Assessment of the effect of the non-volatile wine matrix on the volatility of typical wine aroma compounds by Headspace-Solid Phase Microextraction-Gas Chromatography Analysis

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Abstract

With the aim of knowing the effect of the whole non-volatile wine matrix composition on the volatility of typical wine aroma compounds, five types of wine matrices (young white, young red, oak aged red, Cava sparkling and a sweet wine) representing a wide range of wine compositions, were previously deodorized and reconstituted to the same ethanol concentration and aromatized with a mixture of 36 aroma compounds at 5 levels of concentration. Slopes of regression lines, obtained by solid phase microextraction-gas chromatography-mass spectrometry, were compared to the slopes calculated for the same compounds in a control wine, with no matrix effect. The main observed effect was a reduction in the slopes, or a retention effect, that was larger for the reconstituted sparkling wine, which showed between 11% and 69% lower slopes than the control wines for compounds such as ethyl hexanoate and octanoate and the terpenic compound nerol. In addition, an increase in the slope, or a "salting out" effect in the most compositional complex reconstituted aged-red and sweet wines was also noticed for some volatiles (2-methylbutyrate, butyl and hexyl acetate, 5-methyl furfural) with very low boiling point or low hydrophobic constant values.

Key words: wine matrix, aroma, volatility, Head Space-Solid Phase Microextraction-Gas Chromatography-Mass Spectrometry.

1. Introduction

Aroma is one of the main characteristics in defining the quality of wines. Therefore, many works in the scientific literature have been devoted to the identification and quantification of the key aroma compounds responsible for specific aromatic nuances in wines (Campo, Ferreira, Escudero, & Cacho, 2005; Escudero, Campo, Farina, Cacho, & Ferreira, 2007; Ferreira, López, Escudero, & Cacho, 1998; Ferreira, Ortín, Escudero, López, & Cacho, 2002; Guth, 1997; Kotseridis & Baumes, 2000). However, aroma perception of a wine is strongly influenced by the way indigenous aroma molecules distribute between the gas and liquid phases, which is characterised by the partition coefficient. Partitioning of volatile substance between the liquid and gas phases is mainly governed by aroma compound volatility and solubility (Voilley, 2006). These physicochemical properties are expected to be influenced by wine constituents present in the medium, such as polysaccharides, mono- and disaccharides, polyphenols and proteins among others (Pozo-Bayón & Reineccius, 2009). The interaction between aroma molecules and wine non-volatile compounds might influence aroma release and ultimately the ortho- and retro-nasal aroma perception.

Many wine matrix non volatile components (carbohydrates, proteins, polyphenols) come from the skins and the pulp of the grapes and from the cell wall of the fermentation yeast. In addition, ethanol, produced during wine fermentation, represents a mayor wine matrix component. The great importance in considering the wine matrix in the perception of some important wine aroma compounds has been recently evidenced by Pineau, Barbe, Van Leeuwen and Dubourdieu (2007), when showing that the odour threshold of β-damascenone in hydroalcoholic solution was over 1000 fold higher than in a reconstituted red wine.

Some research has been devoted to study interactions between aroma compounds and specific wine matrix constituents. Dufuour and Bayonove (1999b) confirmed the existence of hydrophobic interactions between catechins and some types of aroma compounds, and in some more recent studies it has been shown that gallic acid (in 1% ethanol solution) significantly decrease the volatility of 2-methoxypyrazine, while naringine at the same level had little effect (Aronson & Ebeler, 2004).

The effect of wine polysaccharides and mainly those derived from yeast cell walls such as mannoproteins, on the volatility of aroma compounds has been also proved (Langourieux & Crouzet, 1997; Lubbers, Charpentier, Feuillat, & Voilley, 1994; Lubbers, Voilley, Feuillat, & Charpentier, 1994). The extent of this effect depends on the type of mannoprotein and even on the yeast strain (Chalier, Angot, Delteil, Doco, & Gunata, 2007). Moreover, the different effect of yeast macromolecules released by different types of inactive yeast preparations usually used to enhance fermentations on the volatility of typical wine aroma compounds has been recently shown (Pozo-Bayón, Andújar-Ortiz, Alcaide-Hidalgo, Martín-Álvarez, & Moreno-Arribas, 2009).

Ethanol, the main wine matrix component, has the capacity to modify the solution polarity, thus altering the gas-liquid partition coefficient. The effect of increasing amounts of ethanol decreasing wine aroma volatility has been very well documented (Conner, Birkmyre, Paterson, & Piggott, 1998; Escalona, Piggott, Conner, & Paterson, 1999; Hartmann, McNair, & Zoecklein, 2002; Robinson, Ebeler, Heymann, Boss, Solomon, & Trengove, 2009; Rodríguez-Bencomo, Conde, Rodríguez-Delgado, García-Montelongo, & Pérez-Trujillo, 2002; Whiton & Zoecklein, 2000).

However, most of the studies on the effect of wine matrix components on the volatility of aroma compounds have been carried out using artificial wine matrices, usually aqueous or hydroalcoholic solutions, containing a very limited number of wine

components and spiked with several types of aroma compounds. Although this can be a valuable approach to know the role of some specific matrix components, the results rarely could be extrapolated to real wines because of their great compositional complexity and wide variety of volatile chemical classes. In an attempt to have more information related to the effect of wine matrix composition on aroma volatility, Robinson et al. (2009) have recently presented an interesting full factorial design to asses the matrix effects of ethanol, glucose, glycerol, proline and catechin, on the volatility of 20 wine aroma compounds, in which they corroborated previous results related to the great effect of ethanol followed by glucose, and the little effect of catechin, glycerol and proline.

However, the effect of the whole non volatile composition from real wine matrices on representative wine volatile compounds has not been study so far. Therefore, the objective of this work has been to study the effect of five types of wine matrices representing a wide range of wine compositions, which were previously deodorized and reconstituted to the same ethanol concentration, on representative chemical groups of wine aroma compounds. To do so, the comparison of the regression lines obtained by HS-SPME-GCMS in each reconstituted matrices has been performed and the results are discussed based on the physicochemical characteristics of the aroma compounds and on the chemical composition determined in each wine matrix.

