*Manuscript
Click here to download Manuscript: Manuscript.docx

Sensory, olfactometric and chemical characterization of the aroma potential of Grenache and Tempranillo winemaking grapes

Yohanna ALEGRE, María-Pilar SÁENZ-NAVAJAS, Purificación HERNÁNDEZ-ORTE and Vicente FERREIRA*

Laboratory for Aroma Analysis and Enology (LAAE), Department of Analytical Chemistry, Universidad de Zaragoza, Instituto Agroalimentario de Aragón (IA2) (UNIZAR-CITA), c/Pedro Cerbuna 12, 50009 Zaragoza, Spain

**corresponding author:* Vicente Ferreira (vferre@unizar.es); Tel: 34 976 762067

Yohanna alegre: yohannaalegre@unizar.es

María-Pilar Sáenz-Navajas: maria@saenz.info

Purificación Hernández-Orte: puhernan@unizar.es

Abstract

Reconstituted polyphenolic and aromatic fractions (PAFs) from 33 different Grenache and Tempranillo grapes were incubated in strict anoxia (75ºC x 24 h). Obtained hydrolyzates were characterized by sensory analysis, gas chromatography-olfactometry (GC-O) and gas chromatography-mass spectrometry (GC-MS).

Five different aroma categories emerged. Grenache may develop specific tropical fruit/citric, kerosene and floral and Tempranillo toasty-woody and red-fruit characteristics. Those notes seem to mask alcoholic and fruit-in-syrup descriptors and the common vegetal background. Twenty-seven odorants were detected by GC-O. GC-MS data showed a clustering closely matching the one found by sensory analysis, suggesting the existence of 5 specific metabolomic profiles behind the 5 specific sensory profiles. Overall results suggest that 3-mercaptohexanol is responsible for tropical fruit/citric, TDN for kerosene, volatile phenols for woody/toasty, β-damascenone and massoia lactone, likely with Z-1,5-octadien-3-one for fruit-in-syrup and alcoholic notes. Nine lipid-derived unsaturated aldehydes and ketones may be responsible for the vegetal background.

Keywords: *Aroma precursors, glycosides, aging, sensory properties, norisoprenoids, lipid-derived aroma, terpenols, volatile phenols*

Running title: Aroma potential of Grenache and Tempranillo grapes

1. Introduction

The aroma of wine is the result of perceptual interactions between a relatively wide array of aroma compounds. While major wine volatiles are byproducts of yeast fermentation, it has been recently suggested that up to 27 relevant wine aroma compounds have direct origin in grape specific precursors (Ferreira & Lopez, 2019). These specific precursors are mainly glycosides (Gunata, Bitteur, Brillouet, Bayonove, & Cordonnier, 1988; Hjelmeland, Zweigenbaum, & Ebeler, 2015; Williams, Strauss, Wilson, & Massy-Westropp, 1982) and glutathionyl and cysteinyl conjugates (Darriet, Tominaga, Lavigne, Boidron, & Dubourdieu, 1993; Fedrizzi, Pardon, Sefton, Elsey, & Jeffery, 2009; Peyrot des Gachons, Tominaga, & Dubourdieu, 2002), but there are also other types of grape non-volatile molecules able to act as wine aroma precursors. Relevant examples are S-methylmethionine which is the main specific precursor of dimethyl sulfide (DMS) (Loscos, Segurel, Dagan, Sommerer, Marlin, & Baumes, 2008), different polyols which after different dehydrations and chemical rearrangements can rend terpenols (Williams, Strauss, & Wilson, 1980) or nor-isoprenoids (Winterhalter, 1991), and also acids or hydroxyacids which during aging will form ethyl esters or lactones (Ferreira & Lopez, 2019). Aroma molecules derive from these specific precursors at different rates depending on the number and difficulty of the chemical changes required to form the aroma molecule from the precursor. For instance, linalool and geraniol are released from their glycosidic precursors very fast, because only the glycosidic bond between glucose and the aroma molecule has to be broken since the aroma molecule is directly the “aglycone” (Strauss, Wilson, Gooley, & Williams, 1986; Wilson, Strauss, & Williams, 1984). Consequently, these aroma molecules are more easily found in young wines, while aged wines contain decreased levels of these two aroma compounds. In an intermediate category there is, among others, β-damascenone.

Its release takes more time because the formation of the aroma molecule requires, at least, a dehydration and a chemical rearrangement, in addition to the cleavage of the glycosidic precursor. This aroma molecule tends to reach maxima levels after some aging (Slaghenaufi & Ugliano, 2018; Waterhouse, Sacks, & Jeffery, 2016). The extreme case is constituted by some other aroma molecules, such as TDN, DMS, guaiacol or vanillin, whose levels increase continuously with aging. In the case of DMS this happens because the cleavage of the precursor is very slow at wine pH. In all the other cases, it seems that there is a complex net of chemical reactions required to form the aroma molecules.

In any case, the assessment of this aroma potential is not straightforward and researchers have used strategies combining either enzymatic hydrolysis, harsh acid hydrolysis or both simultaneously (Delfini, Cocito, Bonino, Schellino, Gaia, & Baiocchi, 2001; Loscos, Hernández-Orte, Cacho, & Ferreira, 2009). Enzymatic hydrolysis using glycolytic enzymes is the most efficient strategy at breaking aglycones (Hampel, Robinson, Johnson, & Ebeler, 2014; Liu, Zhu, Ullah, & Tao, 2017), but some of the most relevant wine odorants, such as β-damascenone, β-ionone or TDN are not even formed (Loscos, Hernández-Orte, Cacho, & Ferreira, 2009). Precursors other than glycosides cannot be either determined using this type of hydrolysis. On its side, harsh acid hydrolysis makes it possible to assess β-damascenone, β-ionone, TDN and different types of precursors, but labile molecules such as linalool or geraniol, are nearly completely degraded (Loscos, Hernández-Orte, Cacho, & Ferreira, 2009). Levels of volatile phenols released are also very low and often unrelated to those found by enzymatic hydrolysis. Best results, at least from the sensory point of view, are obtained by slow acid hydrolysis mimicking wine aging (Francis, Sefton, & Williams, 1992; Loscos, Hernandez-Orte, Cacho, & Ferreira, 2010; Sefton, Francis, & Williams, 1993).

The problem of this strategy is that takes long time, since aroma development can take as long as 7 aging weeks (Alegre, Arias-Pérez, Hernández-Orte, & Ferreira, 2020). Furthermore, often aroma notes related to oxidation or to the degradation of carotenoids are noted, suggesting that results are far from optimal.

Recently, it has been observed that if the aging is carried out in complete anoxia and in the presence of grape polyphenols (polyphenolic and aroma fractions or PAFs), there is an intense aroma development which includes sensory nuances closely related to some typical wine aroma nuances. Most remarkably, the aroma development observed after 24h at 75ºC was relatively similar to that observed after seven weeks at 45ºC, both from the sensory, olfactometric and chemical points of view (Alegre, Arias-Pérez, Hernández-Orte, & Ferreira, 2020).

In this context, the main hypothesis of the present work is that the accelerated anoxic aging of reconstituted PAFs extracted from different lots of Tempranillo and Grenache grapes, will produce intense aroma fractions of different sensory characteristics integrated by aroma molecules derived from the different specific aroma precursors contained in grapes. For that, the PAFs-based strategy will be applied to Grenache and Tempranillo winemaking grapes from different origins and states of ripeness. The aroma developed will characterized by sensory analysis, GC-Olfactometry and quantitative GC in order to obtain a first assessment about the diversity of the aroma nuances developed and of the nature of the aroma compounds responsible for those grape-derived aroma nuances.

2. Materials and methods

2.1. Chemicals

ACS quality absolute ethanol was obtained from Panreac (Barcelona, Spain), pure water was purchased from a Milli-Q purification system (Millipore, USA) and LiChrosolv quality Methanol and HPLC quality dichloromethane were obtained from Merck (Darmstadt, Germany).

Sep Pak C18 silica, prepacked in 10 g cartridges were obtained from Waters (Ireland). LiChrolut EN resins cartridges were purchased from Merck (Darmstadt, Germany). A VAC ELUT 20 station supplied by Varian (Walnut, Creek, USA) was used to carry out a semiautomated solid phase extraction. L-tartaric acid, sodium chloride, $NaHCO_3$ and ammonium sulfate were supplied by Panreac (Barcelona, Spain). The Internal Standard solution contained 3-octanone, 2-octanol and 3,4-dimethylphenol.

Samples. The study was carried out with Grenache and Tempranillo grapes from different high quality Spanish producers (Dominio Pingus, Bodegas Ramón Bilbao, Bodega Vega Sicilia, Bodega Viñas del Vero, and Bodega Ilurce) belonging to 3 winemaking areas (Ribera del Duero: D, Somontano: S, and Rioja: R). Samples were coded with three identifiers. The first refers to the degree of ripening: unripe (u) samples were taken one week before vintage, ripe (r) samples were harvested at the optimal point of ripeness, and overripe (o) were collected one week after optimal ripeness. The optimal moment of harvest was determined based on Cromonenos® methodology (Kontoudakis, Esteruelas, Fort, Canals, & Zamora, 2010). The second identifier refers to the variety (T=Tempranillo, G=Grenache), and the third to the regional origin and specific vineyard plot: D1-D4 for DO Ribera del Duero, S1-S4 for DO Somontano and R1-R9 for DOCa Rioja vineyard plots.

2.2. Preparation of ethanolic musts (mistelles)

Ten kilograms of grapes were taken from different areas of north Spain, from two varieties (Grenache and Tempranillo) at one, two or three ripeness states in relation to the optimal date of vintage and depending on climate conditions and vine state. A total number of 33 different lots of grapes were collected (Table 1). Grapes were kept at 5 ºC during the transport from the vineyard to the experimental cellar in the Institute of Grapevine and Wine Sciences (ICVV, Logroño, La Rioja). Grapes were first destemmed and crushed in the presence of 5 g/hL of potassium metabisulfite and 15% (w/w) of ethanol to prevent oxidation and fermentative processes, and to accelerate extraction. After seven days macerating at 13 ºC, the ethanolic must (mistelle) was pressed, filtered and stored at 5 ºC in the dark.

2.3. Extraction of phenolic and aromatic fractions (PAFs)

The extraction of the phenolic and aromatic fractions (PAFs) was carried out as described by Alegre et al. (Alegre, Arias-Pérez, Hernández-Orte, & Ferreira, 2020). Attending to the procedure, 750 mL of ethanolic must (mistelle) were dealcoholized in a rotatory evaporator at 23 ºC and a pressure of 20 mbar, to a final volume of around 410 mL containing less than 3% (v/v) ethanol. This volume was percolated through a 10 g prepacked Sep Pak C18 cartridge (previously conditioned with 44 mL of methanol followed by 44 mL of milli-Q water with 2% of ethanol). Sugars, amino acids, acids and ions were removed by washing with 88 mL of milli-Q water at pH 3.5. The cartridge was then dried by letting air pass through and the polyphenolic and aroma precursor fractions (PAFs) were recovered by elution with 100 mL of absolute ethanol.

2.4. Accelerated hydrolysis

PAFs were then reconstituted to their original volume (750 mL) with water containing 5 g/L of tartaric acid to form a model wine (rPAF) containing 13.3% (v/v) ethanol and pH adjusted to 3.5. Then, 180 mL of these rPAFs were introduced into the anoxic chamber and distributed into three-60 mL WIT™ (*wine-in-tube*) tubes which were closed within the chamber and were further bagged into two consecutive thermo-sealed plastic bags. The bags were of certified oxygen permeability and contained an activated charcoal with an oxygen scavenger (AnaeroGen from Thermo Scientific Waltham, Massachusetts, United States) as described by Vela et al. (Vela, Hernandez-Orte, Franco-Luesma, & Ferreira, 2018). The bagged rPAFs were then taken out of the anoxic chamber and put into an oven for incubation at 75 °C for 24 hours to form arPAFs. Released aroma compounds were then analyzed by sensory analysis, gas chromatography-olfactometry (GC-O) and gas chromatography-mass spectrometry (GC-MS).

2.5. Sensory characterization of hydrolysates

The hydrolysates obtained from rPAFs (arPAFs) were submitted to two different sensory tasks. In the first, the 33 arPAFs were subjected to a sorting task in which judges were asked to group samples according to odor similarities. Then, one arPAF was selected out of each one of the formed groups as the most representative one. These five arPAFs were submitted to a more complete sensory description via flash profile methodology by semi-trained panelists. Both sensory tasks were conducted between October and December 2018. In all cases, samples were taken out of the fridge at 5 °C one hour before the sensory tasks. Ten-mL samples were poured 30 minutes prior to the session and served in normalized (German Institute for Normalisation, DIN) dark wine glasses (Sensus, Schott Zwiesel, Germany) labeled with random three-digit codes and covered with plastic Petri dishes. The order of presentation of samples was different for each participant attending to a randomized order. Samples were served at room temperature and evaluated in a ventilated and air-conditioned tasting room at around 20

°C under ambient light. Participants were not informed about the nature of the samples nor the objective of the study.

