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3 **Impact of the non-volatile wine matrix composition on**

4 **the retronasal aroma release during wine consumption**

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ABSTRACT

The impact of the non-volatile wine matrix composition on the retronasal aroma released during the consumption of wine has been evaluated. For this purpose, a tailored made retronasal aroma trapping device (RATD) was used to entrap the exhaled breath of six panelists previously trained in a specific consumption procedure. Five compositional different wines (white wine, sparkling white wine, young red wine, aged red wine and a sweet wine), all of them, except the sweet wine, adjusted to the same ethanol content and aromatized with a mixture of four targeted volatile compounds were evaluated. Aroma release data were submitted to multivariate statistic analysis in order to relate wine chemical composition and aroma release during wine drinking. Results showed inter-individual differences and a clustering of panelists among lower and higher aroma releasers, which was in agreement with differences in their breathing capacity. A significant influence of the matrix composition in the low aroma releasers group during wine consumption was observed. The consumption of red wines provoked a significant higher aroma release than the consumption of white and sweet wines. From the chemical composition determined in the wine samples (pH, total acidity, total polyphenols, neutral polysaccharides, residual sugar and nitrogenous compounds), the amount of total polyphenols was better correlate with the observed effect. It is postulated that these compounds might be involved in the formation of a product coating on the throat and pharynx during drinking.

KEY WORDS: wine, non-volatile wine matrix, retronasal aroma release, wine drinking

INTRODUCTION

The hedonic behavior behind wine consumption is greatly influenced by wine aroma, which is one of the most outstanding characteristics for explaining wine quality. Wine aroma is composed by hundreds of volatile compounds which depending on their concentration and chemical structure will have a higher or lower impact on the overall flavor profile of a wine.^{1, 2} The characterization of these compounds and the elucidation of their sensory relevance for wine aroma have been the focus of many interesting works³⁻⁷ and taking into account the magnificent of this task, it is likely these works will continue in the future.

However, we already know that only the characterization of the overall flavor composition of a food (for example the headspace profile) does not directly correlate with the perceived sensations during consumption.⁸ The process of aroma release during food consumption is a sequential process, which starts when the food is smelled (orthonasal aroma) and that continues during the processing of the food in the mouth. Within the oral cavity, volatile compounds are released from the food and from here the breathing flow carries them to the olfactory region where are perceived. This is a dynamic and complex process known like retronasal aroma in which not only the physicochemical characteristic of the compounds, but other physiological factors such as the breathing flow, the presence of saliva and the tongue movements are also involved.^{8, 9} For liquid foods, such as wine, although the processing in the mouth is not as critical, there are some factors such as the formation of a coating on the pharyngeal mucosa, the flow rates, temperature, etc., that should be considered as important aspects

for modulating the aroma released and available for the olfactory receptors during consumption.⁹⁻¹³

In spite that retronasal aroma is directly related to flavor perception and it is a key modulator for food consumption and food preferences, there are not many scientific works directed to understand aroma release during wine consumption. This type of study would need new analytical approaches based on the monitorisation of the aroma released during drinking.¹ One possible approach is the use of artificial devices to simulate the drinking process. Following this *in vitro* approach, Genovese and collaborators¹⁴ studied the influence of saliva on wine aroma release. Although undoubtedly, the use of artificial devices has considerable advantages (better control of the variables of the study, many experimental repetitions, no requirements of human subjects avoiding ethical considerations, etc), they cannot mimic the real situation during wine consumption, therefore, they do not take into consideration all the physiological factors involved on aroma release (such as swallowing), making difficult their correlation with sensory perception. Other approaches to monitor aroma release during the *in vivo* food consumption, such as the use of real time monitoring (breath by breath analysis) by using mass spectrometric techniques such as atmospheric pressure ionization or proton transfer reaction mass spectrometric techniques (API-MS and PTR-MS respectively),^{15, 16} or the use of aroma trapping devices of the exhalation breath during consumption,¹⁷⁻¹⁹ which are often employed to study aroma release in many types of liquid and solid foods, have been very little applied in the wine field. Only Lasekan et al.,²⁰ applied an exhaled odor trapping device to investigate the aroma compounds released during the consumption of palm wine.