2. Experimental

2.1. Wines Samples

Five commercial wine samples representative of different wine matrix composition were selected for this study: a young Chardonnay white wine, a young Beaujolais red wine, an old oak aged Tempranillo red wine, a Cava wine (Spanish sparkling wine

manufactured by the traditional method) and a sweet biological aged wine made from Pedro Ximénez grapes.

2.2. Reconstituted Wines

2.2.1. Deodorization procedure

One hundred twenty mL of each wine were deodorized by introducing the wines for 20 minutes in an ultrasound bath, following by the addition of 15 g of Amberlite XAD-2 from Supelco (Bellefonte, PA, USA) and stirring for 1h. Wines were filtered through glass wool. The whole procedure was repeated twice. This procedure allowed the complete elimination of all the aroma compounds in the wines (confirmed by SPME-GC-MS analysis).

2.2.2. Wine Reconstitution

Eight mL of each wine contained in 20 mL vials (Agilent Technologies, Palo Alto, CA, USA) were completely dried in a lyophilizer (Labconco, KA, MS, USA). A total of 60 samples were prepared by using this procedure (12 per each wine type). The dried wines were weight to calculate the repeatability of the liophylization process.

The residue after lyophilisation was reconstituted with an hydroalcoholic solution (120 mL L^{-1}) to a final volume of 8 mL and spiked with the volatile mixture at five different concentration levels (Table 1). Duplicates of each reconstituted wines were prepared following this procedure.

Besides the 5 types of reconstituted wine matrices, a control wine representing a sample with "no matrix effect" was prepared by mixing ethanol (120 mL L^{-1}), 4 g L^{-1} tartaric acid (Panreac, Barcelona, Spain) and adjusting the pH to 3.5 with NaOH (Panreac).

2.3. HS-SPME procedure

Forty μL of an internal standard solution (3,4 dimethylphenol, 400 mg L^{-1}) and 2.3 g of NaCl were added to each vial of reconstituted wine. Previously, different compounds

were essayed to be used as internal standards for this study, taking in consideration their stability along the experiment (low variations in absolute areas due to wine matrix, time and volatile concentrations added to the wines), therefore avoiding as much as possible, the correction of the matrix effects, which was the main objective of this study. The vials were sealed with PTFE/Silicon septum (Supelco). The extraction was automatically performed by using a CombiPal system (CTC Analytics AG, Zwingen, Switzerland) provided with a 50/30 µm DVB/CAR/PDMS fiber of 2 cm length (Supelco). The samples were previously incubated for 10 minutes at 50 ºC and the extraction was performed in the headspace of the vial for 30 minutes at 50 ºC. The desorption was performed in the injector of the GC chromatograph (Agilent 6890N) in splitless mode for 1.5 minutes at 270 ºC. After each injection the fiber was cleaning for 30 minutes avoiding any memory effect. All the analyses were performed in duplicate (one injection per sample vial).

2.4. Gas Chromatography–Mass Spectrometry analysis

An Agilent MSD ChemStation Software was used to control the system. For separation, a Carbowax 20M fused silica capillary column (30 m x 0.25 mm i.d. x 0.25 µm film thickness) Quadrex Co. (Woodbridge, CT, USA) was used. Helium was the carrier gas (1 $ml \cdot min^{-1}$). The oven temperature was programmed as follows: 40 ºC as initial temperature, held for 5 minutes, followed by a ramp of temperature at 4 ºC min^{-1} to 240ºC an then held for 15 minutes.

For the MS system (Agilent 5973N), the temperatures of the manifold and transfer line were 150 and 230 ºC 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 450 amu) and Sim mode for some specific compounds. The signal corresponding to a specific ion of quantification was calculated by the data

system. **Table 1** detailed the studied compounds, retention times, ion of quantification and detection mode, boiling point, hydrophobic constant and linear concentration range studied for each compound. Quantitative data were obtained by calculating the relative peak area in relation to that of the internal standard (3,4-dimethylphenol).

2.5. Chemical Matrix Composition

2.5.1. Total nitrogen, free amino acids and peptides

Total nitrogen was determined by the Kjeldahl method using a heating digestor unit, a SMS Scrubber and an UDK-142 automatic distillation unit (Velp Scientifica, Usmate, Italy).

Free amino acids and peptides plus free amino acids were determined by the methods 5 and 1, respectively, published by Doi, Shibata and Matoba (1981). A spectrophotometer DU 70 (Beckman Coulter Inc., Brea, CA, USA) was used for both determinations.

2.5.2. Neutral Polysaccharides and residual sugars.

The concentration of neutral polysaccharides was determined by the phenol sulphuric method, according to Segarra, Lao, López Tamames and De La Torre Boronat (1995). Residual sugars (glucose and fructose) were determined following the OIV method (OIV, 1990).

2.5.3. Total polyphenols

Total polyphenols were determined by the Folin-Ciocalteau method and spectrophotometric measured at 670 nm (Singleton & Rossi, 1965).

2.5.4. Total acidity and pH

Total acidity was determined by titration with NaOH 0.1 N and pH was determined using a pHmeter (Mettler, Toledo, Barcelona, Spain).

2.5. Statistical analysis

Linear regression to establish the calibration curves of each aroma compound in the 5 types of reconstituted and control wines and the lack of fit test to judge the adequacy of the models were performed. In addition, for each aroma compound the slope from the calibration curve of each wine was compared to that of the control wine. STATGRAPHICS Centurion XV program, version 15.2 (2006, Statistical Graphics Corporation, Manugistics Inc., MD, www.statgraphics.com) was used for data processing.