2.5.1. Sorting task

Participants: a volunteer sensory panel comprised of 22 wine-science researchers and established winemakers (27% men and 73% women ranging in age from 25 to 63, with an average of 37 years old) participated. They had extended experience in wine production and tasting (average of 13 years) and were considered to be experts according to Parr, Heatherbell, and White (2002).

Procedure: Participants were presented simultaneously with 35 samples: 33 arPAFs plus 2 replicate samples (R_uGS4 and R_rTR1) to assess the reproducibility of the panel. Panelists were then asked to sort the arPAFs based on odor similarity by grouping glasses on the table. Participants could form as many groups as they wished (minimum of two groups and maximum of 34). Upon completion, they recorded the three-digit codes of the samples belonging to each group on a paper sheet and were asked to describe the groups they formed with their own words (maximum of two terms per group). At this step, participants were allowed to smell again, but not to modify the groups they had already established in the previous task.

Data analysis: Results from each panelist were pooled into an individual similarity matrix (arPAFs × arPAFs) in which 0 meant that two arPAFs were sorted in different groups and 1 that were in the same group. Individual matrices from all judges were summed to form the global similarity matrix. A multidimensional scaling (MDS) analysis was carried out with this global similarity matrix to get a spatial representation of the samples (Schiffman, Reynolds, & Young, 1981). Further hierarchical cluster analysis (HCA) with the Ward criteria was performed on the MDS coordinates. The five clusters identified by truncating the tree diagram were consolidated by aggregation around mobile centers. The sample closest to the gravity center of the cluster was selected as the most representative for each cluster. Analyses were carried out using XLSTAT software (version 2014.2.02).

For the terms derived from the description of the groups, an initial list was built with all the terms elicited by participants. This list was first reduced by omitting words with hedonic or emotional character (e.g. pleasant, easy, classic, different…) and adverbs (e.g., very, barely, extremely…). For remaining words, a lemmatization process was performed, i.e., words sharing the same lemma or root (e.g., sour, sourness) were grouped in the same category. Finally, all terms were grouped in categories according to semantic similarities. This process was performed individually by three experienced researchers, who through a triangulation task (Abric, 2003) achieved a final consensual list of terms. The frequency of quotation of each term was calculated and only terms cited by at least 23% of the panel (>4 participants) were considered.

2.5.2. Flash profile

Participants: a sensory panel comprised of 12 participants (25% men and 75% women ranging in age from 25 to 63, with an average of 35 years old) attended the sessions. They were all stuff members of the Laboratorio de Analisis del Aroma y Enología (LAAE, Universidad de Zaragoza) with extended experience in wine aroma description (average of 10 years) and considered to be experts according to (Parr, Heatherbell, & White, 2002).

Procedure: The five arPAFs selected in the sorting task (-I uGS1, uGR3, rTR1rTR5, andoD2) were sensory characterized by flash profile in duplicate. The methodology followed involved three different steps: 1) generation of descriptors, 2) panel training, and 3) description of samples. Therefore, panelists were firstly asked to provide descriptors that differentiate the five samples. They could give as many attributes as they wanted. Then, during an inter-session, all the descriptors were pooled to create a global list. The panel coordinator created aroma references for the descriptors in the list in order to train the panelists (Table A.1 of supplementary material). References were prepared in ethanolic solutions (15% v/v) and different arPAF matrices to simulate the sensory space studied. During the training, panelists were asked to associate the references to the descriptors in the global list. Panelists were qualified when they were able to correctly identify at least 80% of the references. Finally, they were given the global list of descriptors, not intending to reach a consensus, but to allow them to refine or complete the list they provided in the first step. Panelists were asked to score the five samples on each of the descriptors they had chosen. Each descriptor was rated in a non-structured 10-cm length scale anchored with the words ‘absence’ on the left end, and ‘high intensity’ on the right end. For each panelist, all arPAFs were presented simultaneously in a different and random order in duplicate in two sessions held in different days (one replicate by session). Data from each panelist was compiled in an individual data matrix (attribute in columns and arPAFs in rows), and pooling the 24 individual matrices (responses of 12 panelists in duplicate), a global data matrix was formed. This was further submitted to generalized procruster analysis (GPA). In order to visualize the relationships between attributes and arPAFs, only attributes cited by at least five panelists (20% of the panel) were used. GPA analyses was performed with XLSTAT software (version 2014.2.02; Addinsoft, NY, USA).

2.6. Quantification of aroma compounds

Volatile compounds released from precursors were extracted using a solid phase extraction (SPE) cartridge, as described by López et al., (Lopez, Aznar, Cacho, &

Ferreira, 2002). The SPE bed consisted on 65 mg of LiChrolut EN resins packed in a one mL polypropylene SPE cartridge. The sorbent was conditioned with two mL of dichloromethane, two mL of methanol and two mL of milli-Q water containing 12% (v/v) of ethanol. Then, 15 mL of the arPAF, to which 100 µL of ethanolic internal standard solution (2-octanol, 3-octanone, 3.4-dimrthylphenol and 2-octanol) had been added, were passed through the cartridges at 2 mL/min. The bed was then washed with 1.5 mL of an aqueous solution 30% in methanol and 1% in $NaHCO_3$. After this, the resins were dried by letting air pass through them and aroma compounds were finally eluted with 600 µL of dichloromethane containing 5% methanol (v/v).

Two µL of this extract were injected in a QP2010 gas chromatograph equipped with a quadrupole mass spectrometer detector from Shimadzu (Japan) following the method proposed by Oliveira et al., (Oliveira, 2019). The column, a DB-WAXetr (30 m x 0.25 mm with 0.5 µm film thickness), was from Agilent (USA). Helium (1.26 mL/min) was the carrier gas. The initial oven temperature was 40 °C, kept for 5 min, then raised at 1°C/min to 65ºC, then at 2 °C/min to 220 °C and finally hold for 50 min. Injection was made in splitless mode at 250ºC, splitless time was 1.5 min, and during the injection a pressure pulse of 4 bar was applied. The mass analyzer was set in single ion monitoring mode (SIM) and the complete list of m/z ratios selected for each compound as well as their retention time are shown in Table A.2 of supplementary material. The quantification was performed by interpolating the SI-normalized peak area in the calibration straight lines containing at least three different concentration levels of each compound.

GC-MS data and sensory data (frequency of citation of each attribute) were merged in a matrix and a two-dimensional principal component analysis (PCA) was carried out. Sensory data were considered simple illustrative variables, but did not take any role in the factorization process. XLSTAT software (version 2014.2.02; Addinsoft, NY, USA) was used.

2.7. Gas chromatography- olfactometry (GC-O)

The aroma compounds present in the arPAFs samples selected in the sorting task were isolated and preconcentrated using a dynamic headspace sampling technique producing extracts representative of orthonasal olfaction (San-Juan, Pet'ka, Cacho, Ferreira, & Escudero, 2010). For this, 80 mL of sample were transferred to a specifically designed bubbler flask, where without agitation nor bubbling, the headspace was purged by a 100 mL/min stream of pure N_2 for 200 min. Volatiles were trapped in a 400 mg LiChrolut EN SPE bed contained in a three mL polypropylene SPE cartridge installed on top of the bubbler flask. Then, the SPE cartridge was removed from the system, dried with N_2 and volatiles were eluted with 3.2 mL of dichloromethane containing 5% methanol. The extract was concentrated to 100 µL under a stream of pure nitrogen and 1 µL was further injected in the GC-O system. This was a Trace GC gas chromatograph (ThermoQuest, Milan, Italy) with a sniffing port ODO-I (SGE, Ringwood, Australia) and a flame ionization detector (FID). The column was a DB-WAX (30 m x 0.32 mm i.d. x 0.5 mm film thickness) from J&W (Folsom, CA, USA), preceded by a deactivated precolumn (3 m x 0.32 mm i.d.) supplied by Supelco (Bellefonte, PA). The carrier gas, hydrogen, was used at a constant flow rate of 3.5 mL/min. Injection was in splitless mode (60 s splitless time). Detector and injector temperatures were 250 ºC. The sniffing port was heated to prevent the condensation of high boiling point compounds, and it was equipped with a humidifier filled with deionized water. The temperature program used was 40 ºC for 5 min, increased by 4 ºC/min to 100 ºC and then 6 ºC/min to 220 ºC, keeping this temperature during 10 min. The olfactometry signal was obtained by using a panel of 6 trained judges (83% women and 17% men from 25 and 34 years, median =

28 years) from the laboratory staff. The sniffers annotated the time, odor description and odor intensity (0 = not detected; 1 = weak odor, 2 = clear odor; 3 = extremely strong odor, half values allowed) when they detected an aroma. Identification was carried out by comparing odor descriptors, chromatographic retention indexes in the DB-Wax and DB5 columns and Mass Spectra with those of pure reference compounds.

GC-O data from the six panelists were compiled and, for each detected odorant, a GCO score was obtained by calculation of the modified frequency in percentage (% MF), using the formula proposed by Dravnieks (Dravnieks, 1985):

$$\%MF = \sqrt{\%F\ x\ \%I}$$

where F (%) is the aromatic attribute detection frequency expressed as a percentage and I (%) is the average intensity expressed as a percentage of the maximum intensity. Those odorants not reaching a maximum %MF of 40 % in any of the studied samples were considered noise and were eliminated.

3. Results and discussion

Phenolic and aromatic fractions (PAFs) extracted from 33 different lots of grapes from Grenache and Tempranillo were reconstituted in synthetic wine and further submitted to accelerated hydrolysis at 75 ºC for 24h in strict anoxic conditions. Most samples developed strong aromatic nuances. The aroma developed by the different samples was characterized by sensory analysis, GC-O and GC-MS.

3.1. Sensory characterization

The first sensory study consisted of a sorting task aimed at grouping samples attending to their odor properties. Results of the sorting task are summarized in the dendrogram shown in Figure 1. The labels (descriptors) most frequently used by the judges to describe the clusters created in the sorting task are also given. It can be first observed that the replicate samples introduced as controls (R_uGS4; and R_rTR1) are plotted together in the dendrogram, supporting the consistency of the panel. It can be also observed that the sensory task identified five different sensory categories split into two major groups (group A: clusters 1-2 and group B: clusters 3-5), each one containing samples predominantly from a single variety. Thirteen out of the 16 samples belonging to clusters 1 and 2 are from Grenache, while 16 out of 19 in the other three clusters are from Tempranillo. The two clusters integrated in the main group A (cluster 1+2) were described as “tropical fruit/citrus” and “floral” for cluster 1 and as “floral” and “fruit in syrup” for cluster 2, suggesting that “floral” is an attribute more specific of Grenache. For group B (clusters 3-5), containing mainly Tempranillo, three other sensory categories were identified. Cluster 3 was mainly described as “woody-toasty”, “red fruit” and “black fruit”, and “fruit in syrup”; cluster 4 as “vegetal”; and cluster 5 as “vegetal” and “fruit in syrup”. Remarkably, the cluster does not reveal any relevant effect of geographic precedence or of the degree of ripeness.

One sample per sensory category was selected as the most representative for each cluster (from cluster 1: uGS1; from cluster 2: uGR3; from cluster 3: oTD2; from cluster 4: rTR5; and from cluster 5: rTR1) for a deeper sensory characterization using flash profile. Results of this study are summarized in the GPA maps given in Figure 2. The two first components accumulate 35% and 29% of the original variance, respectively. A first observation from the distribution of samples observed in Figure 2a is that the varietal distribution obtained in the previous sensory task, is not identified here. In fact, the two samples from Grenache are plotted in extreme positions in the first component. This apparent contradictory result should be attributed to the complementary nature of this second sensory task, which aims quantifying sensory descriptors in dissimilar samples, while the sorting task aims to classify samples. Nevertheless, most descriptors used in the sorting task in Figure 1 were further cited in the flash profile (Figure 2) and the sensory profiles obtained are relatively equivalent as will be seen.