On the other hand, in the last years the influence of the non-volatile matrix composition has been pointed out as an outstanding factor influencing wine aroma release.²¹⁻²⁷

Using specific wine non-volatile components^{22, 25-27} or the whole wine matrix composition^{23, 24} has been possible to determine the interaction effect between wine compounds and specific wine volatiles. Even the effect of these interactions on the sensory characteristics of wines has been shown.^{28, 29} Most of these analytical studies have been done in static conditions, which, although very valuable, do not represent the retronasal aroma delivery of volatiles during a real wine consumption situation.

Therefore, taking these considerations in mind, the objective of this work has been to determine the impact of the non-volatile wine matrix composition on the retronasal aroma released during the consumption of wine. To achieve the objective of this work a tailored made retronasal aroma trapping device (RATD) was used to trap the exhaled breath of six panelists previously trained in a specific consumption procedure. Five compositional different wines aromatized with a mixture of volatile compounds at the same concentration were evaluated and aroma release data were submitted to multivariate statistic analysis in order to relate wine chemical composition and aroma release during wine drinking.

MATERIAL AND METHODS

Wine samples

Five commercial Spanish wines representative of different winemaking technologies and with different matrix compositions were selected for this study: a young Verdejo white wine (**WH-W**), a young Tempranillo red wine (**YR-W**), a 4-years-aged (16 months in oak barrels) Tempranillo red wine (**AR-W**), a Cava white wine (Spanish sparkling wine manufactured by the traditional method) (**SP-W**) and a sweet biologically aged wine made from Pedro Ximénez grapes (**SW-W**).

To avoid as far as possible the effect of ethanol on the volatility of aroma compounds, all wines were adjusted to the same ethanol content (13.5% v/v) except the sweet wine that was kept in its initial concentration (15% v/v). All the wines were spiked with a solution of 4 *food-grade* aroma compounds (50 mg/ L) from Sigma-Aldrich (Steinheim, Germany) characterized by presenting different physicochemical properties (Table 1). The initial concentration of these aroma compounds in the original wines was also previously determined.

Retronasal Aroma Trapping Device (RATD)

A tailored made retronasal trapping device (RATD) was employed for this study. Briefly, this glass device allowed trapping the exhaled breath after wine consumption into a Tenax trap thanks to a nosepiece coupled to a hollow tube in which the trap was fitted. A vacuum pump connected to a rotameter allowed to have a steady flow through the trap. Finally, a flowmeter let us to know the exact flow through the trap. In preliminary experiments, an optimization and validation of the effectiveness of this device for the purposes of this study was performed. The retention time, ion of quantification, the range of concentration essayed for each volatile compound and regression lines, together with the values of the residual standard deviation (s) and the determination coefficient (R^2), which are estimators of the adequacy of the regression models, are presented in table 2. In addition, the inter- and intra-traps variability are presented. Adequate relative standard deviation between analysis, very low values of s and satisfactory values for the regression coefficients were obtained for the essayed compounds: isoamyl acetate ($R^2=0.964$), ethyl hexanoate ($R^2=0.991$), linalool ($R^2=0.985$) and β -phenylethanol ($R^2=0.978$).

Retronasal aroma trapping procedure during wine consumption

Six volunteers (2 males y 4 females) between 26-34 years of age previously trained in the retronasal aroma trapping procedure participated in this study. They were instructed not to eat, drink or smoke 2 h before the experiments. They had no known illnesses and self-reported normal and olfactory and gustatory functions. Before each experiment panelists should clean their mouths and rinse with a bicarbonate solution. The monitorisation of the oral cavity of the panelists for the four compounds of interest was performed before each analysis.