3. Results and discussion

3.1. Non-volatile wine matrix composition

The results obtained from the analysis of wine matrix components (amino acids, peptides, total nitrogen, residual sugars, total polyphenols and neutral polysaccharides) and some other physicochemical characteristics such as total acidity, pH and the weight of the non-volatile residue of the five wines under study are presented in **Table 2.** The % (w/w) of wine residue (compared to the whole volume of wine in the vial) after lyophilisation, was calculated as the average residue weighted in 12 vials of the same type of wine. The lower deviation in this parameter (RSD < 3.25 %) shows that the lyophilisation process was very reproducible for all the wines. As can be seen, the non-volatile residue was lower for white and sparkling wines, being 1.9 and 1.8 %, respectively. The sweet wine showed the highest non-volatile residue (34.6 %), mainly because of their great amount of sugars. In addition, this wine, showed the highest values of nitrogen compounds (total nitrogen, amino acids and peptides). However, the sweet wine presented lower total acidity (3.07 g L^{-1} tartaric acid) and in consequence, higher pH (4.59) compared to the other ones. Besides of the sweet wine, it is also remarkable the high level of residual sugars determined in the aged-red wine, 9.34 g L^{-1},

compared to the other non-sweet wines. In addition, aged wines (old red wine and mainly sparkling wine) showed the highest peptide content. The release of peptides because of the slow hydrolysis of proteins during wine aging has been extensively described (Martínez-Rodríguez, Carrascosa, Martín-Álvarez, Moreno-Arribas, & Polo, 2002; Martínez-Rodríguez & Polo, 2000). White and sparkling wines showed the lowest polyphenol content (230 and 125 mg L^{-1} gallic acid, respectively), while as expected, the young and the old red wines showed the highest values (1820 and 2142 mg L^{-1} gallic acid, respectively). Besides the sweet wine, which showed, as it was said before, the highest pH (4.59), the pH of the rest of the wines, was however barely similar, between 3.02 for the sparkling wine and 3.55 for the aged-red wine. These results are showing great differences in the composition of the five types of wines, which may distinctively affect the volatility of the aroma compounds.

3.2. Comparison between the regression parameters calculated in the reconstituted and control wines.

The influence of ethanol in the volatility of aroma compounds was not considered in this study, since it has been extensively demonstrated (Conner, Paterson, & Piggott, 1994; Escalona et al., 1999; Hartmann et al., 2002; Robinson et al., 2009; Rodríguez-Bencomo et al., 2002). Therefore, the ethanol concentration was kept the same in all the reconstituted and control wines.

To evaluate the effect of the whole non-volatile composition on the volatility of the aroma compounds, regression lines for the 36 volatile compounds using two replicates at five level of concentration for each of the 5 reconstituted and control wines were calculated. In total 216 regression lines with 5 points and in duplicate were carried out for this study. The slopes from the regression lines obtained in the five reconstituted wines were compared to the slopes calculated for the same compounds in a control wine

244 formed by ethanol and tartaric acid, therefore considering that it did not show any
245 matrix effect.
246 The slopes of the regression lines obtained with the control and reconstituted wines are
247 shown in **Table 3**. The table also shows the residual standard deviation (s) and the
248 determination coefficients (R^2) which are estimators of the adequacy of the regression
249 models. In addition, to judge the adequacy of the linear models, the F-ratio for lack of
250 fit was calculated (Massart, Vandeginste, Deming, Michotte, & Kaufman, 1990). As can
251 be seen, in general, most of the studied aroma compounds showed R^2 higher than 0.99
252 and very low values of residual standard deviation, in fact, the residual standard
253 deviation expressed as a percentage of the mean value (s/y*)* was lower than 15 % for
254 most of the compounds (data not shown).
255 The comparison between the slopes for the aroma compounds in the reconstituted and
256 control wines is also shown in **Table 3**. In this table, compounds in bold showed
257 statistically significant differences in the slopes between both types of wines after the
258 application of two-sample t-test. In general, in the reconstituted aged-red wine, a higher
259 number of volatile compounds showed differences in the slopes compared to the control
260 wine. The white wine showed on the contrary, the lowest differences in the slopes.
261 Besides of the type of wine matrix composition, depending on the type of aroma
262 compound more or less differences compared to the control wine were also noticed. For
263 example, some chemical groups, such as C13 nor-isoprenoids and some volatile
264 phenols, lactones and furanic compounds exhibited important differences in the slopes
265 in most of the reconstituted wines compared to the control wine. Most of them have
266 been described as key aroma compounds in different types of wines (Chatonnet,
267 Dubourdieu, & Boidron, 1992; Ferreira, Jarauta, Ortega, & Cacho, 2004; Mendes-Pinto,
268 2009; Pollnitz, Pardon, & Sefton, 2000). In addition, the slopes of other compounds,

such as the esters ethyl decanoate and isoamyl acetate, benzyl alcohol, terpinen-4-ol, and the bencenic compound methyl vanillate, showed significant differences in the reconstituted wines compared to those in the control wines. However, some chemical groups such as esters and alcohols did not show as much differences between reconstituted and control wines. These results are showing an interaction between the wine non volatile composition and the aroma compounds that not only depend on the wine matrix composition but on the type and physicochemical characteristics of the aroma compounds.

3.3. Interaction between non volatile composition and aroma compounds

To better understand the interaction between the aroma compounds and the non volatile composition, **Table 4** shows the results of the comparison of the slopes of the reconstituted and control wines expressed as percentage. This value can be negative or positive, depending on the slope was lower or higher, respectively, than that calculated in the control wine. In this table, only those compounds, which slopes showed statistical significant differences and values higher than 10% compared to the slopes in the control wine, have been presented in bold.