In the task, eight descriptors emerged as the most relevant to describe the samples. In order of use: “alcoholic” (cited by 70% of panelists), “fruit in syrup” (63%), “vegetal” (50%), “kerosene” (40%), “tropical fruit/citrus” (40%), “woody/toasty” (29%), “red fruit” (29%) and floral (21%). Attributes differ attending to their ability to discriminate samples, as can be observed in the GPA planes shown in Figures 2b, 2c and 2d. The most discriminant attributes are those occupying narrow areas of the plane, since specifically define one or two samples. By contrast, those more widely distributed in the plane are similarly used to define all the samples, indicating that represent common attributes. Attending to this criterion, attributes can be ranked into three categories: highly discriminant, discriminant and common. Highly discriminant attributes are characteristic of only one sample and occupy a quite narrow area of the plane. This is the case of “Tropical fruit/ citrus”, “woody/toasty” (Figure 2b), and “kerosene” (Figure

2c). Discriminant attributes are found in one of the halves of the plane, as can be observed in the cases of “alcoholic” (upper half, Figure 2d), “floral” (right half, Figure 2c), and “red fruit” (down half, Figure 2d). The attribute “Fruit in syrup” is slightly less discriminant, since 73% of the times is found in the left half (Figure 2c) while the attribute “vegetal” is not discriminant at all. As can be seen in Figure 2b, it is evenly distributed within the plane, indicating that it is a common characteristic of all the samples.

The sample uGS1, which was representative of the first cluster, is projected on the right part of the plot in Figure 2a, indicating that it was described mainly with the terms "tropical fruit/citrus", and " kerosene", which are exclusive attributes for this sample, and as “floral”, and “red fruit”, which are attributes shared with other samples. The sample uGS1 is also the single one lacking the attribute “fruit in syrup”, and scores very low in “alcoholic”. This is mostly in agreement with results from the sorting task. The sample oTD2, the representative of the third cluster in Figure 1, is identified as the second most different in this task. This sample is mainly described with terms such as toasty-woody (exclusive attribute), “red fruit” (shared with the previous one) and “fruit in syrup” (shared with all samples in the left plane). Samples rTR1, representative of cluster 5, and uGR3, representative of cluster 2 were mainly described as “alcoholic” and “fruit in syrup”. Finally, rTR5, representative of the cluster 4 was described with “alcoholic” and “vegetal” notes.

It is remarkable that the attribute “alcoholic” is present in the three samples which do not have specific sensory notes (uGR3, rTR1 and rTR5). Since these wine models did not contain major fermentation volatiles, such as higher alcohols, this attribute was likely an exclusive characteristic of ethanol which was similarly present in all the samples. This suggests, that only some of the odorants present in samples uGS1and oTD2, likely also those ones responsible for their exclusive sensory characteristics, are able to mask the aroma (sweet, alcohol) and chemesthesic (pungent, harsh, hot) notes of alcohol. Furthermore, it can be hypothesized, that the “fruit in syrup” character is at least in part the result of the interaction between alcohol and odorants of fruity character, and that only the odorants specifically present in uGS1, likely the ones contributing to its exclusive “tropical fruit/citric” character, can mask. A similar observation was made when the addition of a small amount (1 ng/L) of a green odorant (4-methyl-4-mercaptopentanone) to an aromatic reconstitution reproducing the aroma of a white wine from Macabeo changed the aroma from sweet, alcoholic, synthetic to fresh fruit (Escudero, Gogorza, Melus, Ortin, Cacho, & Ferreira, 2004).

Therefore, from the sensory point of view, grapes from Tempranillo and Grenache contain aroma precursors able to develop a common vegetal character, general fruity characteristics at quite different levels of intensity and a differential set of sensory descriptors. Fruity notes likely become integrated with ethanol into the “fruit in syrup” aroma descriptor. The differential set of sensory descriptors, includes terms such as “tropical fruit/citric”, “kerosene”, “toasty-woody”, “floral”, and “red fruit”. Some of these sensory descriptors were at levels enough to mask the sensory characteristics of ethanol and are likely implied in the specific aromatic profiles of the varieties. Acknowledging the preliminary character of this study, Grenache grapes seem to be able to specifically develop “tropical fruit/citric” and maybe also “floral” characteristics, while Tempranillo grapes seem to be able to develop a specific “woody-toasty” character.

3.2.GC-O analysis

In order to identify the odorants responsible for the distinctive descriptions between clusters, the 5 arPAFs studied by flash profiling were also submitted to GC-O. Data from the study are summarized in Table 2, which shows the 27 different odor zones detected by the panel. Twenty-five odorants were identified as responsible for those odor zones with different levels of certainty. In 21 of the cases a single odorant seems to be responsible for the odor zone; in two others, marked with a 1 superscript in the table, there remains some doubts about the presence of additional odorants in the odor zone, since the odor descriptors of the identified odorants do not completely explain the odor descriptors given by the panel. In one of the odor zones, two odorants were identified. Additionally, no odorants could be identified in the odor zones with polar retention indexes at 1012, 1109 and 1779.

The 25 identified odorants can be classified attending to their biochemical origin into 5 different categories: lipid-derivatives with 11 members, phenol-derivatives (5 members), terpenes (4 members), nor-isoprenoids (2 members) and miscellaneous (3 members). Within the lipid-derivatives category there are 7 unsaturated aldehydes, 2 unsaturated ketones and 2 lactones. Lipid derivatives are molecules with either 9 (six of them), 8 (two of them), 10 (two of them) or 6 (just one) carbon atoms. Within the phenol-derivatives category, there are 4 volatile phenols and ethyl cinnamate. Among terpenes, linalool, linalool oxide, dihydromyrcenol and α-terpineol were identified. The two nor-isoprenoids are β-ionone and β-damascenone, and among the miscellaneous category, phenylacetaldehyde, 3-mercaptohexanol and furaneol were found. The former is an amino acid derivative, the second one is the product of the hydrolysis of different glutathionyl- and cysteinyl precursors, and the third one is a sugar derivative.

Odorants in the table are ranked attending to the difference between the maxima and minima scores. This parameter, given in the last column, is an indication of the potential ability of an odorant to introduce sensory differences, so that most discriminant should be ranked first. Nevertheless, it should be noted that in those cases in which GC-O scores are close to saturation, such as Z-2-nonenal, this parameter can underestimate the discriminating ability of the odorant. In any case, attending to this criterion, the table reveals that the odorants potentially most discriminant between the five representative samples are poorly known compounds which in fact could not be quantified, two even identified, in the present study. Three out of the four most discriminant odorants are maxima in the sample representative of cluster 1 and, on the basis of their sensory descriptors, the two first odor zones in the table should be responsible for the specific tropical fruit and citrus character of samples in this cluster. The first odorant is 3-mercaptohexanol, which is an extremely powerful and well-known grape-derived odorant. Its presence, however, was not expected because the hydrolysis of the different precursors is assumed to occur exclusively via specific β-lyase activities of yeast (Roland, Schneider, Razungles, & Cavelier, 2011). It can be argued that it is an artifact formed by the relatively high temperatures at which the hydrolysis took place, but it was also found when the hydrolysis was carried out at 45ºC (Alegre, Arias-Pérez, Hernández-Orte, & Ferreira, 2020) and in earlier studies, Darriet et al showed that it could be released by acid catalysis in the presence of ascorbic acid (Darriet, Tominaga, Demole, & Dubourdieu, 1993). On the other hand, it is known that its precursors can be present in Grenache at mg/L levels (Concejero, Peña-Gallego, Fernandez-Zurbano, Hernández-Orte, & Ferreira, 2014), so that less 0.1% cleavage would suffice for its detection. The odor zone eluting at IR1464 also had a grapefruit and citrus character, and two odorants compatible with this odor were identified: linalool oxide and dihydromyrcenol. A third potentially discriminant odorant, the strawberry smelling compound eluting at 1109, was also maxima in this sample. The table also reveals the presence of two discriminant odorants maxima in the sample representative of cluster 4 (vegetal odor) and scoring high also in the representative of cluster 5 (vegetal and fruit).

These two odorants are the unidentified solvent-smelling with IR 1012 and the mushroom-blood-metal smelling Z-1,5-octadien-3-one. This last compound has been recently shown to play a role in the perception of dry fig and geranium nuances in musts (Allamy, Darriet, & Pons, 2017). Both compounds may play a role in the perception of vegetal notes most clearly identified in clusters 4 and 5. Another discriminant odorant was identified as phenylacetaldehyde, and scored maxima in the sample representative of cluster 2 (floral). Other floral smelling odorants also scored high in this sample, such as linalool, ethyl cinnamate or β-ionone. On the other hand, many of the lipid derivatives, such as E,E-2,4-decadienal, E-2-nonenal, Z-2-decenal, E,E-2,4-nonadienal or Z-3-hexenal, have quite limited ranges of variability in the GCO scores, which suggests that these odorants derived from lipids are a common constitutional background in all samples contributing to vegetal notes.

3.3. Quantitative data

The 33 samples were also analyzed quantitatively by GC-MS. Targeted compounds included those found relevant in previous studies (Loscos, Hernandez-Orte, Cacho, & Ferreira, 2010; Oliveira & Ferreira, 2019) and belonged to five different chemical categories: norisoprenoids, terpenoids, lactones, volatile phenols, vanillin derivatives and ethyl esters. Unfortunately, some remarkable odorants identified by GC-O in Table 2 could not be quantified, well because of the low concentration at which they are found, well because they require specific analytical procedures involving chemical derivatization or selective isolation. Overall, 30 different aroma compounds could be quantified. Results are summarized in Table 3 and the complete set of results is given as supplementary material. Quantitative data were processed by one-way ANOVA considering as factors the sensory cluster, grape variety, geographical precedence and degree of ripeness. The most influential factor was the sensory cluster for which all aroma compounds except furaneol, varied significantly with differences in many cases of large magnitude, as can be seen in Table 3. The second most influential factor was grape variety, for which 24 out of the 30 aroma compounds varied significantly, in some cases also with large differences, as can be also seen in the table. By contrast, the factor with smallest influence in the dataset was the degree of ripeness, for which only one compound reached significance (supp. material). The factor geographical origin, had a small but significant influence on the levels of 16 aroma compounds (supp. material). Nevertheless, the real influence of this factor cannot be well assessed since the experiment was not adequately balanced, but results in any case suggest that its influence is much smaller than that of the variety.

These observations are further supported by the PCA carried out with quantitative data, as can be seen in the plot in Figure 3. Samples are distributed in the plane following exactly the same five clusters identified in the sensory sorting task. This close similarity between the sensory and chemical spaces is quite infrequent in wine flavor chemistry, and decidedly suggests that the sensory classes identified in the sorting task, are the consequence of quite specific profiles of volatiles. Since some aroma relevant molecules detected in the GC-O experiment have not been quantified, it seems that those profiles of volatiles reflect the existence of specific metabolic patterns. Additionally, and comparing to the difficulties found in wine to correlate sensory and chemical spaces, it can be hypothesized that major fermentation volatiles largely complicate and distort the relationship between the chemical and the sensory spaces.

In order to facilitate the discussion of results and, in particular, in order to focus the discussion on the odorants most relevant from the sensory point of view, the two last columns in Table 3 contain the odor thresholds of the quantified odorants and the ratios OAVmax/OAVmin. Such ratios are indicative of the potentiality of the odorant to introduce sensory differences within the pool of samples. If such ratios are calculated including only those OAVs>1 (strict criterion), the odorants potentially responsible for higher sensory variability are β-damascenone, TDN, linalool, limonene, furaneol and 4-vinylphenol, whose ratios are higher than 2. If the ratios are calculated including all those OAVs>0.2 (conservative criterion), then massoia lactone also shows a high discriminating potential reaching a 5.2 ratio. Odorants with some ability (ratio <2 but >1.3) to introduce sensory differences attending to these ratios are also β-ionone, geraniol, 1,8-cineole, guaiacol and 4-vinylphenol. The highest ratios measured for furaneol are due to spurious very large concentration values registered in some individual samples. This is the most polar and difficult to extract compound in the list, so that such extreme behavior could be attributed to limitations of the analytical method.

The plot in Figure 3 basically states that Grenache samples are found at the far-right part of the plane, split into two major groups, one at the North (coinciding with cluster 2 in the sorting task) and a second at the South (cluster 1), two other samples (uGR4 and uGR3) more centered and a single odd sample (uGS3) in the left part of the plane. Samples from Tempranillo are all of them but three (uTD2, rTD2 and oTR5), at the left part of the plane, split into three groups corresponding to the clusters 3, 4 and 5 identified in the sorting task (Figure 1). Then, considering Figure 3 and data in Table 3, it can be said that samples from Grenache are richest in norisoprenoids (except ionones), terpenoids (except limonene) and vanillin derivatives, while those of Tempranillo are richest in most volatile phenols. This has to be relevant from the sensory point of view, first because differences affect to relatively large number of compounds having similar aroma properties (terpenols, vanillins, volatile phenols) whose sensory effects will be cooperative; second because some of the components have high OAVmax/OAVmin ratios, in particular β-damascenone, TDN, linalool and massoia lactone, which are maxima in Grenache and 4-vinylphenol which is maxima in Tempranillo.