The drinking procedure consisted in mainly two steps. In the first one, 20 mL of wine were provided with a syringe to the panelist, who kept it into the mouth during 10 seconds with the lips closed. After this time, the panelist was committed to swallow and naturally breathing by the nose through the glass nosepiece during 20 seconds more. The procedure was repeated until the consumption of 100 mL of wine. The experiments were done in duplicate. The same procedure was followed with the original control wines (without aroma added), in order to know the amount of aroma initially present in the wines that could be trapped with this device. These results were used to correct the aroma release data.

Analysis of volatile compounds

The aroma compounds from the expiration breathe by the nose and trapped into the Tenax cartridges were desorbed with 6 mL of a hexane/diethyl ether solution (1:1). Thirty µl of an internal standard (3-octanol) were added, and then, the sample was concentrated using a nitrogen stream to a final volume of 200 µl and analyzed in the GC-MS. A volume of 8 µL of the concentrated breath extract was injected in a cool injection system unit (CIS), (Gerstel, Mülheim an der Ruhr, Germany) in the solvent vent mode. The injector temperature was from -80 °C until 270 °C.

The identification of volatile compounds was carried out with a Gas Chromatograph Agilent 6890N coupled to a quadrupole Mass Detector Agilent 5973. After injection on the CIS, volatile compounds were separated on a Supra-Wax polar capillary column (60 m $\times$ 0.25mm i.d. $\times$ 0.50 μ m film thickness) from Konik (Barcelona, Spain). Helium was the carrier gas at a flow rate of 1 mL/min. The oven temperature was initially held at 50 °C for 2 min, then increased at 8 °C/min to 240 °C and held for 15 min.

For the MS system (Agilent 5973N), the temperatures of the transfer line, quadrupole and ion source were 270, 150 and 230 °C respectively. Electron impact mass spectra were recorded at 70 eV ionization voltages and the ionization current was 10 μ A. The acquisitions were performed in Scan (from 35 to 350 amu) and Sim modes. The identification of compounds was based on the comparison of retention times and mass spectra. The mass spectra were compared with those from NIST 2.0 database. Relative peak areas (RPAs) were obtained by calculating the relative peak area in relation to that of the internal standard. The use of RPAs data to express aroma release was sufficient for this type of analysis as the aim of the work was to compare the extent of aroma release between wine samples.

Wine matrix composition

Total nitrogen, free amino acids and peptides

Total nitrogen was determined by the Kjeldahl method using a heating digester unit, an SMS scrubber and a UDK-142 automatic distillation unit (Velp Scientifica, Usmate, Italy). Free amino acids and peptides plus free amino acids were determined by methods 5 and 1 respectively of Doi et al.³⁰ A DU 70 spectrophotometer (Beckman Coulter, Brea, CA, USA) was used for both determinations.

Neutral polysaccharides and residual sugars

Neutral polysaccharides were determined by the phenol/sulfuric acid method according to Segarra et al.³¹ Residual sugars (glucose and fructose) were determined by the OIV method.³²

Total polyphenols

Total polyphenols were determined by the Folin–Ciocalteu method and spectrophotometric measurement at 670 nm.³³

Total acidity and pH

Total acidity was determined by titration with 0.05 mol/L NaOH and pH was determined using a pH meter (Mettler Toledo, Barcelona, Spain).

Statistical analysis

The statistical methods used for data analysis were: linear regressions to establish the regression parameters for each aroma compound released after wine drinking and the lack of fit test to judge the adequacy of the linear models; cluster analysis (the square Euclidean distance was taken as a measure of the proximity between two samples and Ward's method was used as linkage rule) to check the grouping of panelists depending on their aroma release performance; one-way ANOVA to test the influence of matrix composition on aroma release and to test compositional differences between wines; least significant difference (LSD) test for mean comparison; principal component analysis (PCA) to examine the relationship between compositional parameters and wine matrices; and correlation analysis to determine the existence of correlations between each of the compositional variables and the aroma release data of the four aroma compounds. The STATISTICA program for Windows version 7.1 was used for data processing (StatSoft, Inc., 2005, www.statsoft.com).