As can be seen in the table, the main observed effect is a reduction in the slopes calculated in the reconstituted wines compared to the control wine. This reduction could be considered as a retention effect of certain volatile by the non volatile wine matrix composition, as has been previously noticed in model systems (Dufour & Bayonove, 1999a; Dufour et al., 1999b; Dufour & Sauvaitre, 2000; Escalona, Homman-Ludiye, Piggott, & Paterson, 2001; Hartmann et al., 2002). Interestingly, this effect was higher in the case of the reconstituted sparkling wine, which for some esters such as ethyl hexanoate and octanoate and the terpenic compound nerol, shows between 11 % and 69 % lower slopes in the reconstituted sparkling wine than in the control wine. Although,

none of the non-volatile compounds determined in the wines were in higher proportion in this type of wine compared to the other four (**Table 2**), the reconstituted sparkling wine showed a quite large amount of nitrogen compounds, such as amino acids, peptides and total nitrogen. The latter parameter could be also indirectly indicating a relevant amount of protein, specifically mannoproteins from yeast origin, very abundant in aged sparkling wines (Núñez, Carrascosa, González, Polo, & Martínez-Rodríguez, 2005) which have been found to specifically bound several types of aroma compounds (Chalier et al., 2007). In addition, the old red wine showed lower slopes for many volatile compounds compared to the slopes in the control wine. These differences in the slopes, ranged between 12 % and 73 % lower than the control for β-citronellol and vinylphenol respectively. The youngest wines, such as the white and young red wine showed a smaller retention effect. Surprisingly, in spite of the higher complexity of the sweet wine composition, it did not show the expected higher retention effect. It is also important to underline, that the reduction in the slopes (or retention effect) noticed for many volatile compounds in the reconstituted wines compared to the control wine, was much higher than the reduction showed in some recent studies performed in model wine systems supplemented with glucose, catechin, glycine and proline or a combination of all of them (Robinson et al., 2009). This is indicating large differences, and possibly, an undervaluation of the retention effect observed when studding wines supplemented with a reduced number of matrix components compared to considering the whole and truly non volatile composition of the wines.

In addition to the retention effect, an increase in the slope in the reconstituted wine compared to the control wine was also noticed for some volatiles. This effect means an increase in the volatility for some compounds in presence of specific non-volatile compounds that is also called a “salting out” effect. In **Table 4,** the compositional more

319 complex reconstituted aged-red wine and sweet wine seemed to induce in a higher
320 extent this effect. It is interesting to underline that this effect seems to be more evident
321 for certain esters, such as ethyl 2-methylbutyrate, butyl, and hexyl acetate, and other
322 compounds such as 5-methyl furfural, all of them are compounds with very low boiling
323 point or low Log *P* value (**Table 1**). Mono- and disaccharides in solution are known to
324 structure water molecules thus decreasing the amount of free water in the matrix,
325 therefore increasing the concentration of aroma compounds in the remaining available
326 free water, which in turns affects the apparent partition equilibrium of the volatile
327 compounds in favour of the gas phase (Delarue & Giampaoli, 2006). In addition to
328 mono or disaccharides other small soluble compounds such as amino acids, may also
329 induce a salting out effect in wine (Pozo-Bayón et al., 2009).
330 Depending on the aroma chemical class and examining the differences observed
331 between the slopes in the reconstituted and control wines (**Table 4**), it was possible to
332 observe some similar trends between compounds from the same chemical class and
333 their behaviour in the five reconstituted wines.
334 3.3.1. Esters
335 In general, in white and sparkling wines, a reduction in the slope for many esters
336 compared to the control wine was found. However, the aged red and the sweet wines,
337 show retention and salting out phenomena. The higher amount of sugars and other
338 soluble compounds in these wines might be the responsible for the observed effect
339 (Delarue et al., 2006).
340 Among linear ethyl esters, the most hydrophobic compound, ethyl decanoate, (Log *P* =
341 4.79) showed the highest retention effect in all wines, possibly due to a higher
342 interaction with the wine matrix. The higher polarity of ethyl hexanoate (Log *P* = 2.83)

343 and octanoate (Log *P* = 3.81) also seemed to be involved in their higher retention by
344 wine matrix.
345 Although, ethyl cinnamate presented a hydrophobic constant, Log *P* = 2.85, similar to
346 that of the ethyl hexanoate, (Log *P* = 2.83), the behaviour of both compounds presented
347 some differences. The interactions π-π of aromatic cycle with other electron unsaturated
348 systems of the matrix may explain the higher retention of ethyl cinnamate, in white and
349 aged-red wines (Jung & Ebeler, 2003).
350 Interestingly, small esters which shows low boiling points and relatively low Log *P*
351 values, such as ethyl butyrate, ethyl 2-methylbutyrate, isobutyl acetate, and butyl acetate
352 showed in general very low interaction with any of the studied wine matrices.
353 3.3.2. Alcohols
354 These group of compounds were not affected as much by the non volatile composition
355 as other chemical groups. C6 alcohols, 1-hexanol, cis-3-hexen-1-ol and trans-3-hexen-1-
356 ol showed similar hydrophobic constant (Log *P* = 1.61-1.82), and therefore similar
357 behaviour. Only a slight retention effect (15-16 %) for both alkenols in sparkling wine
358 and a "salting out" effect (14 %) for 1-hexanol in aged-red wine was observed. In the
359 case of aromatic alcohols, β-phenylethyl alcohol and benzyl alcohol, only showed
360 retention effects in the case of sparkling wine, being more important for the more
361 hydrophobic compound, β-phenylethyl alcohol (Log *P* = 1.57). However, benzyl
362 alcohol (Log *P* = 1.08) presented a "salting out" effect for white (31 %) and aged-red
363 (17 %) wines.
364 3.3.3. Terpenes
365 In all the reconstituted wines, except in the white wine, most of the terpenes showed a
366 retention effect. The slopes calculated in the wines were between 13 % and 69 % lower
367 than in the control wine. The white wine however, did not show any retention effect,