Going into more detail with the help of Table 3, the two Grenache clusters clearly differ because cluster 2 contains highest levels of β-ionone, β-damascenone, linalool, limonene (second highest) and of massoia lactone, while cluster 1 contains highest levels of TDN. The high contents of TDN in Grenache has been recently observed (Oliveira & Ferreira, 2019). These compositional differences explain the floral and fruit in syrup character of samples in cluster 2, and the specific kerosene attribute of samples in cluster 1 (Figure 2), but cannot explain the tropical fruit and citrus character of samples in cluster 1. Attending to the olfactometric study in Table 2, these should be attributed to 3-mercaptohexanol, linalool oxide and dihydromyrcenol which were not quantified. Among the Tempranillo clusters, cluster 4, is characterized by its minima contents in most aroma compounds. It contains highest levels of guaiacol, eugenol, and 2,6-dimethoxyphenol, but only the former is barely above threshold. This would explain that samples in this cluster were characterized only by vegetal and alcoholic notes, which are the general background notes, as was seen in Figure 2. Samples in cluster 5 also have close to minima contents in most aroma components, but have higher levels than those of cluster 4 in β and α-ionones, and in β-damascenone. This, together with the presence of Z-1,5-octadien-3-one could explain their fruit in syrup character, in addition to the vegetal and alcoholic notes.

Finally, samples in cluster 3 have an intermediate composition to those of clusters 2 and 5. They have higher levels of volatile phenols, particularly of vinylphenols, and smaller levels of terpenes, vanillin derivatives, massoia lactone and β-damascenone than those of samples in cluster 2. They also have, except for most volatile phenols, higher levels of aroma compounds than samples in cluster 5. The higher levels of volatile phenols would explain the woody/toasty character of samples in cluster 3. Attending to previous results (San Juan, Ferreira, Cacho, & Escudero, 2011), it can be hypothesized that the red fruit character and the lack of alcoholic character would be the consequence of a smaller fruit in syrup character, because of the smaller levels of massoia lactone, β-damascenone than samples in cluster 2 and smaller levels of Z-1,5-octadien-3-one and higher levels of fruity odorants than samples in cluster 5.

4. Conclusions

Hydrolyzates obtained from PAFs extracted from grapes from Tempranillo and Garnacha have aromas classified into five different sensory categories with a common vegetal background character. Grenache-related categories may have specific tropical fruit/citric, kerosene and floral characteristics, while Tempranillo-related may develop specific toasty-woody and red fruit sensory notes. Specific sensory notes seem to mask alcoholic and fruit in syrup aroma descriptors which would be also common.

The GC-O profiling of representative samples revealed that 3-mercaptohexanol, linalool oxide and dihydromyrcenol, two unidentified odorants, phenylacetaldehyde and Z-1,5-octadien-3-one are potentially the most discriminant odorants of the data set. A large group of powerful lipid-derivatives including 7 unsaturated aldehydes, 2 unsaturated ketones and 2 lactones, having 9 (6 of them), 10, 8 (2 of them each) or 6 (just 1) carbon atoms, may be responsible for the vegetal background and have also implications in the fruit in syrup perception. Other identified odorants were 4 volatile phenols, ethyl cinnamate, β-ionone and β-damascenone, linalool and α-terpineol and furaneol.

The PCA derived from quantitative data (30 odorants, including only 12 out of the 27 detected by GC-O) showed a clustering perfectly matching the one found by sensory analysis, which suggests the existence of 5 specific metabolomic profiles behind the 5 specific sensory profiles. Quantitative data confirm that Grenache is richest in norisoprenoids (except ionones), terpenoids (except limonene) and vanillin derivatives, while those of Tempranillo are richest in most volatile phenols.

The integration of all data suggests that 3-mercaptohexanol, maybe together with linalool oxide and dihydromyrcenol, would be responsible for the tropical fruit/citrus character, that TDN is responsible for kerosene notes and that volatile phenols, notably guaiacol and 4-vinylphenol, would be responsible for the woody/toasty character. It is also suggested that β-damascenone and massoia lactone, likely with Z-1,5-octadien-3-one would be main contributors to fruit in syrup and alcoholic notes and would mask red fruit character.

Acknowledgments

Work funded by the Spanish MCIU AGL2017-87373-C3-1R. Y.A. and LAAE acknowledge the Diputación General de Aragón for a predoctoral fellowship, as well as the European Social Fund.

Conflict of interest

The authors declare that they have no conflict of interest.

References

Alegre, Y., Arias-Pérez, I., Hernández-Orte, P., & Ferreira, V. (2020). Development of a new strategy for studying the aroma potential of winemaking grapes through the accelerated hydrolysis of phenolic and aromatic fractions (PAFs). *Food Research International, 127*, 108728.

Allamy, L., Darriet, P., & Pons, A. (2017). Identification and organoleptic contribution of (Z)-1,5-octadien-3-one to the flavor of *Vitis vinifera* cv. Merlot and Cabernet Sauvignon musts. *Journal of Agricultural and Food Chemistry, 65*(9), 1915-1923.

Concejero, B., Peña-Gallego, A., Fernandez-Zurbano, P., Hernández-Orte, P., & Ferreira, V. (2014). Direct accurate analysis of cysteinylated and glutathionylated precursors of 4-mercapto-4-methyl-2-pentanone and 3-mercaptohexan-1-ol in must by ultrahigh performance liquid chromatography coupled to mass spectrometry. *Analytica Chimica Acta, 812*, 250-257.

Darriet, P., Tominaga, T., Demole, E., & Dubourdieu, D. (1993). Evidence of the presence of a 4-mercapto-4-methylpentan-2-one precursor in *Vitis vinifera* Sauvignon blanc grape variety. *Comptes rendus de l'academie des sciences series III-Sciences de la vie-Life sciences, 316*(11), 1332-1335.

Darriet, P., Tominaga, T., Lavigne, V., Boidron, J. N., & Dubourdieu, D. (1993). Mise en évidence dans le raisin de *Vitis vinifera J.* (var. Sauvignon) d'un précurseur de la 4-méthyl-4-mercaptopentan-2-one. *Comptes rendus de l'Académie des sciences, 316*, 1332-1335.

Delfini, C., Cocito, C., Bonino, M., Schellino, R., Gaia, P., & Baiocchi, C. (2001). Definitive evidence for the actual contribution of yeast in the transformation of neutral precursors of grape aromas. *Journal of Agricultural and Food Chemistry, 49*(11), 5397-5408.

Dravnieks, A. (1985). *Atlas of odor character profiles*. Philadelphia, PA: ASTM.

Escudero, A., Gogorza, B., Melus, M. A., Ortin, N., Cacho, J., & Ferreira, V. (2004). Characterization of the aroma of a wine from Maccabeo. Key role played by compounds with low odor activity values. *Journal of Agricultural and Food Chemistry, 52*(11), 3516-3524.

Fedrizzi, B., Pardon, K. H., Sefton, M. A., Elsey, G. M., & Jeffery, D. W. (2009). First identification of 4-S-glutathionyl-4-methylpentan-2-one, a potential precursor of 4-mercapto-4-methylpentan-2-one, in Sauvignon blanc juice. *Journal of Agricultural and Food Chemistry, 57*(3), 991-995.

Ferreira, V., & Lopez, R. (2019). The actual and potential aroma of winemaking grapes. *Biomolecules, 9*(12), 818.

Francis, I. L., Sefton, M. A., & Williams, P. J. (1992). Sensory descriptive analysis of the aroma of hydrolysed precursor fractions from Semillon, Chardonnay and Sauvignon blanc grape juices. *Journal of the Science of Food and Agriculture, 59*(4), 511-520.

Gunata, Y. Z., Bitteur, S., Brillouet, J. M., Bayonove, C., & Cordonnier, R. E. (1988). Sequential enzymic hydrolysis of potentially aromatic glycosides from grape. *Carbohydrate Research, 184*, 139-149.

Hampel, D., Robinson, A. L., Johnson, A. J., & Ebeler, S. E. (2014). Direct hydrolysis and analysis of glycosidically bound aroma compounds in grapes and wines: Comparison of hydrolysis conditions and sample preparation methods. *Australian Journal of Grape and Wine Research, 20*, 361-377.

Hjelmeland, A. K., Zweigenbaum, J., & Ebeler, S. E. (2015). Profiling monoterpenol glycoconjugation in *Vitis vinifera* L. cv. Muscat of Alexandria using a novel putative compound database approach, high resolution mass spectrometry and collision induced dissociation fragmentation analysis. *Analytica Chimica Acta, 887*, 138-147.

Kontoudakis, N., Esteruelas, M., Fort, F., Canals, J. M., & Zamora, F. (2010). Comparison of methods for estimating phenolic maturity in grapes: Correlation between predicted and obtained parameters. *Analytica Chimica Acta, 660*(1-2), 127-133.

Liu, J., Zhu, X. L., Ullah, N., & Tao, Y. S. (2017). Aroma glycosides in grapes and wine. *Journal of Food Science, 82*(2), 248-259.

Lopez, R., Aznar, M., Cacho, J. F., & Ferreira, V. (2002). Determination of minor and trace volatile compounds in wine by solid-phase extraction and gas chromatography with mass spectrometric detection. *Journal of Chromatography A, 966*(1-2), 167-177.

Loscos, N., Hernandez-Orte, P., Cacho, J. F., & Ferreira, V. (2010). Evolution of the aroma composition of wines supplemented with grape flavour precursors from different varietals during accelerated wine ageing. *Food Chemistry, 120*, 205-216.

Loscos, N., Hernández-Orte, P., Cacho, J. F., & Ferreira, V. (2009). Comparison of the suitability of different hydrolytic strategies to predict aroma potential of different grape varieties. *Journal of Agricultural and Food Chemistry, 57*(6), 2468-2480.

Loscos, N., Segurel, M., Dagan, L., Sommerer, N., Marlin, T., & Baumes, R. (2008). Identification of S-methylmethionine in Petit Manseng grapes as dimethyl sulphide precursor in wine. *Analytica Chimica Acta, 621*(1), 24-29.

Oliveira, I. (2019). *Contribution of wine microorganisms to the aroma composition of wine and its sensory impact.* Analytical Chemistry (Ph.D. thesis), University of Zaragoza.

Oliveira, I., & Ferreira, V. (2019). Modulating fermentative, varietal and aging aromas of wine using non-*Saccharomyces* yeasts in a sequential inoculation approach. *Microorganisms, 7*(6).

Parr, W. V., Heatherbell, D., & White, K. G. (2002). Demystifying wine expertise: olfactory threshold, perceptual skill and semantic memory in expert and novice wine judges. *Chemical Senses, 27*(8), 747-755.

Peyrot des Gachons, C., Tominaga, T., & Dubourdieu, D. (2002). Sulfur aroma precursor present in S-glutathione conjugate form: Identification of S-3-(hexan-1-ol)-glutathione in must from *Vitis vinifera* L. cv. Sauvignon blanc. *Journal of Agricultural and Food Chemistry, 50*(14), 4076-4079.

Roland, A., Schneider, R., Razungles, A., & Cavelier, F. (2011). Varietal thiols in wine: Discovery, analysis and applications. *Chemical Reviews, 111*(11), 7355-7376.

San-Juan, F., Pet'ka, J., Cacho, J., Ferreira, V., & Escudero, A. (2010). Producing headspace extracts for the gas chromatography–olfactometric evaluation of wine aroma. *Food Chemistry, 123*(1), 188-195.

San Juan, F., Ferreira, V., Cacho, J. F., & Escudero, A. (2011). Quality and aromatic sensory descriptors (mainly fresh and dry fruit character) of Spanish red wines can be predicted from their aroma-active chemical composition. *Journal of Agricultural and Food Chemistry, 59*(14), 7916-7924.

Schiffman, S. S., Reynolds, M. L., & Young, F. W. (1981). *Introduction to multidimensional scaling theory, methods, and applications*. New York: Academic Press.

Sefton, M. A., Francis, I. L., & Williams, P. J. (1993). The volatile composition of Chardonnay juices: a study by flavor precursor analysis. *American Journal of Enology and Viticulture, 44*(4), 359-370.

Slaghenaufi, D., & Ugliano, M. (2018). Norisoprenoids, sesquiterpenes and terpenoids content of Valpolicella wines during aging: Investigating aroma potential in relationship to evolution of tobacco and balsamic aroma in aged wine. *Frontiers in chemistry, 6*, 66-66.

Strauss, C. R., Wilson, B., Gooley, P. R., & Williams, P. J. (1986). Role of monoterpenes in grape and wine flavor. In T. H. Parliment & R. Croteau (Eds.), *Biogeneration of Aromas*, vol. 317 (pp. 222-242): Washington, DC: American Chemical Society.

Vela, E., Hernandez-Orte, P., Franco-Luesma, E., & Ferreira, V. (2018). Micro-oxygenation does not eliminate hydrogen sulfide and mercaptans from wine; it simply shifts redox and complex-related equilibria to reversible oxidized species and complexed forms. *Food Chemistry, 243*, 222-230.