RESULTS AND DISCUSSION

Retronasal aroma release during wine consumption

Different published works have shown that wine matrix might exert an important role on aroma release in static headspace conditions.^{21-24, 34, 35} However, the relevance of this effect in a real wine consumption situation has not been evaluated so far. In this work, we have focused on the aroma compounds released during wine consumption in the so-called expiration breath, since it might be a good representation of the aroma compounds which will interact with the olfactory system.¹⁷ Therefore, to study the influence of the wine matrix non-volatile composition on aroma release during wine drinking, five compositional different wines (white, sparkling wine, red, aged red and sweet wine) were spiked (at the same concentration), with a mixture of four aroma compounds characteristics of the wine aroma profile and with different physicochemical properties (isoamyl acetate, ethyl hexanoate, linalool and β -phenylethanol). The type of aroma compound and concentration was selected in order to make an easy-to-drink wine, preventing the possible tiredness and rejection of the wine by the panelists and avoiding analytical problems (sensitivity). To overcome the effect of ethanol, which has been shown, might significantly affect the partition coefficient of the aroma compounds^{22, 36-39}, all the wines, except the sweet wine were adjusted to the same ethanol concentration (13.5 % v/v). The differences in ethanol content between the sweet wine (15 % v/v) and the rest of the wines was of 2.5 % (v/v), which is unlikely might exert a significant effect on the release of the aroma compounds.³⁹ Wines were consumed following a systematic procedure in order to minimize inter-individual differences. Previous training sessions were performed with the panelists to get them familiar with the procedure. During the experiment, a relatively small amount of wine, typically consumed with a meal (100 mL) was provided. Two repetitions of the same wine were

performed in the same day but in different seasons (leaving at least 2 hours between replicates).

As it was described in material and methods, a tailored-made breathing trapping device, previously optimized and validated for this purpose was employed. Although this type of device did not allow the monitorisation of the retronasal aroma release in real time such as the breath by breath analysis using mass spectrometric techniques (PTR-MS; APCI-MS), other important advantages such as the unequivocally identification of the compounds of interest, the possibility of having a concentrated breath extract,¹⁷ and the facility to adapt it to any laboratory with a relatively low economical investment make it very interesting for the purposes of this type of studies.

The average data (relative peak areas) corresponding to each of the aroma released by the six panelists, independently of the wine type, is depicted in Figure 1. Previously, the same consumption procedure was employed to evaluate the five wines without spiked aromas, and only traces of the four aroma compounds were detected. However, all the release data were corrected taking into consideration these results. As can be observed in the figure, in spite that all the panelists followed a strict consumption procedure, for each single aroma compound, large differences in the released patterns between individuals were found. Interestingly, these differences seemed to be constant toward the same compound type. For example, the inter-individual release pattern of the two esters, isoamyl acetate and ethyl hexanoate was quite similar; panelist #1, released the highest amount of these compounds, while panelist #6 always released the lowest. However, when considering a more chemically different compound, such as linalool, and β -phenylethanol, this pattern was different. For instance, linalool was higher released by panelists #1, #2, and #4, while the rest of subjects released almost the same amount. In addition, depending on the type of compound, differences between

panelists were less pronounced, and for example, the release of β -phenylethanol was more or less the same for all of them. The existence of inter-individual differences on aroma release patterns during food consumption have been extensively described, both in solid⁴⁰⁻⁴² and liquid food-matrices^{8, 10, 11, 43} and could be attributed to anatomical and physiological differences between panelists (respiratory flows, saliva composition, oral and pharyngeal mucosa, etc.).