368 which is in agreement with its simpler matrix composition, more similar to that of the
369 control wine. In red and sparkling wines, the cyclic terpenes, terpinen-4-ol but mainly
370 α-terpineol showed a slight lower retention effect, compared to the non-cyclic ones
371 (linalool, nerol and β-citronellol), revealing the important effect of the molecular
372 chemical structure in the interaction with some non-volatile compounds (Heng et al.,
373 2004; Semenova, Antipova, & Belyakova, 2002). However, in the sweet wines, non-
374 cyclic terpenes (linalool, nerol and β-citronellol) did not show any effect probably due
375 to retention effect compensate the "salting-out" effect of sugar (Robinson et al., 2009).
376 Interestingly, aged red-wine showed lower retention than young red wine, which may
377 be due to the differences in the type of polyphenols, that have been shown may interact
378 with terpenic compound in ethanol or aqueous solutions. The polymeric polyphenols,
379 more abundant in aged wines, have lower retention capacity than monomeric
380 polyphenols. This fact has been described by Dufour et al. (1999b), who observed
381 higher retention of limonene by catechin than by tannin.
382 Although the main observed effect for terpenes was a retention by the non-
383 volatile composition, β-citronellol in white wine, showed higher slopes in the
384 reconstituted than in the control wine, therefore an increase in its volatility or a salting
385 out effect was noticed. None explanation based on the composition parameters analysed
386 in this wine seems to explain this effect; however, other non analysed matrix chemical
387 components may be the responsible for the observed effect.
388 3.3.4. C13 nor-isoprenoids
389 Among the C-13 norisoprenoids studied, the most hydrophobic β-damascenone (Log *P*
390 = 4.21) showed the highest retention effect in all the reconstituted wines except in the
391 white wine. The retention effect was lower for the α-ionone, which showed lower log P
392 value (3.85). However, β-ionone with the same Log *P* and boiling point than α-ionone

393 did not show any significant retention effect. This is showing the great specificity for
394 some interactions between these compounds and some non volatile compounds of the
395 wine matrix.
396 3.3.5. Volatile phenols
397 Volatile phenols presented similar hydrophobic constants, ranging from *Log P* = 2.29
398 for eugenol to *Log P* = 2.58 for 4-ethylphenol. Among them, 4-ethylphenol and 4-
399 ethylguaiacol did not show any important effect due to the matrices studied. However,
400 eugenol and 4-vinylphenol presented in all the wines a noticeable retention effect. For
401 eugenol this effect was similar for all wines (between 18 and 26 %). However, 4-
402 vinylphenol presented great differences among the wine matrices. While white and
403 sparkling wines showed a slight retention effect (≈20 %), red wines showed a strong
404 retention effect (slope 73-83 % lower than in the model solution). This strong retention
405 effect for red wines could be due to important π-π interactions because of the high
406 content in polyphenols of these wines (Jung et al., 2003). Vinylphenols have been
407 associated to off-flavours produced by spoiling microorganism in red wines (Chatonnet
408 et al., 1992), and on the basis of these results, the polyphenol content of wines might
409 contribute to the extent of this effect. Sweet wine, with lower content of total
410 polyphenols and higher in sugars than red wines, may compensate the high retention
411 effect of polyphenols with the "salting-out" effect due to the high contents in sugars.
412 The lower retention in white wines could be due to the low concentration of
413 polyphenols found in these wines (<230 mg L^{-1} gallic acid).
414 3.3.6. Benzenic compounds
415 Methyl and ethyl vanillate showed retention effect in most of the studied wines that
416 could be due to their relative high hydrophobic constants (Log *P* = 1.82 and 2.32
417 respectively). However, vanillin only showed statistically significant effects for the

418 sparkling wine (40 %). The hydrophobic constant of vanillin (Log *P* = 1.21) is the
419 lowest of the three compounds, therefore this could be explaining the minor
420 hydrophobic interactions compared to the respective methyl and ethyl esters.

421 3.3.7. Lactones and furanic compounds

422 The furanic compound 5-methyl-furfural, showed in all wine matrices a salting out
423 effect, exhibiting in all cases higher slopes in the reconstituted than in the control wine.
424 This compound presented the lowest Log *P* value (0.63) from all the volatile
425 compounds under study. In addition this compound exhibited a "salting out" effect
426 independently on the wine type, thus confirming the great importance of the
427 hidrophobicity of the molecule in explaining the retention effects with the non volatile
428 wine matrix compounds. The behaviour of both whiskey lactones was barely similar in
429 red and sparkling wines, showing a slight retention effect (9-21 %). On the contrary,
430 *trans*-whiskeylactone (15 %) showed a slight "salting out" effect in the white wine.

431 3.3.8. Acids

432 Only the behaviour of octanoic acid was studied. This compound exhibited a relatively
433 high hydrophobicity (Log *P* = 3.03), but only presented statistically significant effects
434 in white and sweet wines. In both wines a "salting out" effect was observed, showing an
435 increase in its slope between 46-47 % compared to the control wine. Although in the
436 case of sweet wine, the higher amount of sugars might be the responsible for the
437 observed effect, in the case of white wines, none explanation based on the composition
438 parameters analysed seems to explain this effect.

439 3.4. Principal Component Analysis

440 As it has been evidenced, the interaction effect (retention or salting out) observed for
441 the aroma compounds in the different wine matrices, strongly depended on the type of
442 matrix and on the physicochemical characteristics of the volatile compound. Therefore,

443 to obtain straightforward relationships between the behaviour of a compound and the
444 composition of each matrix is very difficult. Nonetheless, in order to gain insight on the
445 relationships between the type of aroma compound and the interactions with the wine
446 non-volatile composition, a principal component analysis (PCA) considering the slopes
447 for all volatile compounds in the six wines and their compositional parameters was
448 carried out. From this treatment four main principal components (PC) were obtained.
449 The first principal component (PC1) explained 33.27% of data variation and presented
450 higher correlation values with hexyl acetate (-0.736), β-phenylethyl acetate (-0.837),
451 linalool (-0.715), nerol (-0.761), methyl vanillate (0.861), ethyl vanillate (0.866) and
452 octanoic acid (-0.743). Moreover, several compositional parameters determined in the
453 matrices were correlated with PC1, such as the non-volatile residue (-0.705), amino
454 acids (-0.727), pH (-0.825) and total nitrogen (-0.728). The second principal component
455 (PC2), explained 27.51 % of data variation and correlated with the volatile compounds,
456 ethyl 2-methylbutyrate (-0.740), isobutyl acetate (-0.765), β-phenylethyl alcohol
457 (0.713), terpinen-4-ol (0.825), β-citronellol (0.791), β-damascenone (0.938), α-ionone
458 (0.981), 4-ethylguaiacol (0.920), trans-whiskey lactone (0.808) and cis-whiskey lactone
459 (0.749). The third principal component (PC3) explained 22.06 % of data variation and
460 correlated with ethyl cinnamate (0.797) and isoamyl acetate (0.749). Finally, the fourth
461 principal component (PC4), which explained a 13.62 % of data variation correlated with
462 ethyl decanoate (-0.882), eugenol (-0.822) and 5-methylfurfural (0.801). Therefore only
463 PC1 was correlated with the compositional parameters. **Figure 1** shows the
464 representation of the six types of matrices in the plane defined by the first and second
465 principal components (PC1 and PC2) which explained 61 % of data variation. As can be
466 seen in the figure, PC1 showed high and positive values for the sparkling wine; while
467 on the contrary, it showed high but negative values for the sweet wine. Control, white

