 a	0.8 a	0.7 a	0.6 a	0.9 b
β -citronellol	1.4 a	1.1 a	1.1 a	1.3 a	1.4 a	1.3 a	1.3 b	1.1 a	1.6 c
Nerol (cis-geraniol)	0.9 b	0.7 a	0.8 ab	0.9 a	0.9 a	0.9 a	0.9 ab	0.8 a	1.1 b
Esters									
Ethyl propanoate	37.8 a	36.1 a	39.7 b	39.1 a	41.8 a	41.2 a	38.6 b	37.1 a	36.8 a
Isobutyl acetate	19.2 b	16.8 a	18.9 b	19.0 a	20.0 a	19.2 a	18.5 c	17.2 a	17.8 b
Ethyl butanoate	58.9 b	52.0 a	60.6 b	58.5 a	63.0 a	58.2 a	57.5 c	53.5 a	55.5 b
Ethyl 2-methylbutanoate	34.8 b	28.7 a	32.1 ab	32.3 a	34.4 a	32.0 a	31.3 c	28.8 a	30.2 b
Butyl acetate	48.0 a	45.9 a	48.4 a	48.6 a	51.6 a	50.3 a	47.8 a	43.6 a	47.6 a
Isoamyl acetate	100.1 b	89.2 a	96.6 ab	94.7 a	104.4 a	88.8 a	94.1 a	82.9 a	94.1 a
Ethyl hexanoate	184.0 b	150.7 a	164.7	169.9 a	176.4 a	164.3 a	151.0 a	136.4 a	153.7 a
Hexyl acetate	112.4 b	93.1 a	101.1	104.7 a	108.3 a	101.7 a	91.8 a	82.6 a	93.6 a
Ethyl octanoate	65.9 b	46.6 a	50.1 a	56.5 a	59.7 a	53.3 a	42.2 ab	38.2 a	43.5 b
Ethyl decanoate	35.4 b	19.5 a	19.3 a	25.0 a	26.4 a	22.2 a	17.8 a	15.3 a	17.8 a
Diethyl succinate	1.0 a	0.9 a	0.9 a	1.1 a	1.2 a	1.1 a	1.8 a	1.9 a	2.5 b
Beta-phenylethyl acetate	11.5 a	11.6 a	11.0 a	13.3 a	13.9 a	13.7 a	12.7 ab	11.8 a	14.9 b
Ethyl dodecanoate	29.2 a	14.0 a	11.8 a	13.0 a	14.3 a	11.2 a	9.9 c	6.1 a	7.9 b
Alcohols									
Isobutanol	0.2 a	0.3 a	0.4 a	0.3 a	0.3 a	0.3 a	0.5 a	0.5 a	0.5 a

1-butanol	1.6 a	1.6 a	2.0 a	1.7 a	1.7 a	1.7 a	1.8 a	1.7 a	1.9 a
Isoamyl alcohol	68.0 a	77.3 b	75.9 b	78.7 a	80.7 a	79.6 a	83.0 a	78.4 a	85.3 a
1-hexanol	9.2 a	9.9 a	9.9 a	10.4 a	10.6 a	10.7 a	10.7 a	9.7 a	11.3 a
<i>trans</i> -3-hexen-1-ol	1.1 a	1.1 a	1.2 a	1.1 a	1.2 a	1.2 a	1.2 ab	1.1 a	1.3 b
<i>cis</i> -3-hexen-1-ol	0.9 a	0.9 a	1.1 b	1.0 a	1.0 a	0.9 a	1.0 ab	0.9 a	1.2 b
Benzyl alcohol	0.2 a	0.2 a	0.3 a	0.3 a	0.3 a	0.3 a	0.3 a	0.3 a	0.4 b
β -phenylethyl alcohol	1.9 a	1.9 a	1.9 a	2.1 a	2.6 a	2.5 a	2.1 a	2.3 a	3.2 b
Lactones/Furanic									
Furfural	2.9 a	2.7 a	3.2 b	2.1 a	2.3 a	2.1 a	2.7 a	2.6 a	3.3 b
5- methylfurfural	1.2 a	1.1 a	1.2 b	1.1 a	1.2 a	1.2 a	1.1 a	1.1 a	1.4 b
γ -butyrolactone	0.3 a	0.3 a	0.3 a	0.3 a	0.4 a	0.4 a	0.5 a	0.4 a	0.6 a
Furfuryl alcohol	0.1 a	0.1 a	0.1 b	0.1 a	0.2 a	0.2 a	0.1 a	0.1 a	0.2 a
<i>trans</i> -whiskey lactone	0.6 a	0.5 a	0.5 a	0.6 a	0.7 a	0.6 a	0.6 a	0.6 a	0.8 b
<i>cis</i> -whiskey lactone	0.4 a	0.4 a	0.3 a	0.4 a	0.5 a	0.4 a	0.4 a	0.4 a	0.6 b
γ -nonalactone	0.2 a	0.2 a	0.2 a	0.2 a	0.3 b	0.3 b	0.3 a	0.2 a	0.3 b
Volatile phenols									
2-methoxy,4-ethylphenol	1.0 a	1.0 a	0.9 a	1.1 a	1.2 a	1.2 a	1.1 a	1.1 a	1.4 a
Eugenol	0.1 a	0.2 a	0.1 a	0.2 a	0.2 b	0.2 b	0.1 a	0.2 ab	0.2 b
4-ethylphenol	0.7 a	0.7 a	0.6 a	0.8 a	0.9 a	0.9 a	0.7 a	0.8 a	1.1 b
C13-norisoprenoids									
β -damascenone	1.2 a	1.2 a	1.1 a	1.4 a	1.4 a	1.4	1.3 ab	1.1 a	1.5 b
α -ionone	1.9 a	1.6 a	1.5 a	2.0 a	2.0 a	1.9	1.8 ab	1.5 a	2.1 b
β -ionone	2.9 b	2.5 ab	2.2 a	3.1 a	3.1 a	3.1	2.8 a	2.5 a	3.4 b
Acids									
Hexanoic acid	0.6 b	0.5 a	0.7 b	0.8 a	0.8 a	0.7	0.3 a	0.5 a	0.9 a
Octanoic acid	1.2 a	1.1 a	1.1 a	1.6 a	2.2 a	1.5	1.8 a	1.5 a	2.0 a
Decanoic acid	nd	nd	nd	nd	nd	nd	nd	nd	nd