Waterhouse, A. L., Sacks, G. L., & Jeffery, D. W. (2016). Isoterpenoids. In A. L. Waterhouse, G. L. Sacks & D. W. Jeffery (Eds.), *Understanding wine chemistry*). Wiley.

Wilson, B., Strauss, C. R., & Williams, P. J. (1984). Changes in free and glycosidically bound monoterpenes in developing muscat grapes. *Journal of Agricultural and Food Chemistry, 32*(4), 919-924.

Williams, P. J., Strauss, C. R., & Wilson, B. (1980). Hydroxylated linalool derivatives as precursors of volatile monoterpenes of muscat grapes. *Journal of Agricultural and Food Chemistry, 28*(4), 766-771.

Williams, P. J., Strauss, C. R., Wilson, B., & Massy-Westropp, R. A. (1982). Novel monoterpene disaccharide glycosides of *Vitis vinifera* grapes and wines. *Phytochemistry, 21*(8), 2013-2020.

Winterhalter, P. (1991). 1,1,6-Trimethyl-1,2-dihydronaphthalene (TDN) formation in wine. I. Studies on the hydrolysis of 2,6,10,10-tetramethyl-1-oxaspiro[4.5]dec-6-ene-2,8-diol rationalizing the origin of TDN and related C13 norisoprenoids in Riesling wine. *Journal of Agricultural and Food Chemistry, 39*(10), 1825-1829.

Figure captions:

Figure 1. Dendrogram showing the classes derived from the sorting task carried out on the 35 hydrolyzates obtained from 33 PAFs (plus two replicates, marked with R_). Samples in bold are those selected for further flash profiling. Codes: u, r or o, refers to underripe, ripe or overripe; T or G, refers to Tempranillo or Grenache; R, S or D, refers to Rioja, Somontano or Duero (geographical origin); the last number refers to the specific vineyard within the region.

Figure 2. Projections of samples (Figure 2a) or of the different variables (Figures 2b, 2c and 2d) in the plane formed by the two first dimensions obtained in the generalized procruster analysis (GPA) carried out on the sensory data obtained in the Flash profiling. Only attributes cited by at least 20% on the panelist were used.

Figure 3. Projection of samples and variables in the PCA plane obtained from exclusively GC-MS quantitative data. Sensory variables are projected as illustrative variables but did not take part in the analysis. The superimposed circles delimit the clusters identified in the sensory sorting task shown in Figure 1.

***Highlights (for review)**

1. Phenolic and Aromatic Fractions (PAFs) from grapes develop strong aromas
2. 5 different sensory categories of hydrolyzates. 27 odorants detected by GCO
3. Grenache: tropical fruit, kerosene, floral. Tempranillo: toasty/woody, red-fruit
4. Excellent fitting of sensory and chemical spaces. Main odorants:
5. 3-mercaptohexanol, nor-isoprenoids, lipid derivatives, volatile phenols, terpenes

Table 1. Thirty-three different lots of grapes collected at three different moments (underripe: u. ripe: r. and overripe: o). two different varieties (Tempranillo: T and Grenache: G) and from three different regions (Ribera del Duero: D. Rioja: R and Somontano. S) and 17 different vineyard plots (D1-D4. R1-R9. S1-S4)

Codes	Ripeness state	Variety	Denomination of Origin	Vineyard plot
uTD1	underripe	Tempranillo	DO Ribera del Duero	D1
rTD1	ripe	Tempranillo	DO Ribera del Duero	D1
oTD1	overripe	Tempranillo	DO Ribera del Duero	D1
uTD2	underripe	Tempranillo	DO Ribera del Duero	D2
rTD2	ripe	Tempranillo	DO Ribera del Duero	D2
oTD2	overripe	Tempranillo	DO Ribera del Duero	D2
rTD3	ripe	Tempranillo	DO Ribera del Duero	D3
rTD4	ripe	Tempranillo	DO Ribera del Duero	D4
uTR1	underripe	Tempranillo	DOCa Rioja	R1
rTR1	ripe	Tempranillo	DOCa Rioja	R1
oTR1	overripe	Tempranillo	DOCa Rioja	R1
uTR2	underripe	Tempranillo	DOCa Rioja	R2
rTR2	ripe	Tempranillo	DOCa Rioja	R2
uGR3	underripe	Grenache	DOCa Rioja	R3
rGR3	ripe	Grenache	DOCa Rioja	R3
oGR3	overripe	Grenache	DOCa Rioja	R3
uGR4	underripe	Grenache	DOCa Rioja	R4
rGR4	ripe	Grenache	DOCa Rioja	R4
rTR5	ripe	Tempranillo	DOCa Rioja	R5
oTR5	overripe	Tempranillo	DOCa Rioja	R5
rTR6	ripe	Tempranillo	DOCa Rioja	R6
oTR6	overripe	Tempranillo	DOCa Rioja	R6
rTR7	ripe	Tempranillo	DOCa Rioja	R7
rGR8	ripe	Grenache	DOCa Rioja	R8
rGR9	ripe	Grenache	DOCa Rioja	R9
uGS1	underripe	Grenache	DO Somontano	S1
rGS1	ripe	Grenache	DO Somontano	S1
uGS2	underripe	Grenache	DO Somontano	S2
rGS2	ripe	Grenache	DO Somontano	S2
uGS3	underripe	Grenache	DO Somontano	S3
rGS3	ripe	Grenache	DO Somontano	S3
uGS4	underripe	Grenache	DO Somontano	S4
rGS4	ripe	Grenache	DO Somontano	S4

Table 2. Summary of the GC-O experiment carried out on the five PAF-derived hydrolyzates selected as representative of each of the clusters found in the sensory sorting task. Retention indexes in polar (DB-Wax) and non polar (DB-5) stationary phases, odor description, identity and GC-O scores (modified frequency in %) ranked attending to the difference between the maxima and minima scores.

RI polar	RI non polar	description	Compound*	uGS1 cluster 1	uGR3 cluster 2	oTD2 cluster 3	rTR5 cluster 4	rTR1 Cluster 5	max-min
1859	1131	Grapefruit, tropical, guava, green	3-mercaptohexanol[b]	92..8	69.7	71.4	16.7	76.1	76.1
1464	1070	Grapefruit, citrus, floral, sweet	linalool oxide[a] + dihydromyrcenol[c]	67.7	21.5	25.5	0	25.5	67.7
1012		Solvent, ketone	n.i. 1012	9.6	28.9	58.9	73.6	63.6	64
1109		Strawberry, acid, caramel, strawberry-cream	n.i. 1109	50.9	28.9	33.3	16.7	0	50.9
1675	1049	Citrus, bitter almond, green, flower, nuts, cardboard	Phenylacetaldehyde[1b]	37.3	60.9	19.2	6.8	43	54.1
1381	986	Mushroom, blood, metal, iron	*Z*-1.5-octadien-3-one[b]	0	0	26.4	40.8	50	50
1958	1488	Floral, spicy, strawberry candy, rose	β-ionone[a]	26.4	41.9	10.8	0	21.5	41.9
1593	1159	Vegetable, green, cucumber, peas, flower	*E.Z*-2.6-nonadienal[a]	60.8	19.2	49.1	62.7	53.8	43.5
1562	1095	Floral, paint, herbal, citrus	linalool[a]	31.2	44.1	0	0	6.8	44.1
1779		citrus, floral, grapefruit, fruity, sweet	n.i. 1779	32.3	48.1	16.7	6.8	6.8	41.3
2147		floral, toasted, hand cream	ethyl cinnamate[a]	13.6	43.0	9.6	13.6	0	43
1873		spices, clove, smoked, bacon	guaiacol[a]	38..2	66.7	74.5	41.9	62.7	36.3
1307	979	mushroom, humidity	1-octen-3-one[a]	58.9	57.9	70.7	36.3	49.1	34.4
2007		rubber, plastic, dust, earth	o-cresol[a]	38.5	23.6	23.6	15.2	50.5	35.3
2053	1058	caramel, strawberry candy, sugar cotton	furaneol[a]	54.9	67.7	35.4	33.3	40.8	34.4
2020		grilled meat, butter, cream, fried, rubber	y-nonalactone[1a]	31.2	45.6	9.6	20.4	13.6	36
2287	1359	barbecue, fried corn, spicy, toasted	2.6-dimethoxyphenol[a]	19.2	31.2	41.9	9.6	11.8	32.3
1822	1332	rancid, oily, toasted, spicy	*E.E*-2.4-decadienal[b]	66.7	58.9	58.9	40.8	45.1	25.9
2099	1077	stable, horses, manure, animal pee, leather	m/ρ-cresol[a]	58.9	66.3	47.1	33.3	45.1	33
1835	1388	apple compote, raspberry jam	β-damascenone[a]	88.2	88.2	90.5	65.4	82.5	25.1
1543	1165	cucumber, fatty, rancid, carmine	*E*-2-nonenal[a]	68.0	78.2	69.7	66.3	56.1	22.1
1734	1192	floral, sweet, anise, green, citrus	α-terpineol[a]	38.5	40.8	26.4	21.5	24.5	19.3
1621	1253	rancid, paper, cucumber, plastic, mat	*Z*-2-decenal[b]	56.9	60.9	60.9	47.1	66.3	19.2
1710	1224	fat, raw bread, wood, toasted, fried, wax	*E.E* -2.4-nonadienal[b]	52.7	43	50	40.8	43	11.9
2260	1484	coconut, fruity, toasted, spicy, lactic	massoia lactone[a]	68.0	86	86	79.1	80.5	18
1147	800	grass, stem, plant, green	*Z*-3-hexenal[a]	75.5	73.6	83.3	72.6	75.5	10.7
1513	1150	rancid, paper, cardboard, fatty, cucumber	*Z*-2-nonenal[a]	91.3	89.8	92.2	87.4	95	7.6

n.i.. not identified.*Reliability of the identification. [a]retention indexes, odor and mass spectrometry equal to those of the pure standard; [b]as a but Mass Spectrum could not be properly recorded; cas a but data were obtained from literature (pure standard not available). 1 indicates that a second unidentified odorant may be also present within the odor zone

Table 3. Average (±standard deviation) concentrations of compounds (expressed in $\mu g\ L^{-1}$) found in hydrolyzated reconstituted PAFs. Data are segregated attending to the sensory clusters identified by sorting task or to grape variety. F quotients found in the corresponding one-way ANOVAs. Different letters indicate significant differences between sensory clusters according to Fischer post-hoc test. The two last columns of the table Sensory thresholds and potential sensory discrimination abilities are also given as the ratios OAVmax/OAVmin with the condition OAV>1 and between brackets with the condition OAV > 0.2.