and red-young wines exhibited very similar values for PC1, while the aged-red wine was between the above mentioned wines and the sweet wine. Therefore, PC1 is mainly showing a separation between wines because of their differences in the non-volatile matrix composition. In addition those volatile compounds positively and negatively correlated to PC1 showed the highest differences in behaviour depending on the matrix composition. PC2, showed, however, higher differences between white and control wines from the rest of the wine types. All the volatile compounds associated to PC2 showed a very different behaviour in white wine than in the other four types of wines. While volatile compounds positively correlated to PC2 showed none or a "salting out" effect in the white wine, they showed the opposite effect on the other four matrices. On the contrary, ethyl 2-methylbutyrate and isobutyl acetate, negatively associated to PC2 showed a slight retention effect in the white wine, and the opposite effect in the other four types of wines. Therefore, PCA evidences specific aroma compounds which behaved more differently depending on the matrix composition, in which the white wine, compositionally more similar to the control wine, showed the highest differences towards the aroma compounds compared to the other four matrices.

4. Conclusions

This study has shown that the non volatile composition of wines strongly influences the volatility of wine aroma compounds. Two opposite effects, a retention effect, therefore a decreasing in the amount of aroma in the headspace and a "salting out" effect, meaning an increase in the volatility of some aroma compounds were observed depending on the non volatile matrix composition. In addition, the aroma chemical class and mainly its physicochemical properties (volatility and Log *P* value) strongly influence this behaviour. On the basis of our results, many odour threshold values

493 calculated in simply hydroalcoholic solutions and usually employed to evaluate the
494 odour importance of specific volatile compounds might have been over- or infra-
495 estimated. New experiments will be carrying out to verify the importance of these
496 interactions on the sensory aroma perception of wines.
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675 FIGURE CAPTIONS

676

677 **Figure 1**. Representation of the reconstituted wines in the plane defined by the two first

678 principal components obtained from PCA.

Table 1
Volatile compounds, retention time, ions of quantification, physicochemical characteristics and range of concentration essayed in the present study.

Compound	CAS Number	Retention Time (min)	Ion Q (m/z)	Boiling point (ºC)	Log *P* [i]	Concentration range studied (mg L^{-1})
Isobutyl acetate [a]	110-19-0	4.61	56	116.5	1.78	0 - 0.675
Ethyl butyrate [a]	105-54-4	5.19	71	121.5	1.85	0 - 1.456
Ethyl 2-methylbutyrate [a]	7452-79-1	5.64	57	133	2.26	0 - 0.803
Butyl acetate [b]	123-86-4	6.22	43	126.1	1.78	0 - 0.713
Isoamyl acetate [b]	123-92-2	7.78	70	142.5	2.25	0 - 1.619
Ethyl hexanoate [c]	123-66-0	11.54	88	167	2.83	0 - 2.356
Hexyl acetate [b]	142-92-7	13.08	56	171.5	2.83	0 - 2.394
1-Hexanol [a]	111-27-3	16.32	56	157.6	2.03	0 - 2.200
trans-3-Hexen-1-ol [a]	928-97-2	16.64	67	156.5	1.61	0 - 0.875
cis-3-Hexen-1-ol [d]	928-96-1	17.29	67	156.5	1.61	0 - 0.888
Ethyl octanoate [b]	106-32-1	19.12	127	208.5	3.81	0 - 2.124
Linalool [b]	78-70-6	22.40	93	198	2.97	0 - 0.498
5-Methylfurfural [b]	620-02-0	23.03	109	187	0.67	0 - 1.475 [j]
Terpinen-4-ol [b]	2438-10-0	23.80	93	209	3.26	0 - 0.665
Ethyl decanoate [c]	110-38-3	25.63	101	241.5	4.79	0 - 0.931
α-Terpineol [b]	10482-56-1	26.68	59	217.5	2.98	0 - 0.433
β-Citronellol [b]	106-22-9	28.69	69	224	3.91	0 - 1.563 [j]
Nerol [b]	106-25-2	29.55	69	225	3.56	0 - 7.838 [j]
β-Damascenone [e]	23726-93-4	29.98	69	274-275	4.21	0 - 0.425 [j]
β-Phenylethyl acetate [b]	103-45-7	29.85	104	232.6	2.30	0 - 1.531
α-Ionone [b]	127-41-3	30.73	93	259-263	3.85	0 - 0.228 [j]
Benzyl alcohol [a]	100-51-6	31.47	79	205.3	1.10	0 - 1.563 [j]
trans-Whiskey lactone [a]	80041-01-6	31.68	99	260.63	2.00	0 - 0.868 [j]
β-Phenylethyl alcohol [c]	60-12-8	32.32	91	218.2	1.36	0 - 7.838 [j]
β-Ionone [b]	79-77-6	33.00	177	262.93	3.84	0 - 0.240
cis-Whiskey lactone [a]	80041-00-5	33.38	99	260.63	2.00	0 - 0.682 [j]
γ-Nonalactone [a]	104-61-0	35.10	85 [h]	243	2.08	0 - 0.413
4-Ethylguaiacol [f]	2785-89-9	35.27	137 [h]	236.5	2.38	0 - 0.868 [j]
Octanoic acid [g]	124-07-2	36.22	60	239	3.05	0 - 4.656 [j]
Ethyl cinnamate [c]	103-36-6	37.60	131	271	2.99	0 - 0.825
Eugenol [a]	97-53-0	38.47	164 [h]	253.2	2.27	0 - 0.400
4-Ethylphenol [a]	123-07-9	38.76	107 [h]	217.9	2.58	0 - 0.803
3,4-dimethylphenol [g] (IS)	95-65-8	39.78	107 [h]	-	-	-
4-Vinylphenol [f]	2628-17-3	43.53	120 [h]	209.22	2.41	0 - 0.432
Vanillin [a]	148-53-8	46.87	151 [h]	285	1.21	0 - 0.903
Methyl vanillate [f]	3943-74-6	47.65	151 [h]	286	1.82	0 - 0.198
Ethyl vanillate [f]	617-05-0	48.19	196 [h]	292	2.31	0 - 0.733