859 All values (area: arbitrary unit) are divided by a factor of 10.000. Different letters (a-c) for the same aroma compound in the same wine type (synthetic, white, red) denote
860 statistical differences among saliva types after applying LSD test

861 **Table 6.** Average aroma release values and LSD test in the wines determined by dynamic HS-SPME-GC/MS at t = 10 (post oral-phase).

	Synthetic wine			White wine			Red wine		
	Water	Artificial saliva	Human saliva	Water	Artificial saliva	Human saliva	Water	Artificial saliva	Human saliva
Terpenes									
α -pinene	20.6 a	0.2 a	19.0 a	6.8 b	1.6 a	0.7 a	8.8 b	1.2 a	3.7 a
β -pinene	21.6 a	0.1 a	18.4 a	9.8 b	2.8 a	1.1 a	17.5 b	3.6 a	7.5 a
Limonene	11.9	1.3 a	9.7 a	5.5 c	3.6 b	2.2 a	4.2 c	1.7 a	2.7 b
Linalool	4.4 a	4.9 a	4.6 a	4.5 a	4.5 a	5.0 b	4.3 a	4.1 a	4.3 a
Terpinen-4-ol	3.6 a	3.8 a	3.4 a	3.5 a	3.5 a	3.6 a	3.4 a	3.2 a	3.3 a
α -terpineol	3.1 a	3.5 a	2.9 a	2.0 a	2.1 a	2.3 a	1.9 a	1.8 a	1.8 a
β -citronellol	4.1 a	4.0 a	3.7 a	3.8 ab	3.5 a	4.0 b	3.6 a	3.4 a	3.5 a
Nerol (cis-geraniol)	2.3 a	2.3 a	2.4 a	2.1 ab	2.0 a	2.4 b	2.2 a	2.1 a	2.2 a
Esters									
Ethyl propanoate	28.7 a	32.5 b	31.1 a	32.1 a	32.3 a	32.3 a	30.4 a	28.9 a	30.2 a
Isobutyl acetate	17.6 a	19.1 a	18.5 a	18.5 a	19.5 b	18.6 a	17.7 a	17.0 a	17.3 a
Ethyl butanoate	61.8 a	65.2 a	66.6 a	64.2 a	66.3 a	62.7 a	60.0 a	56.2 a	59.2 a
Ethyl 2-methylbutanoate	35.0 a	37.9 a	36.4 a	35.4 ab	36.9 b	34.8 a	33.5 b	31.9 a	32.2 ab
Butyl acetate	52.3 a	59.7 b	55.8 a	55.4 a	58.3 b	56.3 a	54.1 a	52.8 a	52.9 a
Isoamyl acetate	117.5	124.9 a	120.2 a	118.1 a	123.0 a	117.9 a	110.5 a	110.6 a	108.4 a
Ethyl hexanoate	256.6	264.6 a	257.3 a	242.0 a	248.2 a	240.6 a	216.9 a	206.5 a	210.6 a
Hexyl acetate	159.9	163.8 a	159.2 a	150.4 a	153.4 a	149.5 a	133.3 a	126.1 a	128.8 a
Ethyl octanoate	100.9	94.7 a	94.2 a	84.6 a	83.5 a	83.2 a	63.9 a	59.5 a	60.7 a
Ethyl decanoate	57.2 a	45.8 a	45.2 a	45.0 a	42.1 a	42.6 a	31.4 a	28.2 a	28.7 a
Diethyl succinate	2.2 a	2.1 a	2.1 a	2.3 a	2.2 a	2.4 a	4.2 a	3.7 a	4.0 a
Beta-phenylethyl acetate	25.6 a	29.5 a	26.7 a	28.3 a	26.6 a	28.8 a	26.9 a	25.5 a	25.8 a
Ethyl dodecanoate	50.5 a	31.7 a	30.3 a	25.6 b	21.0 a	20.3 a	17.1 b	10.7 a	12.8 a
Alcohols									
Isobutanol	0.2 a	0.3 a	0.3 a	0.3 a	0.3 a	0.3 a	0.5 a	0.5 b	0.5 a