To further investigate if we could have a trend in the aroma release pattern, which could help us in the interpretation of the results of this study, a cluster analysis with the data corresponding to the aroma released by the 6 panelists during the consumption of the five different wines was performed. Figure 2 shows the dendrogram obtained from this analysis in which clearly two groups of aroma releasers can be observed. A first group formed by panelists #1, #2 and #4, and a second comprising panelists #3, #5 and #6. Both groups could be called as higher and lower releasers groups respectively. A similar trend on flavor release was observed during the consumption of liquid emulsions⁴³ and these differences between higher and lower releasers were also in agreement with previous works.^{40, 41}. Although a higher number of individuals might be needed to confirm this trend, the preliminary results derived from this study indicated two behaviors on the aroma release during wine consumption.

Among the anatomical and physiological differences between panelists that might be responsible for the different observed trends on aroma release, the variations in the flow rate between subjects might be an explanation. It has been suggested that a greater respiratory rate could contribute to bringing more volatiles to the upper air ways, and consequently, more volatiles could be present in the expired air of the panelists⁴²⁻⁴⁴. However, in another study performed *in vivo* and *in vitro*, Well et al.,¹³ shown that an increase in flow rate resulted in a decrease in the aroma release.

To determine whether the breathing capacity might have an influence on the grouping of panelists in higher and lower releasers, two breathing related parameters corresponding to the vital capacity (VC) and forced vital capacity (FVC) were calculated. The vital capacity (VC) can be defined as the volume of air breathed out after the deepest inhalation,⁴⁵ while the forced vital capacity (FVC) is the determination of the vital capacity from a maximally forced expiratory effort.^{46, 47} In fact, FVC is the most basic maneuver in spirometry tests.^{46, 47} Both parameters were estimated considering different individual physiological variables such as age, sex, ethnic group and height of the panelists. These data are shown in Table 3. As can be seen, effectively, the highest predicted FVC and VC values corresponded to panelists from the first group (higher releasers), while the second group, the lower releasers, also showed the lowest FVC values. These results suggest that although other physiological variables might also affect the rate of aroma release during the consumption of wine, in the experimental conditions of this study, we have found a direct relationship between respiratory rate and aroma compounds in the expired air of the panelists, which might influence the sensory perception of wine aroma.

Effect of wine matrix composition on aroma release

Considering the two types of behaviors between panelists regarding the aroma release during wine consumption (higher and lower releasers), release data (relative peak areas) obtained from both groups of panelists were separately treated and submitted to one-way ANOVA in order to know if the non-volatile wine matrix composition could have a significant effect on aroma release during wine consumption. Surprisingly, we noticed that the effect of wine matrix was different depending on the group of panelists. For the higher releaser group, only the release of linalool was influenced by matrix composition ($0.05 < P < 0.1$). This result seemed to be related with some analytical constraints of

the trapping device, such as the possible saturation of the Tenax trap during the experiment with higher releasers, which could have masked the wine matrix effect. However, considering the lower releaser group, all the compounds were significant influenced by matrix composition; these results are shown in figure 3 together with the results corresponding to the application of the LSD test with the five types of wine matrices. It is important to highlight that the differences on aroma release also depended on the type of aroma compound considered (e.g. we observed more differences on the release of linalool towards the different wines than on the release of ethyl hexanoate); however, independently on the type of aroma compound considered, there was a clear trend, in which always higher aroma release was observed during the consumption of the two red wines (**YR-W** and **AR-W**) compared to the consumption of white wines (**WH-W** and **SP-W**) and the sweet wine (**SW-W**). As can be seen in figure 3, the highest release of isoamyl acetate, ethyl hexanoate, and linalool was during the consumption of young red wine, while β -phenylethanol was greater released during the consumption of aged red wine.

Therefore, in spite of the inter-individual differences between panelists, the influence of wine matrix composition on the amount of aroma released during wine consumption was evidenced. This finding is in agreement with previous studies in the literature which have already demonstrated using static conditions, the existence of interaction between non-volatile wine matrix components and aroma compounds, which might affect the release of aroma compounds into headspace.^{23, 25-27, 34} Moreover, the effect of wine matrix on aroma release has been shown in *in vitro*-dynamic conditions,¹⁴ being this the first time that this effect is proven in an *in vivo*, real consumption situation.