IS: Internal Standard
Manufacturer: [a] Aldrich, [b] Fluka, [c] Merck, [d] Sigma, [e] Firmenich, [f] Lancaster, [g] Scharlau
[h] Determined in SIM mode
[i] Hydrophobic constants (Log *P*) obtained from EPI Suite (EPA)
[j] In some wines the linear range did not include the whole range of concentration essayed

Table 2
Chemical composition of the five wine matrices studied

Compound	Wine Matrices				
	White	Young-red	Aged-red	Sparkling	Sweet
Non-volatile residue (g)	0.145 (0.005)	0.170 (0.004)	0.213 (0.006)	0.136 (0.004)	3.177 (0.039)
% Non-volatile residue (w/w)	1.9	2.2	2.7	1.8	34.6
pH	3.2 (0.01)	3.48 (0.03)	3.55 (0.04)	3.02 (0.01)	4.59 (0.01)
Total acidity (mg L^{-1} tartaric acid)	5.82 (0.03)	5.71 (0.03)	5.28 (0.00)	5.54 (0.05)	3.07 (0.03)
Total Polyphenols (mg L^{-1} gallic acid)	230 (4)	1820 (21)	2142 (220)	125 (6)	1088 (31)
Neutral polysaccharides (mg L^{-1} mannosa)	1816 (31)	3019 (161)	5754 (80)	2795 (114)	360583 (4256)
Residual sugars (mg L^{-1})	3502 (96)	4633 (74)	9337 (29)	4913 (124)	708285 (17325)
Total Nitrogen (mg L^{-1} N)	195.6 (2.4)	104.6 (7.5)	255.4 (1.4)	174.2 (0.6)	929.1 (29.4)
Amino acids + peptides (mg L^{-1} N)	52.9 (0.8)	43.3 (0.1)	74.1 (6.7)	62.1 (2.7)	240.6 (12.9)
Amino acids (mg L^{-1} N)	27.6 (1.5)	13.6 (0.1)	33.3 (2.0)	23.3 (0.6)	97 (1.6)
Peptides (mg L^{-1} N)	25.4	29.7	40.8	38.7	143.6

Average of two determinations except for non-volatile residue (average of 12 lyophilised vials)
SD in brackets

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Table 3
Slopes, determination coefficients (R^2) and residual standard deviations (s) of the calibrations in the reconstituted wines and in the control wi

Compound	White			Young red			Aged-red			Sparkling	
Esters	Pte	R^2	s	Pte	R^2	s	Pte	R^2	s	Pte	R^2
Ethyl butyrate	0.678	0.984	0.089	0.776	0.996	0.045	**0.913**	0.995	0.061	0.693	0.989
Ethyl 2-methylbutyrate	1.364	0.980	0.109	**1.677**	0.994	0.066	**2.032**	0.992	0.093	1.470	0.987
Ethyl hexanoate	**3.931**	0.983	0.879	4.230	0.971	1.200	4.300	0.972	0.079	**3.995**	0.986
Ethyl octanoate	**6.518**	0.980	1.456	7.085	0.993	0.734	**8.326**	0.989	1.362	**6.535**	0.991
Ethyl decanoate	**10.971**	0.974	1.161	17.377	0.987	1.500	**14.816**	0.989	0.922	**16.573**	0.995
Ethyl cinnamate	**3.316**	0.996	0.126	3.911	0.995	0.154	**3.374**	0.991	0.185	4.148	0.991
Isobutyl acetate	0.278	0.985	0.016	**0.337**	0.996	0.010	**0.383**	0.993	0.014	0.303	0.994
Butyl acetate	1.090	0.984	0.069	**1.199**	0.996	0.030	**1.442**	0.993	0.054	1.074	0.993
Isoamyl acetate	**1.122**	0.965	0.242	**1.210**	0.996	0.074	**0.670**	0.990	0.101	**1.063**	0.988
Hexyl acetate	2.827	0.959	0.971	**3.130**	0.990	0.496	**3.574**	0.988	0.670	2.686	0.985
β-Phenylethyl acetate	**6.323**	0.993	0.564	6.152	0.997	0.331	6.103	0.999	0.213	**5.386**	0.996
Alcohols											
1-Hexanol	0.304	0.984	0.060	**0.335**	0.996	0.028	**0.358**	0.993	0.047	**0.286**	0.990
trans-3-Hexen-1-ol	**0.098**	0.988	0.006	0.107	0.998	0.003	0.117	0.996	0.004	**0.093**	0.993
cis-3-Hexen-1-ol	0.103	0.985	0.008	0.117	0.997	0.003	0.126	0.997	0.004	**0.100**	0.993
Benzyl alcohol	**0.117**	0.995	0.006	**0.087**	0.992	0.008	**0.104**	0.996	0.006	**0.077**	0.986
β-Phenylethyl alcohol	0.269	0.991	0.097	0.224	0.994	0.089	0.250	0.984	0.167	**0.200**	0.992
Terpenes											
Linalool	2.228	0.971	0.132	**1.851**	0.996	0.034	2.124	0.992	0.069	**1.260**	0.987
Terpinen-4-ol	1.740	0.971	0.137	**1.392**	0.991	0.059	**1.465**	0.991	0.069	**1.130**	0.991
α-Terpineol	2.618	0.984	0.104	**2.449**	0.983	0.091	2.594	0.993	0.069	2.515	0.985
β-Citronellol	**2.862**	0.991	0.070	**1.733**	0.994	0.045	**1.939**	0.994	0.053	1.280	0.991
Nerol	1.593	0.982	0.090	**0.936**	0.996	0.023	**1.244**	0.983	0.064	0.501	0.988
C13 nor-isoprenoids											
β-Damascenone	10.153	0.993	0.169	**7.999**	0.996	0.013	**7.883**	0.993	0.174	**7.671**	0.996
α-Ionone	8.599	0.991	0.109	**7.573**	0.993	0.101	**7.039**	0.998	0.044	**7.105**	0.996
β-Ionone	**19.092**	0.995	0.235	**19.058**	0.999	0.088	**16.697**	0.999	0.081	17.602	0.999
Volatile phenols											
4-Ethylguaiacol	**1.958**	0.999	0.023	**1.780**	0.999	0.021	**1.751**	0.999	0.013	**1.647**	0.999
Eugenol	**0.591**	0.997	0.008	**0.613**	0.997	0.010	**0.553**	0.993	0.013	**0.599**	0.996