	Sensory Cluster						Variety			Sensory relevance	
	cluster 1 (5G+1T)	**cluster 2 (7G +2T)**	**cluster 3 (4T + 2 G)**	**cluster 4 (4T)**	**cluster 5 (7T + 1G)**	**F**	**Grenache (15 samples)**	**Tempranillo (18 samples)**	**F**	**Sensory threshold**	**OAVmax/ OAVmin[1]**
NORISOPRENOIDS											
β-ionone	1.16±0.05^{a}	1.57±0.09^{c}	1.41±0.13^{b}	1.15±0.19^{a}	1.56±0.10^{c}	20.6*	1.46±0.18	1.37±0.22	0.0	0.09	1.6
α-ionone	0.40±0.02^{b}	0.45±0.02^{c}	0.41±0.02^{b}	0.34±0.04^{a}	0.48±0.02^{d}	27.0*	0.43±0.04	0.43±0.06	0.0	2.6	0
β -Damascenone	25.10±0.07^{d}	30.85±1.1^{e}	21.13±2.2^{c}	11.19±1.4^{a}	17.59±1.7^{b}	153.5*	25.80±5.97^{b}	19.64±6.12^{a}	20.5*	0.05	3.3
TDN	51.59±9.6^{d}	33.78±5.4^{c}	20.07±4.4^{b}	14.32±1.3^{ab}	11.82±2.4^{a}	56.7*	30.34±11.52^{a}	23.93±17.92^{a}	17.8*	2	9.2
Riesling Acetal	0.43±0.05^{d}	0.36±0.05^{c}	0.22±0.03^{b}	0.14±0.00^{a}	0.17±0.02^{a}	67.1*	0.32±0.11^{b}	0.24±0.11^{a}	34.6*	na	n.a.
TERPENES											
β -citronellol	1.83±0.06^{d}	2.02±0.17^{e}	1.58±0.09^{c}	0.90±0.09^{a}	1.13±0.08^{b}	111.0*	1.79±0.40^{b}	1.36±0.36^{a}	32.9*	100	0
geraniol	3.56±0.20^{c}	3.90±0.90^{c}	2.54±0.63^{d}	1.00±0.05^{a}	1.13±0.12^{a}	38.2*	3.34±1.32^{b}	1.93±0.98^{a}	49.2*	20	0 (1.4)
linalool	9.56±0.95^{c}	11.29±1.3^{d}	7.37±1.6^{b}	5.52±0.58^{a}	6.27±0.77^{ab}	30.8*	10.12±2.26^{b}	6.88±1.49^{a}	73.5*	6	2.1 (2.7)
α -terpineol	30.34±2.5^{c}	27.25±5.0^{c}	15.22±6.6^{b}	3.08±0.05^{b}	5.56±1.9^{a}	57.7*	24.01±1.09^{b}	11.96±10.0^{a}	66.6*	250	0
nerol	0.94±0.06^{b}	1.12±0.20^{c}	0.83±0.11^{b}	0.00±0.00^{a}	0.00±0.00^{a}	139.4*	0.88±0.48^{b}	0.42±0.44^{a}	28.9*	300	0
1.8-cineole	1.30±0.03^{c}	1.26±0.06^{c}	1.08±0.09^{b}	1.15±0.06^{b}	1.02±0.11^{a}	16.2*	1.22±0.13^{b}	1.12±0.13^{a}	17.1*	1.1	1.2 (1.5)
r-limonene	11.50±1.8^{a}	22.80±2.3^{b}	22.22±3.6^{b}	24.26±1.1^{b}	28.45±4.5^{c}	26.3*	21.72±5.35^{a}	22.58±7.21^{a}	6.1#	15	2.5 (3.8)
linalool oxide	3.74±0.18^{c}	3.48±0.40^{c}	1.96±0.52^{b}	1.41±0.11^{a}	1.41±0.25^{a}	72.3*	3.06±0.95^{b}	2.03±0.97^{a}	48.2*	na	n.a.
LACTONES											
furaneol	11.34±25	3.00±3.8	1.14±0.31	21.09±42	3.37±8.8	1.0	9.33±21.78	4.07±14.65	0.2	5	17 (84)
massoia lactone	3.52±0.67^{a}	10.12±2.6^{b}	4.67±1.4^{a}	3.38±0.34^{a}	3.79±0.70^{a}	25.6*	7.00±4.22^{b}	4.39±1.28^{a}	7.0#	10	1.4 (5.2)
VOLATILE PHENOLS											
guaiacol	8.15±0.46^{a}	9.39±0.57^{bc}	10.27±1.4^{cd}	11.30±1.0^{d}	9.12±1.3^{ab}	7.3*	9.32±0.96	9.63±1.58	4.1	9.5	1.3 (1.7)
eugenol	0.26±0.02^{a}	0.32±0.05^{a}	0.53±0.09^{b}	0.73±0.05^{c}	0.59±0.06^{b}	63.5*	0.38±0.14^{a}	0.54±0.17^{b}	41.8*	6	0
E-isoeugenol	0.45±0.04^{a}	0.28±0.04^{a}	0.40±0.10^{b}	0.53±0.04^{b}	0.79±0.17^{c}	28.8*	0.40±0.14^{a}	0.56±0.24^{b}	8.9#	6	0
methoxyeugenol	1.52±0.19^{a}	1.94±0.49^{a}	3.26±1.1^{b}	4.60±0.64^{bc}	4.62±1.8^{c}	12.0*	2.16±0.99^{a}	3.84±1.72^{b}	34.8*	1200	0
2.6-dimethoxyphenol	64.83±2.1^{a}	78.56±3.9^{b}	97.46±8.2^{d}	120.96±4.7^{e}	88.57±5.5^{c}	82.1*	80.89±13.94^{a}	92.21±18.58^{a}	19.3*	570	0 (1.1)
m-cresol	0.47±0.02^{e}	0.42±0.04^{d}	0.26±0.02^{c}	0.13±0.00^{a}	0.18±0.02^{b}	183.7*	0.37±0.12^{b}	0.25±0.12^{a}	39.1*	68	0
o-cresol	0.59±0.03^{d}	0.54±0.02^{c}	0.44±0.02^{b}	0.33±0.02^{a}	0.44±0.02^{b}	95.3*	0.51±0.08^{b}	0.45±0.09^{a}	18.8*	31	0
4-ethylguaiacol	0.11±0.01^{b}	0.09±0.01^{a}	0.09±0.00^{a}	0.09±0.02^{a}	0.09±0.01^{a}	7.9*	0.09±0.01	0.10±0.01	0.9	33	0
4-vinylguaicol	8.40±0.72^{b}	8.61±0.72^{b}	9.95±0.56^{c}	6.17±0.96^{a}	6.74±0.60^{a}	27.2*	8.46±1.31^{a}	7.74±1.50^{a}	7.3#	40	0 (1.4)
4-vinylphenol	102.73±14^{a}	91.41±18^{a}	256.52±82^{c}	191.81±14^{b}	187.04±27^{b}	20.7*	115.85±43.41^{a}	194.66±74.26^{b}	26.0*	180	2.1 (6.0)
VANILLIN DERIVATIVES											
acetovanillone	23.19±1.9^{c}	26.44±2.8^{d}	20.03±2.9^{b}	14.08±0.77^{a}	17.45±2.5^{b}	24.3*	24.03±4.66^{b}	18.49±3.51^{a}	48.0*	1000	0
vanillin	92.71±7.2^{d}	98.22±9.8^{d}	76.55±11^{c}	45.47±3.3^{a}	57.48±8.7^{b}	40.9*	89.43±19.96^{b}	66.65±16.96^{a}	51.8*	995	0
syringaldehyde	178.17±2.0^{c}	256.64±27^{d}	200.23±36^{c}	64.55±20^{a}	116.95±15^{b}	63.9*	210.28±68.02^{b}	145.54±57.32^{a}	20.7*	50000	0
MISCELLANEOUS											
ethyl cinnamate	0.12±0.00^{c}	0.14±0.02^{d}	0.09±0.02^{b}	0.18±0.01^{e}	0.05±0.00^{a}	63.0*	0.12±0.04	0.10±0.05	2.3	1.1	0
ethyl 2-hydroxy-4-methylpentanoate	0.06±0.01^{bc}	0.05±0.01^{ab}	0.04±0.00^{ab}	0.07±0.03^{c}	0.04±0.01^{a}	4.8#	0.05±0.02	0.05±0.01	0.0	51	0

*Significant at P<0.0005; #Significant at P<0.05 na: not available

0

Figure(s)
Click here to download Figure(s): Figures.docx

Figure 1.

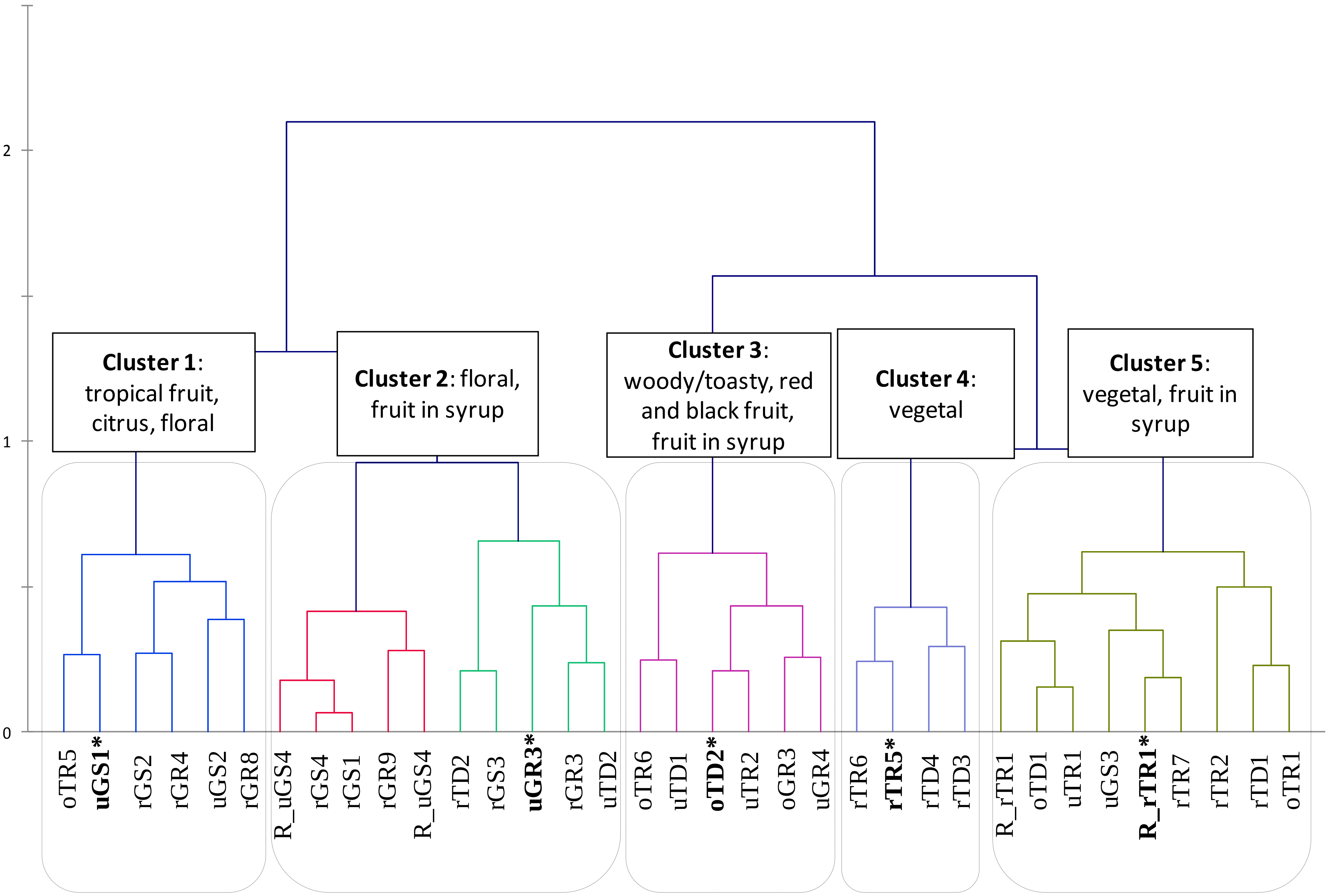
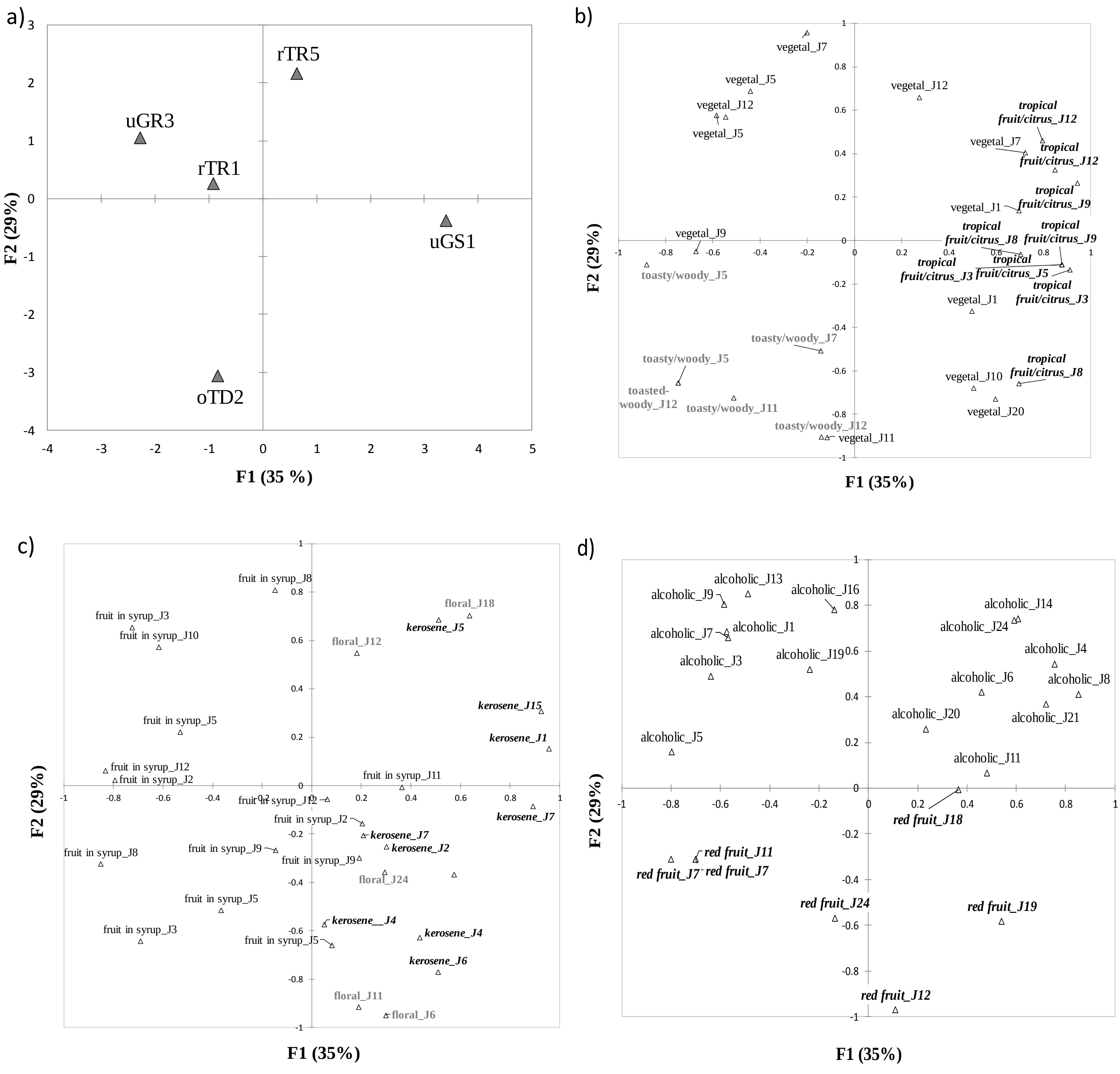
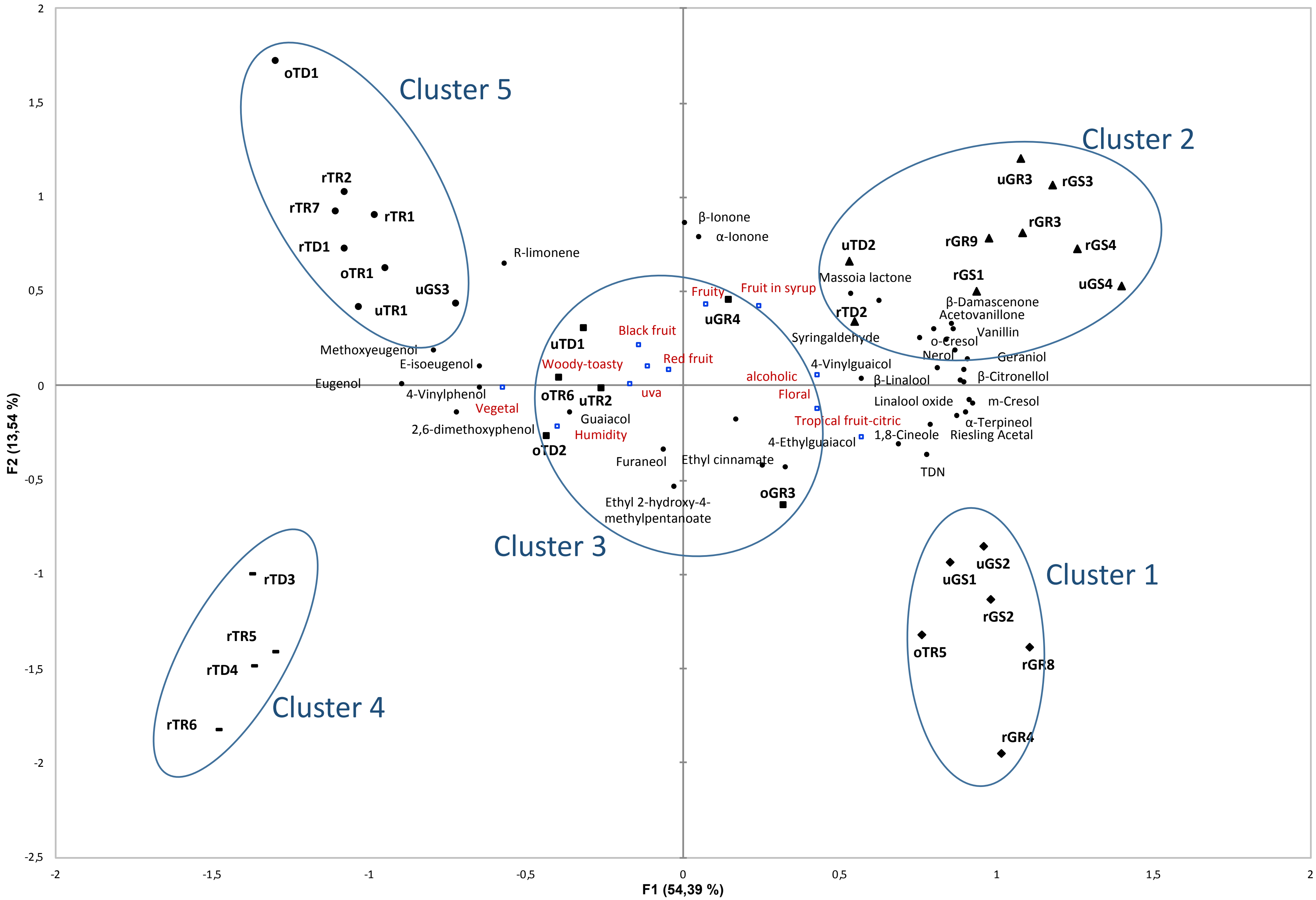