For trying to determine which component/s from the wine matrix were more involved on aroma release during wine consumption, the chemical characterization of the non-

volatile matrix composition of the five wines consumed by the panelists was performed and it is shown in Table 4 together with the results of the LSD test. As can be seen, the 5 wines exhibited significant differences in their composition. As expected, the sweet wine (**SW-W**) was the most different, showing the highest pH (4.12), the lowest value of acidity (3.68 mg tartaric acid/L), and the highest content of neutral polysaccharides (171 g mannose/L) and residual sugars (310 g/L). In addition, this wine showed the highest levels of nitrogen compounds (total nitrogen, amino acids and peptides). In spite of its *a priori*, higher matrix complexity, during the consumption of this wine, we did not observe a significant effect on aroma release, which was more similar to that experienced during the consumption of white wines (**WH-W** and **SW-W**). The lack of a clear interaction effect of this type of wine with typical wine aroma compounds is in agreement with previous results performed in static conditions.²³ It seems, at least, in static conditions, that the higher content of some small molecules in this wine, such as mono- and disaccharides or free amino acids^{21, 48} could be responsible for a “salting out” effect which might compensate the retention effect exerted by other higher molecular weight compounds (such as polyphenols or proteins)^{21, 23} or by an increase in the solution hydrophobicity because of the presence of a large amount of hydrophobic sugars such as fructose.³⁹

The consumption of the two white wines (**WH-W**, **SP-W**) provided in general, a lower aroma release. These wines exhibited the highest values of acidity (5.6-5.29 mg tartaric/L) and therefore lowest pH values (2.99-3.07), although these differences did not seem enough to explain the differences observed on aroma release compared with the aroma release during red wines consumption. In addition, white and sparkling wines showed the lowest values of the majority of non-volatile compounds determined in the samples (polyphenols, polysaccharides, residual sugars, and nitrogen compounds).

However, compared with red wines, the group of compounds that was dramatically different between the two types of samples was the content on total polyphenols. Red wines showed ten times more of these compounds (2010 and 1860 mg gallic acid /L for **YR-W** and **AR-W** respectively) compared to white wines (211 and 173 mg gallic acid/L for **WH-W** and **SP-W** respectively).

To better envisage the compositional differences between the five types of wines, a principal component analysis (PCA) was performed with the data from Table 5. Two principal components were obtained. The first one (PC1), was negatively related ($|\text{loadings}| > 0.82$) with all the compositional parameters (pH, neutral polysaccharides, residual sugars, nitrogen compounds) except total polyphenols, and positively correlated with total acidity (0.92). The PC2 was positively correlated with the total polyphenol content (0.97). Figure 4 shows the representation of the wines on the plane defined by PC1 and PC2. Clearly, PC1 could distinguish between sweet wines, with high and negative values for this component from white and sparkling wines with high and positive values for PC1. Red wines appeared together between sweet and white wines. On the other hand PC2 clearly separate between red wines from the rest. This component was only defined by the polyphenol content, which clearly, as was previously commented, was much higher in these wines than in the rest.

Many works in the literature performed in static conditions, have shown the existence of specific interactions between polyphenols and aroma compounds resulting in a reduction of the aroma released into the headspace.^{25, 34, 39} For instance, it has been shown that monomeric polyphenols, abundant in young-red wines, can interact with terpenes in ethanolic solutions provoking a lower aroma release in *in vitro* conditions.²⁵ Other interactions that have been described involved the galloyl ring of some phenolic compounds and the aromatic ring of some aroma compound^{34, 49}. Anyway, in dynamic

real drinking conditions, as we have used in this study, aroma release seems to be enhanced by the presence of polyphenols. One possible explanation could be that after swallowing, this type of compounds could specifically interact with pharyngeal and/or oesophageal mucosa contributing to the formation of a product coating on the throat and pharynx, which could increase the contact area between air and product, which might favor aroma release.¹⁰ In fact, some works in the literature have already suggested interactions between aroma compounds and oral/ oesophageal mucosa to explain a delay on aroma release, which extent might be dependent on product composition.^{11, 50} The formation of complexes polyphenols-aroma might also interact with the pharyngeal and/or oesophageal mucosa remaining there during an unspecific time, resulting in a greater concentration of aroma molecules to be released by the expiration flows and available for the olfactory receptors.