4-Ethylphenol	1.168	0.997	0.030	**1.153**	0.999	0.005	1.176	0.999	0.009	**1.123**	0.999
4-Vinylphenol	**0.087**	0.998	0.001	**0.019**	0.994	0.000	**0.030**	0.981	0.001	**0.090**	0.996
Benzenic compounds											
Vanillin	0.004	0.986	0.000	0.006	0.994	0.000	0.005	0.994	0.000	**0.007**	0.985
Methyl vanillate	**0.014**	0.988	0.000	**0.019**	0.991	0.000	**0.016**	0.984	0.000	0.023	0.976
Ethyl vanillate	**0.010**	0.991	0.000	0.013	0.990	0.000	**0.011**	0.986	0.001	0.018	0.967
Lactones and furanic compounds											
5-Methylfurfural	**0.569**	0.988	0.053	**0.540**	0.997	0.018	**0.553**	0.992	0.050	**0.484**	0.996
trans-Whiskey lactone	**0.901**	0.997	0.021	**0.714**	0.998	0.019	**0.656**	0.996	0.022	**0.621**	0.998
cis-Whiskey lactone	**0.847**	0.997	0.013	**0.699**	0.999	0.011	**0.663**	0.998	0.012	**0.632**	0.999
γ-Nonalactone	**0.903**	0.997	0.012	**0.886**	0.999	0.008	**0.824**	0.999	0.006	**0.852**	0.999
Acids											
Octanoic acid	**0.753**	0.996	0.101	0.578	0.998	0.067	0.609	0.996	0.115	0.541	0.998

Statistical significant differences between the slopes of each wine respect to the model wine are indicated in bold.

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Table 4

Percentage of slope variation of the volatile compounds in each wine matrix compared to the slopes obtained in the model wine

Compound	White	Young-red	Aged-red	Sparkling	Sweet
Esters					
Ethyl butyrate	-7	6	**25**	-5	9
Ethyl 2-methylbutyrate	-6	**16**	**41**	2	**24**
Ethyl hexanoate	**-12**	-5	-4	**-11**	7
Ethyl octanoate	**-11**	-4	**13**	**-11**	4
Ethyl decanoate	**-48**	-18	**-30**	**-22**	**-27**
Ethyl cinnamate	**-19**	-4	**-18**	1	4
Isobutyl acetate	**-10**	9	**24**	-2	12
Butyl acetate	-3	6	**28**	-5	**13**
Isoamyl acetate	**-15**	-8	**-49**	**-19**	2
Hexyl acetate	-2	8	**24**	-7	**14**
β-Phenylethyl acetate	7	4	3	-9	6
Alcohols					
1-Hexanol	-3	7	**14**	-9	2
trans-3-Hexen-1-ol	**-11**	-3	6	**-15**	-5
cis-3-Hexen-1-ol	-13	-2	6	**-16**	-6
Benzyl alcohol	**31**	-2	**17**	**-13**	9
β-Phenylethyl alcohol	5	-13	-3	**-22**	-10
Terpenes					
Linalool	4	**-13**	-1	**-41**	-6
Terpinen-4-ol	1	**-19**	**-15**	**-34**	**-26**
α-Terpineol	-4	**-10**	-5	-8	**-38**
β-Citronellol	**31**	**-21**	**-12**	**-42**	2
Nerol	-3	**-43**	**-24**	**-69**	5
C13 nor-isoprenoids					
β-Damascenone	3	**-19**	**-20**	**-22**	**-11**
α-Ionone	3	-9	**-16**	**-15**	**-11**
β-Ionone	10	9	-4	1	6
Volatile phenols					
4-Ethylguaiacol	4	-5	-7	**-12**	-7
Eugenol	**-21**	**-18**	**-26**	**-20**	**-21**
4-Ethylphenol	-1	-2	0	-5	-3
4-Vinylphenol	**-23**	**-83**	**-73**	**-20**	**-41**
Benzenic compounds					

Vanillin	-20	20	0	**40**	0
Methyl vanillate	**-33**	-10	**-24**	10	**-29**
Ethyl vanillate	**-33**	**-13**	**-27**	20	**-27**
Lactones and furanic compounds					
5-Methylfurfural	**33**	**26**	**30**	**13**	**20**
trans-Whiskey lactone	**15**	-9	**-17**	**-21**	5
cis-Whiskey lactone	9	**-10**	**-15**	**-19**	6
γ-Nonalactone	**-12**	**-14**	**-20**	**-17**	6
Acids					
Octanoic acid	**46**	12	18	5	**47**

Values statistically significant different (between the wine matrix and the control) and higher than 10 % are indicated in bold

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