Figure 2.



a)
rTR5
uGR3
rTR1
uGS1
oTD2
F1 (35 %)
F2 (29%)
b)
vegetal_J7
vegetal_J5
vegetal_J12
vegetal_J5
vegetal_J12
tropical fruit/citrus_J12
vegetal_J7
tropical fruit/citrus_J12
tropical fruit/citrus_J9
vegetal_J1
vegetal_J9
tropical fruit/citrus_J8
tropical fruit/citrus_J9
toasty/woody_J5
tropical fruit/citrus_J3
tropical fruit/citrus_J5
tropical fruit/citrus_J3
vegetal_J1
toasty/woody_J7
toasty/woody_J5
tropical fruit/citrus_J8
vegetal_J10
toasted-woody_J12
toasty/woody_J11
vegetal_J20
toasty/woody_J12
vegetal_J11
F1 (35%)
F2 (29%)
c)
fruit in syrup_J8
floral_J18
fruit in syrup_J3
kerosene_J5
fruit in syrup_J10
floral_J12
kerosene_J15
fruit in syrup_J5
kerosene_J1
fruit in syrup_J12
fruit in syrup_J2
fruit in syrup_J11
fruit in syrup_J12
kerosene_J7
fruit in syrup_J2
kerosene_J7
fruit in syrup_J8
fruit in syrup_J9
kerosene_J2
fruit in syrup_J9
floral_J24
fruit in syrup_J5
kerosene__J4
fruit in syrup_J3
kerosene_J4
fruit in syrup_J5
kerosene_J6
floral_J11
floral_J6
F1 (35%)
F2 (29%)
d)
alcoholic_J13
alcoholic_J9
alcoholic_J16
alcoholic_J14
alcoholic_J7
alcoholic_J1
alcoholic_J24
alcoholic_J4
alcoholic_J3
alcoholic_J19
alcoholic_J6
alcoholic_J8
alcoholic_J20
alcoholic_J21
alcoholic_J5
alcoholic_J11
red fruit_J18
red fruit_J11
red fruit_J7
red fruit_J7
red fruit_J24
red fruit_J19
red fruit_J12
F1 (35%)
F2 (29%)

Figure 3



Cluster 5
Cluster 2
Cluster 3
Cluster 4
Cluster 1
oTD1
rTR2
rTR7
rTR1
rTD1
oTR1
uGS3
uTR1
R-limonene
β-Ionone
α-Ionone
uGR3
rGS3
rGR3
rGR9
rGS4
uTD2
Massoia lactone
rGS1
uGS4
β-Damascenone
Acetovanillone
rTD2
Fruity
Fruit in syrup
uGR4
Black fruit
Red fruit
Woody-toasty
uTD1
Syringaldehyde
o-Cresol
Vanillin
Nerol
Geraniol
4-Vinylguaicol
β-Linalool
β-Citronellol
alcoholic
Floral
Methoxyeugenol
E-isoeugenol
Eugenol
4-Vinylphenol
Vegetal
oTR6
uTR2
uva
Guaiacol
Humidity
2,6-dimethoxyphenol
oTD2
Linalool oxide
m-Cresol
Tropical fruit-citric
α-Terpineol
1,8-Cineole
Riesling Acetal
4-Ethylguaiacol
Furaneol
Ethyl cinnamate
TDN
Ethyl 2-hydroxy-4-methylpentanoate
oGR3
uGS2
uGS1
rGS2
oTR5
rGR8
rGR4
rTD3
rTR5
rTD4
rTR6
F1 (54,39 %)
F2 (13,54 %)
2
1,5
1
0,5
0
-0,5
-1
-1,5
-2
-2,5

Supplementary Material
Click here to download Supplementary Material: supporting information.docx

Supporting Information for

Aroma potential of Grenache and Tempranillo grapes

Yohanna Alegre, María-Pilar Sáenz-Navajas, Purificación Hernández-Orte and Vicente Ferreira*

Laboratory for Aroma Analysis and Enology (LAAE), Department of Analytical Chemistry, Universidad de Zaragoza, Instituto Agroalimentario de Aragón (IA2) (UNIZAR-CITA), c/Pedro Cerbuna 12, 50009 Zaragoza, Spain

*corresponding author: Vicente Ferreira (vferre@unizar.es); Tel: 34 976 762067

Table of Contents

Table A.1.

descriptor	odor references
alcohol	solution of 15% (v/v) absolute ethanol in water
dried fruit, fruit in syrup	β-damascenone (0.05 µg L^{-1}) + methional (0.5 µg L^{-1}) + phenylacetaldehyde (1 µg L^{-1}) + furfural (14.1 mg L^{-1})
fresh fruit *(tropical fruit, citrus)*	mercaptohexyl acetate (25 ng L^{-1}), 3-mercaptohexanol (60 ng L^{-1})
black fruit *(blackberry, blueberry)*	pool ethyl esters+β-ionone (0.09 µg L^{-1}) + 4-methyl-4-mercaptopentanone (0.8 ng L^{-1})
red fruit *(strawberry, raspberry)*	γ-decalactone (10 µg L^{-1}) + furaneol (5 µg L^{-1})
nuts *(almond, wallnut)**	reference nº 50 of *Nez du vin*
floral *(white flowers, acacia)*	linalool (25 µg L^{-1}) + ethyl cinnamate (1.1 µg L^{-1}) + phenylethyl acetate (250 µg L^{-1})
vegetal-herbaceous *(cut grass, green pepper)*	3-isobutyl-2-metoxipyrazine (2 ng L^{-1}); *Z*-3-hexenal (0.25 µg L^{-1})
vegetal-dried herbs *(hay, tobacco)**	reference nº 50 of *Aromabar of wine scents (premium edition)*
methol-balsamic	1,8-cineole
lactic *(yoghurt, cheese, cream)*	diacetyl (100 µg L^{-1})
toasted *(caramel, roasted coffee)*	furfurylthiol (0.4 ng L^{-1}) + furaneol (5 µg L^{-1}); benzylmercaptan (0.3 µg L^{-1}) + acetylpyrazine (62 µg L^{-1})
animal *(leather, broth)*	4-ethylphenol (35 µg L^{-1})
kerosene	1,1,6-trimethyl-1,2-dihydronaftalen (TDN) (2 µg L^{-1})
moldy	1-octen-3-one (15 ng L^{-1})
oxidation *(backed potato, honey, rotten apple)*	acetaldehyde (500 µg L^{-1}) + methional (0.5 µg L^{-1}) + phenylacetaldehyde (1 µg L^{-1})

*references obtained from commercial aroma kits.

Table A.2.

Compounds	RT	m/z
NORISOPRENOIDS		
β-ionone	74.27	177[a] , 192
α-ionone	69.67	121[a], 93, 192
β-damascenone	67.89	69[a], 19
TDN	63.45	157[a], 142, 172
riesling acetal[*1]	57.05	138[a], 125, 133
TERPENOIDS		
β-citronellol	65.51	69[a], 81, 123
geraniol	69.90	69[a], 123
linalool	52.43	71[a], 93, 121
α-terpineol	61.00	93[a], 121, 136
nerol	67.30	93[a], 68
1,8-cineole	20.96	108[a], 81
r-limonene	20.81	93[a], 67
Cis-linalool oxide	44.50	94[a], 59, 111
Trans-linalool oxide	46.65	94[a], 59, 111
LACTONES		
furaneol	78.98	57[a], 128, 85
massoia lactone	88.50	97[a], 68
VOLATILE PHENOLS		
guaiacol	70.32	109[a], 124
eugenol	85.49	164[a], 149
E-isoeugenol	93.58	164[a], 149
methoxyeugenol	101.67	194[a], 119
2,6-dimethoxyphenol	90.05	154[a], 139
m-cresol	81.98	108[a], 79
o-cresol	77.83	108[a], 79
4-ethylguaiacol	79.00	137[a], 152
4-vinylguaicol	86.77	150[a], 135
4-vinylphenol	95.57	120[a], 91
VANILLIN DERIVATIVES		
acetovanillone	105.43	166[a], 123
vanillin	402.46	155[a], 152, 123
syringaldehyde	120.38	182[a], 181, 167
MISCELLANEOUS		
ethyl cinnamate	83.76	131[a], 176
ethyl 2-hydroxy-4-methylpentanoate	51.93	87[a], 69

[a]Quantitative fragments m/z
*Compounds tentatively quantified using alkanes to determine the retention index
[1]relative area

Table A.3.