Although obtaining straightforward relationships between the aroma release behavior during wine drinking and the effect of wine matrix components is a difficult task because not only the compositional parameters, but also the physicochemical characteristics of the aroma compounds and the physiological parameters are affecting retronasal aroma release, a correlation analysis between compositional parameters (from table 4) of all the wines of the study and the average values of aroma release considering the data from all the panelists was performed. Results showed that the matrix components which better correlated with the aroma release were the total polyphenol content, which were positively correlated with the release of isoamyl acetate ($r = 0.81$), ethyl hexanoate ($r = 0.80$) and linalool ($r = 0.97$). Figure 5 shows this correlation, where is possible to see how red wines showed the highest aroma release values, while the wines with the lowest polyphenol content showed the lowest aroma release for this compound. In spite of the greater aroma release that was observed by β -

phenylethanol during the consumption of AR-W, the release of this compound did not correlate with the total polyphenol content or any other compositional parameter analysed in the samples. This suggests that its release could be more affected by other chemicals of different nature or by specific interaction with different types of polyphenols more characteristics of aged wines (polymerized polyphenols) which will deserve further studies.

In conclusion, results of this work, show that in spite of the interindividual differences due to certain physiological characteristics of the individuals (such as the breathing capacity), there is an influence of the wine matrix composition on the amount of retronasal aroma released during wine consumption, and therefore, available for the olfactory receptors. Among the different major wine matrix components, polyphenols, seem to enhance aroma release during wine drinking, which could be due to the involvement of these compounds on the formation of a wine coating after swallowing which might increase the contact area between exhaled air and product and/or because of the formation of polyphenol-aroma complexes on the surface coating acting as a reservoir of aroma molecules ready to be released by the expiration flows. Oncoming work is directed to study the nature of these interactions and their meaning for the sensory characteristics of the wines.

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440 **Notes**

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FIGURE CAPTIONS

Figure 1. Average values of aroma release (relative peak areas) obtained for each panelist during wine consumption considering the five different wines (**WH-W**, **SP-W**, **YR-W**, **AR-W** and **SW-W**) and two repetitions of the same wine. For better comparison, data for β -phenylethanol are multiplied by a 10 factor. Different background patterns mean different panelists.

Figure 2. Dendrogram showing the grouping of panelists depending on their aroma release performance during the consumption of the five different wine types.

Figure 3. Results of the aroma release during the consumption of the five different wine types for the lower releaser group. Significant differences on aroma release depending on matrix composition are indicated with * $0.05 < P < 0.1$ or ** $P < 0.05$. Different letters across the different wines denotes statistical differences after the application of LSD test.

Figure 4. Representation of the wines on the plane defined by PC1 and PC2 obtained with the non-volatile matrix composition data (table 4).

Figure 5. Correlation between the retronasal release of linalool during wine consumption and the polyphenol content in the five types of wines.

TABLES:

Table 1. Main physicochemical characteristics of the aroma compounds used in the study of aroma release during the consumption of different types of wine matrices.

Compound	Chemical group	Molecular weight (g/ mol)	Log P^a	Boiling point (°C)
Isoamyl acetate	Ester	130.2	2.3	134.9
Ethyl hexanoate	Ester	144.1	2.8	167.0
Linalool	Terpene	152.2	3.4	204.1
β -phenylethanol	Alcohol	122.2	1.6	224.9

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

Table 2. Chromatographic and regression parameters ($y = a + bx$) determined for the added aroma compounds in the breathing trapping experiments.

	RT ^a	Ion Q ^b	Linear regression ^c					Repeatability ^d	
			Linear range (mg/L)	a	b	s	R ²	Inter-cartridge RSD (%)	Intra-cartridge RSD (%)
Isoamyl acetate	11.42	43	25-100	0.2209	0.0126	0.0930	96.4	13.3	6.8
Ethyl hexanoate	13.32	88	0-100	-	0.0142	0.0982	99.1	23.0	14.9
Linalool	18.53	71	0-100	-	0.0053	0.0487	98.5	11.6	11.8
β-phenylethanol	24.35	91	0-90	-	0.0002	0.0018	97.8	19.0	19.5

^a RT = Retention time (minutes); ^b Ion Q = Quantification ion; ^c Linear regression parameters = intercept (a) , slope (b), residual standard deviation (s) and determination coefficient (R²); ^d = Repeatability is expressed as an average of 5 essays performed in the same conditions

Table 3. Predicted Forced Vital Capacity and Vital Capacity Values calculated with Dynamic Measurement Technologies (www.dynamicmt.com) based on Hedenström et al., 1985-1986^{46, 47} and Langhammer et al., 2001,⁴⁵ respectively.

Panelist	FVC		VC	
	Predicted Value (L)	±SD	Predicted Value (L)	±SD
1	4.751	0.430	4.478	0.130
2	5.635	0.860	5.754	0.120
3	4.122	0.430	3.902	0.130
4	5.725	0.860	5.651	0.120
5	4.525	0.430	4.288	0.130
6	4.001	0.430	3.817	0.130

Table 4. Mean $\pm$ standard deviation (SD) values (n=3) of the chemical composition of the five matrices: White wine (**WH-W**), Sparkling wine (**SP-W**), Young red wine (**YR-W**), Aged red wine (**AR-W**), Sweet wine (**SW-W**).

	WH-W		SP-W		YR-W		AR-W		SW-W	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
pH	2.99 ^a	0.0	3.07 ^b	0.0	3.93 ^d	0.0	3.66 ^c	0.0	4.12 ^e	0.0
Total acidity (mg tartaric acid/L)	5.60 ^e	0.1	5.29 ^d	0.1	4.06 ^b	0.0	4.41 ^c	0.1	3.68 ^a	0.0
Total polyphenols (mg gallic acid/L)	211.50 ^a	0.6	173.23 ^a	5.2	2009.67 ^d	52.0	1859.67 ^c	20.9	574.33 ^b	41.1
Neutral polysaccharides (g mannose/L)	1.82 ^a	0.1	0.96 ^a	0.1	2.66 ^a	0.1	2.38 ^a	0.1	171.07 ^b	9.4
Residual sugars (g/L)	1.59 ^a	0.0	1.14 ^a	0.0	3.82 ^b	0.3	2.58 ^{ab}	0.3	310.08 ^c	2.7
Total nitrogen (mg/L)	206.78 ^b	3.8	129.36 ^a	8.3	315.00 ^c	51.1	358.68 ^c	19.4	1010.80 ^d	10.3
Amino acids + peptides (mg N/L)	64.51 ^a	0.7	104.38 ^b	0.9	141.05 ^d	3.2	113.07 ^c	2.5	319.61 ^e	2.4
Amino acids (mg N/L)	36.39 ^a	0.7	38.39 ^a	0.4	69.59 ^c	1.2	58.69 ^b	3.2	67.20 ^c	0.9
Peptides (mg N/L)*	28.12 ^a		65.99 ^c		71.46 ^d		54.39 ^b		252.41 ^e	

Values are the average of three determinations. * This data is indirectly determined as the difference between the analytical determination of amino acids plus peptides and free amino acids, therefore SD values are not included in the table. Different letters across the different wines denotes statistical differences after the application of LSD test.

FIGURE GRAPHICS

Figure 1.

Figure 2.

Figure 3.

Figure 4.

Figure 5.

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