1-butanol	1.9 a	2.4 a	2.4 a	2.4 a	2.5 a	2.4 a	2.3 a	2.4 a	2.3 a
Isoamylic alcohols	92.4 a	116.7 b	102.6 a	113.3 a	118.7 a	117.6 a	112.4 a	117.0 b	112.3 a
1-hexanol	17.5 a	21.2 b	19.0 a	19.6 a	20.3 a	20.3 a	19.5 a	19.1 a	19.2 a
<i>trans</i> -3-hexen-1-ol	2.1 a	2.5 a	2.4 a	2.3 a	2.9 a	2.5 a	2.3 a	2.2 a	2.2 a
<i>cis</i> -3-hexen-1-ol	1.7 a	2.1 b	2.1 b	2.1 a	2.1 a	2.3 a	2.0 a	2.0 a	2.2 b
Benzyl alcohol	0.4 a	0.4 a	0.4 a	0.4 a	0.4 a	0.4 a	0.4 a	0.4 a	0.4 a
β -phenylethyl alcohol	3.8 a	3.8 a	3.7 a	3.5 a	3.4 a	3.9 a	3.9 a	3.2 a	3.7 a
Lactones/Furanic									
Furfural	5.2 a	6.1 b	6.2 b	4.8 b	5.1 c	4.5 a	5.4 a	5.6 a	5.8 a
5- methylfurfural	2.2 a	2.4 a	2.5 a	2.4 a	2.3 a	2.4 a	2.4 a	2.2 a	2.5 a
γ -butyrolactone	0.4 a	0.5 a	0.3 a	0.4 a	0.5 a	0.5 a	0.6 a	0.5 a	0.6 a
Furfuryl alcohol	0.2 a	0.2 a	0.2 a	0.2 a	0.2 a	0.2 a	0.2 a	0.2 a	0.2 a
<i>trans</i> -whiskey lactone	1.4 a	1.4 a	1.3 a	1.3 a	1.3 a	1.4 a	1.4 a	1.3 a	1.3 a
<i>cis</i> -whiskey lactone	0.9 a	0.9 a	0.8 a	0.9 a	0.9 a	0.9 a	1.0 a	0.9 a	0.9 a
γ -nonalactone	0.5 a	0.5 a	0.5 a	0.5 b	0.5 a	0.5 b	0.5 a	0.4 a	0.5 a
Volatile phenols									
2-methoxy,4-ethylphenol	2.4 a	2.4 a	2.3 a	2.4 a	2.2 a	2.5 a	2.3 a	2.2 a	2.3 a
Eugenol	0.3 a	0.3 a	0.3 a	0.3 ab	0.3 a	0.4 b	0.3 a	0.3 a	0.3 a
4-ethylphenol	1.5 a	1.6 a	1.5 a	1.5 a	1.4 a	1.7 a	1.6 a	1.4 a	1.4 a
C13-norisoprenoids									
β -damascenone	3.4 a	3.8 a	3.4 a	3.6 a	3.6 a	3.8 a	3.3 a	3.1 a	3.2 a
α -ionone	5.4 a	5.5 a	4.9 a	5.2 a	5.2 a	5.5 a	4.7 a	4.3 a	4.5 a
β -ionone	7.8 a	8.1 a	7.3 a	8.3 a	8.0 a	8.7 a	7.4 a	6.7 a	6.9 a
Acids									
Hexanoic acid	1.0 a	0.9 a	1.1 a	1.4 a	1.4 a	1.6 b	1.3 b	1.0 a	1.1 a
Octanoic acid	2.5 a	2.1 a	2.6 a	3.6 b	3.1 a	4.0 b	3.0 a	2.5 a	2.4 a
Decanoic acid	nd	nd	nd	nd	nd	nd	nd	nd	nd

862 All values (area: arbitrary unit) are divided by a factor of 10.000. Different letters (a-c) for the same aroma compound in the same wine type (synthetic, white, red) denote
863 statistical differences among saliva types after applying LSD test

Figure 1

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871

2a)

872

2b)

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874 Figure 2

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3a)

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3b)

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880 Figure 3

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4a)

4b)

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886 Figure 4