	Cluster 1: Tropical fruit-citrus, floral						Cluster 2: Floral, fruit in syrup								
	rGR4	oTR5	rGR8	uGS2	uGS1	rGS2	uGS4	rGS4	rGS1	rGR9	rTD2	rGS3	uGR3	rGR3	uTD2
NORISOPRENOIDS															
β-ionone	1.15	1.11	1.15	1.24	1.21	1.12	1.45	1.51	1.46	1.60	1.45	1.64	1.65	1.66	1.65
α-ionone	0.38	0.42	0.40	0.41	0.41	0.39	0.42	0.46	0.46	0.44	0.44	0.48	0.49	0.43	0.43
β-damascenone	24.60	25.84	25.85	24.07	25.20	25.04	32.43	32.26	29.39	31.30	30.27	30.18	29.92	31.79	30.08
TDN	55.46	49.97	68.96	42.71	44.67	47.79	41.48	32.80	32.38	32.14	25.42	32.28	31.28	32.90	43.30
riesling acetal[1]	0.42	0.38	0.53	0.42	0.40	0.45	0.48	0.41	0.36	0.34	0.31	0.36	0.34	0.33	0.32
TERPENOIDS															
β-citronellol	1.84	1.73	1.78	1.87	1.89	1.83	2.19	2.07	1.93	1.95	1.79	2.13	2.09	2.25	1.79
geraniol	3.69	3.28	3.72	3.50	3.39	3.80	4.82	4.51	3.12	3.93	2.77	3.69	3.91	5.43	2.86
linalool	9.21	8.23	9.48	11.11	9.96	9.40	12.38	11.96	11.37	11.52	8.92	12.15	12.02	12.20	9.09
α-terpineol	31.09	26.42	33.49	29.78	29.18	32.07	34.16	31.84	25.47	26.58	19.74	28.63	28.00	30.86	19.93
nerol	0.91	0.90	1.05	0.91	0.96	0.94	1.33	1.24	0.97	1.19	0.76	1.15	1.21	1.34	0.92
1,8-cineole	1.30	1.27	1.29	1.35	1.30	1.30	1.33	1.34	1.32	1.26	1.25	1.27	1.25	1.21	1.14
r-limonene	10.61	10.15	9.89	14.05	13.38	10.93	21.91	22.30	21.08	22.81	18.09	23.47	25.52	25.32	24.72
linalool oxide	3.81	3.65	4.07	3.65	3.70	3.56	4.11	3.76	3.62	3.40	2.88	3.64	3.43	3.56	2.88
LACTONES															
furaneol	62.70	0.93	1.03	1.36	1.15	0.87	1.15	1.48	1.17	8.18	0.63	1.00	10.87	0.97	1.56
massoia lactone	4.49	4.17	3.13	2.74	3.14	3.44	8.45	10.42	8.81	12.04	7.70	13.26	14.35	9.68	6.35
VOLATILE PHENOLS															
guaiacol	8.27	7.92	7.35	8.65	8.31	8.41	9.55	9.30	9.00	8.89	10.01	10.37	8.93	9.77	8.73
eugenol	0.26	0.28	0.24	0.27	0.24	0.28	0.33	0.28	0.25	0.32	0.35	0.28	0.30	0.40	0.37
E-isoeugenol	0.44	0.42	0.41	0.51	0.44	0.50	0.36	0.31	0.24	0.26	0.26	0.30	0.24	0.26	0.25
methoxyeugenol	1.57	1.88	1.44	1.40	1.37	1.44	1.67	1.70	2.10	1.63	3.14	1.94	1.57	1.62	2.09
2,6-dimethoxyphenol	66.70	66.21	61.62	64.36	66.76	63.32	79.43	76.24	74.26	75.16	85.44	81.66	73.84	80.90	80.12
m-cresol	0.49	0.45	0.46	0.49	0.46	0.49	0.47	0.47	0.42	0.40	0.39	0.46	0.41	0.42	0.34
o-cresol	0.61	0.58	0.53	0.59	0.62	0.60	0.53	0.54	0.52	0.53	0.52	0.52	0.58	0.58	0.57
4-ethylguaiacol	0.11	0.12	0.11	0.12	0.11	0.11	0.09	0.10	0.10	0.09	0.09	0.09	0.10	0.09	0.09
4-vinylguaicol	8.07	7.51	9.12	8.86	7.74	9.14	9.11	8.32	8.12	8.96	7.70	8.81	7.64	9.87	8.94
4-vinylphenol	111.83	116.96	111.64	79.22	93.18	103.52	89.99	89.95	102.22	86.13	126.37	97.26	74.97	62.25	93.51
VANILLIN DERIVATIVES															
acetovanillone	22.58	20.03	23.83	25.59	22.56	24.52	29.64	27.27	26.27	25.16	24.96	31.47	26.69	24.65	21.81
vanillin	96.80	81.33	86.80	99.04	93.64	98.65	108.58	101.87	100.14	98.83	89.64	114.86	96.26	90.80	82.98
syringaldehyde	179.54	175.95	178.61	178.99	180.46	175.49	288.44	276.75	270.31	253.79	244.67	291.87	236.33	211.10	236.48
MISCELLANEOUS															
ethyl cinnamate	0.12	0.11	0.11	0.12	0.12	0.12	0.11	0.13	0.12	0.17	0.10	0.16	0.15	0.15	0.14
ethyl 2-hydroxy-4-methylpentanoate	0.08	0.06	0.05	0.05	0.05	0.05	0.04	0.05	0.05	0.05	0.05	0.05	0.04	0.05	0.04

Table A.3. contd.

	Cluster 3: Toasted-woody, red fruit, black fruit, fruit in syrup						Cluster 4: Vegetal				Cluster 5: Vegetal, fruit in syrup							
	oTR6	**oTD2**	**uTR2**	**uTD1**	**oGR3**	**uGR4**	**rTD4**	**rTD3**	**rTR5**	**rTR6**	**oTD1**	**rTR1**	**oTR1**	**uGS3**	**uTR1**	**rTD1**	**rTR2**	**rTR7**
NORISOPRENOIDS																		
β-ionone	1.44	1.30	1.41	1.49	1.22	1.58	1.05	1.07	1.44	1.05	1.66	1.47	1.43	1.52	1.48	1.57	1.69	1.64
α-ionone	0.42	0.43	0.45	0.41	0.38	0.40	0.29	0.36	0.38	0.33	0.51	0.49	0.46	0.45	0.50	0.47	0.48	0.47
β-damascenone	17.52	20.89	19.62	22.93	23.17	22.64	10.92	11.15	13.02	9.68	17.05	17.00	16.50	17.38	15.10	19.17	17.94	20.62
TDN	18.86	24.26	16.82	14.76	26.44	19.31	15.54	14.93	14.23	12.59	7.52	9.58	13.50	13.30	13.39	11.42	14.70	11.15
riesling acetal[1]	0.23	0.24	0.19	0.19	0.22	0.26	0.15	0.15	0.14	0.14	0.13	0.17	0.19	0.19	0.18	0.16	0.18	0.15
TERPENOIDS																		
β-citronellol	1.47	1.55	1.59	1.61	1.73	1.52	0.83	0.96	1.00	0.82	1.08	1.19	1.27	1.20	1.10	1.13	1.04	1.03
geraniol	2.10	2.15	2.19	2.11	3.30	3.41	0.98	0.95	1.01	1.06	0.98	1.08	1.26	1.21	1.27	1.00	1.05	1.18
linalool	6.57	5.83	7.22	6.17	8.54	9.91	5.96	5.72	5.74	4.68	5.68	6.71	7.22	7.53	5.70	5.97	5.71	5.66
α-terpineol	10.24	11.36	12.69	11.06	26.90	19.03	3.01	3.11	3.11	3.07	2.78	5.36	6.32	9.43	5.55	5.03	5.03	5.02
nerol	0.77	0.70	0.84	0.75	0.98	0.93	< D.L	< D.L	< D.L	< D.L	< D.L	< D.L	< D.L	< D.L	< D.L	< D.L	< D.L	< D.L
1.8-cineole	1.00	1.05	1.19	1.16	1.13	0.96	1.18	1.22	1.15	1.07	0.97	1.10	1.16	1.16	1.04	0.93	0.91	0.88
r-limonene	25.79	21.11	20.29	20.31	18.36	27.47	24.07	23.40	25.92	23.64	37.23	25.69	24.11	23.94	25.68	29.30	30.81	30.82
linalool oxide	1.74	1.83	1.74	1.53	2.99	1.97	1.58	1.36	1.35	1.34	0.90	1.45	1.52	1.78	1.52	1.32	1.49	1.30
LACTONES																		
furaneol	1.06	0.86	1.55	1.09	1.46	0.79	< D.L	< D.L	84.35	< D.L	< D.L	1.90	< D.L	25.09	< D.L	< D.L	< D.L	< D.L
massoia lactone	6.50	4.10	3.86	4.60	2.92	6.01	3.13	3.14	3.39	3.85	4.33	5.21	3.63	3.15	3.68	3.15	3.83	3.32
VOLATILE PHENOLS																		
guaiacol	12.15	10.05	9.99	9.38	11.58	8.48	12.31	10.73	10.12	12.04	7.81	9.76	11.19	8.18	8.10	10.29	9.58	8.04
eugenol	0.63	0.60	0.53	0.60	0.41	0.42	0.81	0.72	0.70	0.70	0.70	0.61	0.59	0.53	0.63	0.62	0.51	0.56
e-isoeugenol	0.39	0.33	0.59	0.43	0.33	0.36	0.58	0.53	0.48	0.51	0.69	1.13	0.93	0.68	0.82	0.65	0.77	0.62
methoxyeugenol	3.73	3.48	3.23	4.92	2.15	2.06	5.49	4.63	4.27	4.02	8.64	5.58	4.53	3.10	3.63	4.43	3.52	3.50
2.6-dimethoxyphenol	112.05	99.97	91.82	96.84	95.43	88.67	126.87	116.17	118.25	122.56	80.10	86.97	98.14	83.60	86.82	91.50	90.35	91.09
m-cresol	0.24	0.23	0.24	0.26	0.29	0.28	0.13	0.13	0.13	0.13	0.17	0.20	0.20	0.21	0.17	0.19	0.17	0.17
o-cresol	0.45	0.41	0.45	0.43	0.42	0.46	0.32	0.36	0.32	0.30	0.44	0.48	0.42	0.43	0.40	0.46	0.42	0.44
4-ethylguaiacol	0.09	0.09	0.09	0.09	0.09	0.09	0.11	0.08	0.07	0.10	0.08	0.09	0.09	0.07	0.11	0.09	0.10	0.09
4-vinylguaicol	10.14	9.56	9.78	9.62	11.01	9.60	7.60	5.75	5.85	5.49	6.50	7.33	7.17	6.84	6.95	7.07	6.66	5.42
4-vinylphenol	328.92	370.30	241.06	261.42	163.18	174.24	203.56	176.02	184.61	203.07	225.21	210.63	153.26	144.66	185.03	192.43	198.81	186.32
VANILLIN DERIVATIVES																		
acetovanillone	20.39	17.28	18.54	17.18	23.84	22.97	15.14	13.91	13.31	13.95	20.35	19.81	18.87	19.64	15.39	16.81	14.62	14.13
vanillin	81.61	62.95	72.64	67.04	88.45	86.59	46.97	41.79	43.77	49.35	49.28	67.86	64.15	68.16	57.04	51.79	56.99	44.55
syringaldehyde	220.89	165.73	165.87	172.53	243.45	232.94	53.97	49.98	59.79	94.45	107.46	129.22	135.09	139.23	102.68	115.32	104.49	102.13
MISCELLANEOUS																		
ethyl cinnamate	0.09	0.09	0.08	0.08	0.13	0.08	0.19	0.18	0.19	0.18	0.06	0.06	0.06	0.05	0.05	0.05	0.06	0.05
ethyl 2-hydroxy-4-methylpentanoate	0.05	0.04	0.04	0.05	0.04	0.05	0.05	0.05	0.12	0.06	0.04	0.04	0.04	0.05	0.03	0.03	0.04	0.03

[1]relative area; < D.L. under detection limit.

*Declaration of Interest Statement

Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: