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Impact of two yeast strains on Tempranillo red wine aroma profiles throughout accelerated ageing

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ABSTRACT

This study aimed at determining the changes induced by two *S. cerevisiae* strains, (IONYS wf™ and Lalvin ICV D254™) on the sensory and chemical aroma profiles of Tempranillo wine, after fermentation and after ageing. The 64 aroma molecules determined were grouped attending to sensory and chemical similarity into 17 aroma vectors. Sensory studies included a sorting task and a descriptive analysis by flash profile with a trained panel. Results revealed that, even if ageing is the dominant factor, the strain of yeast introduces significant and consistent differences, both in sensory and aroma vector profiles (11 out 17 affected). Wines made with D254 contained higher levels of ethyl esters, acetic acid, cinnamates and ethyl acetate and lower levels of linear fatty acids, β-damascenone, acetaldehyde, higher alcohols and lactones than those made with IONYS. The first profile was related to black and fresh fruit notes, while the second to white and compote fruits.

Supplementary data can be downloaded through: https://oeno-one.eu/article/view/4732

INTRODUCTION

The use of commercial *Saccharomyces cerevisiae* strains in wineries to achieve alcoholic fermentation is very common nowadays. Apart from facilitating fermentation monitoring and control, it also permits the modulation of wine styles, particularly through modifications of its aroma profile (Pretorius and Bauer, 2002). Although the number of volatile molecules which can be a part of the volatile fraction of wines is very large, it has been suggested that around 70 odour chemicals are those playing major roles in the aromatic properties of wines (De-la-Fuente-Blanco *et al.*, 2020). Most of these compounds can be efficiently modulated by *S. cerevisiae* yeasts since they are fermentation by-products or compounds derived from grape specific precursors (Swiegers *et al.*, 2005).

The most abundant volatile compounds take part in the fermentative aroma profile of wines: higher alcohols, acids, ethyl and acetate esters. They are derived from the transformation of basic nutrients of must through amino acid and fatty acid metabolisms of yeasts. The modulation of the levels of these compounds and, particularly, of the ethyl esters of short and branched acids by the strain in charge of fermentation leads to sensory differences. A higher production of these compounds is supposed to improve the fruity characteristics of wines (Molina *et al.*, 2009).

On the other hand, the profile of the wine varietal aroma is composed of volatiles present at lower concentrations and derived from grape specific precursors. For example, cinnamates, vanillin derivatives, terpenes and lactones participate in the perception of floral notes in wines, even at sub and peri-thresholds levels (Loscos *et al.*, 2007).

Ageing is responsible for important changes in wine aroma profile notably through acid hydrolysis, chemical rearrangements of unstable molecules and esterification processes (Waterhouse *et al.*, 2016). Levels of some fermentative compounds, such as higher alcohols or fatty acids, are usually little affected during bottle ageing, while levels of others can be greatly modified, particularly those ethyl esters of branched short-chain fatty acids and the acetates of higher alcohols. The latter strongly decrease, while the former slowly increase during ageing (Antalick *et al.*, 2014; Cassino *et al.*, 2019; Díaz-Maroto *et al.*, 2005; Makhotkina and Kilmartin, 2012; Marais and Pool, 1980; Ramey and Ough, 1980). Concerning varietal aroma profile, changes

in the profile of terpenoids, sesquiterpenes and nor-isoprenoids have been reported (Simpson and Miller, 1983). Wine ageing is crucial for the apparition and accumulation of some varietal compounds such as TDN (1,1,6-trimethyl-1,2-dihydronaphthalene), responsible for the kerosene off-odour (Rapp *et al.*, 1985; Slaghenaufi and Ugliano, 2018).

However, few studies have been dedicated to studying the sensory impact of yeasts after some time of ageing. Among those, King *et al.* (2011) evidenced a strong sensory impact of seven *S. cerevisiae* yeasts on the aroma of Sauvignon blanc, including after three years of bottle ageing. Long time periods are required to carry out this type of study, which represents an important inconvenience. Accelerated ageing strategies have consequently been employed, being thermal treatment among the most commonly used (Francis *et al.*, 1994; Simpson, 1978; Singleton *et al.*, 1964; Slaghenaufi and Ugliano, 2018). More recently, accelerated ageing was applied in the total absence of oxygen, including sample preparation in an anoxia chamber. Wine ageing during five weeks at 50 ºC in strict anoxia would be roughly equivalent to one year of bottle ageing (Vela *et al.*, 2017). This process has been successfully applied to demonstrate that fermentative and varietal wine aroma profiles could be efficiently modulated between *Saccharomyces*, *Pichia*, *Torulaspora* and *Lachancea* (Oliveira and Ferreira, 2019) and *S. cerevisiae* strain levels (Denat *et al.*, 2021); ageing was crucial for the appreciation of some of these changes.

In the latter study, the volatiles production capacity of 10 *S. cerevisiae* strains was screened in semi-synthetic must of Tempranillo (supplemented with natural aroma precursors and polyphenols extracted from Tempranillo grapes). It resulted in yeasts separation into three clusters according to their volatiles production. The strains IONYS wf™ and Lalvin ICV D254™ belonged to two different clusters. Globally, the latter produced medium quantities of most volatile compounds and higher levels of ethyl esters of branched acids (ethyl isobutyrate and isovalerate); while IONYS was characterised by a maxima production of most volatiles, particularly of lineal ethyl esters (ethyl propanoate, butyrate, hexanoate, decanoate), acetates from higher alcohols (isobutyl, isoamyl, β-phenylethyl acetates), lactones (γ-butyro, octa, nona and decalactone), volatile phenols (guaiacol, 4-vinylguaiacol), terpenes (geraniol, linalool),

ethyl leucate, dihydrocinnamate and very low production of acetic acid.

In this context, the objectives of the present work are to evaluate the impact of these two strains on the sensory characteristics of Tempranillo wines, to assess whether those strain-related sensory characteristics are consistently kept during ageing, and to elucidate the chemical changes in aroma composition potentially responsible for those aroma sensory properties.

MATERIALS AND METHODS

1. Wine elaboration

1.1. Must preparation

Fermentations were carried out in Lallemand Bio experimental winery (Logroño, Spain). Twenty kilograms of Tempranillo grapes (D.O. Ca. Rioja, 2019 vintage) were harvested, destemmed and manually crushed. Potassium metabisulfite was added at 3 g/hL and Yeast Assimilable Nitrogen (YAN) was supplemented by the addition of 160 mg N/L of Nutrient Vit™ (Lallemand S.L., Barcelona, Spain). The must was divided into three-kilogram batches and homogeneously distributed into 5-L fermenter jars.

1.2. Yeast strains

The two *S. cerevisiae* active dry yeast (ADY) strains, IONYS wf™ (IONYS) and Lalvin ICV D254™ (D254) from Lallemand Bio, were rehydrated and added to the must. ADY rehydration (25 g/hL of must) was performed following the recommendations provided by the supplier: during 20 min in water (40 °C) previously added and homogenised with GO-FERM PROTECT™ (30 g/hL), which is a stimulant and protector agent. During the fermentation, viable yeasts were counted every two days by plating diluted fermentation media on WL agar plates added with antibiotics and incubated at 30 °C for 48 hours (Rollero *et al.*, 2018).

1.3. Vinification, wine ageing and samples

Fermentations were performed in triplicates at 19–22 °C. At the end of alcoholic fermentation (reducing sugars < 1 g/L), potassium metabisulfite (40 mg/L of SO_2), Bactiless™ (20 g/hL, Lallemand Bio) and Gecoll Supra® (40 mL/hL, Laffort, France) were added and wines were decanted for 48 hours. Before bottling, wines were further supplemented with potassium metabisulfite (15 mg/L of SO_2). A volume of

approximately 3 L of wine was recovered for each replicate. Wines were then submitted to a process of accelerated ageing for 5 weeks at 50 °C (Oliveira and Ferreira, 2019). A volume of 1.4 L of each replicate was conditioned into a free-O_2 chamber (Jacomex, Dagneux, France), divided into two 720-mL glass containers, closed with screw caps and bagged in high-density plastic bags containing oxygen scavengers AnaeroGenTM (Thermo Scientific, USA). Samples were vertically maintained into the incubator oven so that wine was not in contact with the screw cap and was separated by approximately 20 mL of Argon headspace.

A total of 12 wine samples were generated: six young wines recently fermented with two yeast strains (I: IONYS, D: D254) in triplicate (I-t1a, I-t1b, I-t1c; D-t1a, D-t1b, D-t1c) and the corresponding six wines after accelerated ageing (I-t2a, I-t2b, I-t2c; D-t2a, D-t2b, D-t2c). Each sample manipulation was carried out in strict anoxia. After opening, samples were transferred and kept at 4 °C in 750, 500 and 350-mL green glass bottles closed with a vacuum wine stopper (Vacu Vin, Spain). These samples were chemically and sensory characterised with the methods described in the following sections.

1.4. Conventional oenological analysis

Conventional oenological parameters of grape juice and wines recently fermented were analysed using the following methodologies: glucose and fructose, YAN, free ammonia nitrogen (FAN), lactic and malic acid and acetaldehyde were analysed by enzymatic kits using a Y15 Biosystems auto-analyser (Barcelona, Spain). Total and volatile acidity was determined by potentiometric titration. Free and total sulphur dioxide were analysed by colorimetry and alcohol content was analysed by NIR spectrometry. Fermentations were monitored by daily measurements of glucose and fructose (International Organisation of Vine and Wine, 2019).

2. Chemical characterisation of the volatile composition

2.1. Reagents and standards

Internal standards used for major volatile compounds analysis were 2-butanol (≥ 99 %), 4-methyl-2-pentanol (99 %), 4-hydroxy-4-methyl-2-pentanone (99 %), ethyl heptanoate (99 %) and heptanoic acid (99 %). Internal standards used for trace volatile compounds analysis were 2-octanol (99.5 %), 3-octanone

(99 %) and 3,4-dimethylphenol (99 %). All the chemical standards used in this study (> 98 %) were purchased from Merck, except TDN (80 %), which was synthesised by Synchem UG&Co (Felsberg, Germany). Dichloromethane (DCM) and methanol (≥ 99 %) were supplied by Merck (Darmstadt, Germany).

2.2. Quantification methods

Major volatile compounds (higher alcohols, acetates and ethyl esters, volatile fatty acids – in concentrations from 10 to 200 mg/L) were analysed according to the method described by Ortega, López, Cacho, and Ferreira (2001). It consists of a liquid–liquid micro-extraction with 250 µL of dichloromethane, of 3 mL of sample previously spiked with internal standards and diluted in 7 mL of MiliQ water, added with 4.1 g of $(NH_4)_2SO_4$. Two microliters of this extract were injected at 250 °C in split mode (split-flow: 30 mL/min, split ratio: 1/20), in a gas-chromatograph equipped with a flame ionization detector (FID). The separation was carried out using a DB-20 column (50 m × 0.32 mm, 0.5 mm of film thickness) from J&W Scientific (Folsom, CA, USA), preceded by an uncoated pre-column (2 m × 0.53 mm). The temperature program started at 40 °C for 5 min, raised at 4 °C/min up to 120 °C, at 2 °C/min up to 112 °C, at 3 °C/min up to 125 °C, hold for 5 min, raised at 3 °C/min up to 160 °C and at 6 °C/min up to 200 °C and hold for 30 min. The carrier gas was H_2 at 2.2 mL/min. The detector temperature was set at 250 °C.

To analyse trace volatile compounds (acetate and ethyl esters, vanillin derivatives, volatile phenols, terpenes, norisoprenoids and lactones -in concentrations from 0.1 to 1000 µg/L), solid-phase extraction (SPE) was performed as detailed in the method initially proposed by López *et al.* (2002) and modified by Oliveira and Ferreira, (2019). Fifteen millilitres of sample previously spiked with internal standards were passed through a pre-conditioned 65 mg LiChrolut SPE cartridge, which was then washed, dried and eluted with 0.6 mL of DCM containing 5 % of MeOH (v/v). Quantification of trace compounds was carried out on a QP2010 gas chromatograph equipped with a Shimadzu (Japan) quadrupole mass spectrometer detector, following the method described by Oliveira and Ferreira, 2019. Two microliters of the extract were injected at 250 °C in splitless mode (flow: 4.5 mL/min). The separation was carried out using a DB-WAXetr column (30 m × 0.25 mm, 0.5 µm film thickness) from Agilent (USA), preceded by a 2 m × 0.25 mm

medium-polar uncoated pre-column. Carrier gas was He at 1.26 mL/min. The chromatographic column was firstly set at 40 °C for 5 min, it was then raised at 1 °C/min up to 65 °C, at 2 °C up to 220 °C and hold for 50 min. Ion source and interface temperature were set at 220 °C and 230 °C. MS was set in single ion monitoring mode (SIM).

Concentrations of major and trace compounds were obtained from the relative response factor of each compound related to its corresponding internal standard, obtained from the analysis of a spiked synthetic wine as described in Ortega *et al.* (2001) and Oliveira and Ferreira (2019). As commercial standards were not available for vitispirane and Riesling acetal, they are expressed as relative area.

3. Sensory analysis

3.1. Experimental conditions

The twelve samples were encoded with random 3-digit numbers. Ten-mL samples were presented in normalised (German Institute for Normalization, DIN) dark wine glasses (Sensus, Schott Zwiesel, Germany) covered with Petri dishes and served at room temperature in individual booths. Samples were presented in a randomised order, different for each participant and were only evaluated orthonasally. Sensory evaluation was carried out 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 and were not paid for their participation.

Wines were evaluated following two different sensory strategies during three sessions held on two different days in two different weeks. Session 1 was devoted to nonverbal characterisation (i.e., sorting task); sessions 2 (wines recently fermented) and 3 (wines after accelerated ageing) to a descriptive analysis by flash profile methodology. Sensory analyses were performed by judges with extensive experience in wine sensory analysis and belonging to the laboratory LAAE (Laboratorio de análisis del aroma y enología, Zaragoza, Spain) and ICVV (Instituto de Ciencias de la Vid y el Vino, Logroño, Spain). The sorting task was carried out by twenty judges (13 women and 7 men, from 22 to 55 years, in average 34 ± 9 years) without previous training, and flash profile by fourteen judges (8 women and 6 men, from 22 to 64 years, in average 36 ± 12 years) previously trained on the specific reference attributes of the

present study. Sensory tests including training are detailed in the following parts.

3.2. Non-verbal characterisation: sorting

The twelve samples (the three biological replicates of the two recently fermented and of the two aged wines) were presented simultaneously together with a duplicated control consisting of two samples of a commercial red wine (Tempranillo, 2018). Participants were asked, during a 20-min session, to smell the fourteen samples and to group them according to their aroma similarities; the number of groups formed should be between 2 and 13, both inclusive. Once the sorting was achieved, panellists were asked to describe the groups using 1 to 3 attributes. Panel repeatability was assessed by verifying that the two replicates of control wines were grouped together.

3.3. Descriptive analysis: flash profile (FP)

The twelve samples were evaluated separately on two different days, one for the six recently fermented samples, and a second one for the six aged samples. Each of the two groups of samples were described by FP, which consisted of three 30 min sub-sessions each, held the same day and separated by at least one hour. In the first sub-session, participants were asked to smell the samples orthonasally and generate discriminant descriptors, without number restriction. Then, all descriptors were gathered and grouped in categories by three experienced experimenters independently. The final list of descriptors was obtained by consensus (see Table S1 for (A) recently fermented wines and (B) aged wines). In the second sub-session, panellists were trained with commercial aroma references prepared in ethanol 15 % v/v by LAAE. Panellists were asked to associate the references to the descriptors. Judges able to correctly associate 80 % of the references were qualified. In the third sub-session, qualified panellists evaluated the six samples object of study (one replicate of each sample) together with one sample in duplicate to evaluate panel repeatability. The seven samples were presented simultaneously and panellists were asked to rank the samples for each attribute of the final list on a 10 cm graduated scale (1 cm intervals), from 0 (low intensity) to 10 (high intensity).

4. Statistical analysis

All the statistical analyses were performed using XLSTAT (version 2020.1.3, Addinsoft, New York, USA). The effect of the factors yeast (IONYS and D254) and ageing (young and aged wines) were evaluated using one-way and two-way analysis of variance (ANOVA).

4.1. Volatile compounds quantifications: data treatment

To understand the potential sensory effects linked to ageing or the strain of yeast, concentrations were first normalised by the corresponding Olfactory Thresholds (OTs) to yield Odour Activity Values (OAVs). When the concentration was below the detection limit, it was replaced by the detection limit itself and divided by the corresponding OT. Aroma compounds were arranged into aroma vectors. Aroma vectors are groups of aroma compounds sharing chemical and sensory characteristics, whose sensory action is known to be additive (Ferreira *et al.*, 2021). As a first rough approximation of the intensity of any vector in a given sample, the OAVs of the aroma compounds within the vector were summed. As OAVs are simply concentrations normalised by the threshold, they cannot be used to predict the relative importance of a given odorant or group of odorants in a mixture, since that will ultimately depend on the particular psychophysical functions, which are not known, and on the existence of perceptual interactions between aroma vectors, most of which are also poorly known. However, they can provide a rough estimation of the number of different primary odours present in the mixture at detectable levels, and a reasonable estimation of their relative intensity between samples.

Aroma vector composition is detailed in the Supplementary Material (Table S2). Terpenes were separated into two groups, terpenes 1, mainly present in young wines, and terpenes 2 related to compounds for which ageing time is necessary for their formation. Some compounds presenting specific chemo-sensory characteristics were not grouped and appeared individually. It is the case of acetaldehyde, acetoin, diacetyl, acetic acid, ethyl acetate, β-phenylethyl acetate, β-damascenone, furaneol, rose oxide and TDN. For each aroma vector, OAVs of individual compounds were summed (summed-OAV). Aroma vectors with summed-OAV (or OAV in the case of aroma vector composed of an individual compound) inferior to 0.2 were not considered, since they were probably non-perceptible from a sensory point of view. This arbitrary value has been fixed according to previous studies (San Juan *et al.*, 2012). That was the case for furaneol (not detected), acetoin, rose oxide, vanillin and terpenes 2. For each group of samples (young and aged), a PCA was generated with

the OAVs of the retained aroma vectors as active variables. The potential sensory difference introduced by the strain of yeast in each aroma vector was assessed calculating the ratio between the maximum and minimum average OAVs (average of 3 replicates).

4.2. Sorting task data treatment

Data obtained through the sorting task was summarised in a matrix (sample × sample) obtained by summing the number of times a pair of samples were sorted in the same group. This similarity matrix was submitted to multidimensional scaling (MDS). Hierarchical clustering analysis (HCA) was further calculated on all the dimensions derived from MDS, considering Euclidean distances, Ward's method and automatic truncation. The terms generated for the group description were filtered, eliminating hedonic descriptors, and were submitted to lemmatisation and categorisation. These processes consist of an arrangement of the terms according to their root (lemmatisation) and semantic similarities (categorisation). It was performed individually by three experienced researchers (LAAE). The final list of terms was obtained by triangulation of the three lists generated independently (Abric, 2005). Only compounds with a citation frequency superior to 20 % (equivalent to 4 participants) were considered.

4.3. Flash profile

Generalized Procrustes Analysis (GPA) was performed on individual matrices (sample x attributes) built entering product ranking for each panellist. HCA was calculated on the coordinates obtained.

4.4. Correlation between chemical and sensory data

To investigate the correlations between volatiles quantifications and FP results, one Principal Components Analysis (PCA) was generated for the two groups of samples (recently fermented and aged). All the aroma vectors were considered as active variables. Sample ranking through FP, for the descriptors associated with a citation frequency superior to 20 %, were considered as supplementary variables. Spearman correlation coefficients were also calculated. RV coefficients were calculated to evaluate the degree of similarity between chemical and sensory spaces generated through PCA (chemical variables) and GPA (descriptive variables), respectively.

RESULTS AND DISCUSSION

Fermentations of Tempranillo must were carried out with two *S. cerevisiae* yeast strains. In both cases, fermentation lasted 9 days. Maxima yeast population was reached after 3 days of fermentation at $13.3 \pm 5.8 \times 10^7$ CFU/mL for D254 and at $4.6 \pm 2.0 \times 10^7$ CFU/mL for IONYS, not significantly different. The chemical parameters of the original must and the recently fermented wines are presented in Table 1. As can be seen, the wine fermented by D254 contained slightly but significantly higher amounts of residual sugars than that fermented by IONYS. Nevertheless,

TABLE 1. Conventional oenological parameters of must and wines after alcoholic fermentation carried out by the yeast strains D254 and IONYS.

	must	IONYS	D254
alcohol (% v/v)	-	12.5 ± 0.4	13.0 ± 0.1
glucose and fructose (g/l)*	203 ± 6	0.6 ± 0.1	1.0 ± 0.2
free SO2 (mg/l)	-	4 ± 2	4 ± 2
total SO2 (mg/l)	-	13 ± 4	13 ± 4
pH (20 °c)	3.33 ± 0.02	3.43 ± 0.08	3.48 ± 0.01
total acidity (g/l)	4.3 ± 0.1	7.2 ± 0.1	7.3 ± 0.2
volatile acidity (g acetic/l)*	-	0.15 ± 0.01	0.52 ± 0.02
L-malic acid	2.0 ± 0.1	1.81 ± 0.09	1.74 ± 0.09
lactic acid	-	0.08 ± 0.01	0.09 ± 0.01
acetaldehyde (g/l)	-	54 ± 10	32 ± 16

Significance of the factor yeast is indicated by * (pvalue < 0.05) and - indicates that data is not available.

the low levels measured suggest that alcoholic fermentation was properly performed. No other significant difference was found, except for volatile acidity, whose level is three times smaller for IONYS (0.15 g/L) than for D254 (0.52 g/L). The production of low levels of acetic acid by IONYS in comparison with other commercial yeasts has already been reported in previous studies (Pérez *et al.*, 2018).

1. Volatile composition and aroma vectors

Major and trace volatile compounds were measured after fermentation in the two strains and after anoxic accelerated ageing (5 weeks at 50 °C). Overall, 64 compounds were detected at concentrations above detection limits (data presented as average ± standard deviation is available in supplementary material, Table S3).

Overall, the aroma compounds measured were compiled into 17 aroma vectors with combined OAVs above 0.5 in at least one of the samples. Seven of them are mono-component, and the rest are formed by a mixture of odorants. The most complex aroma vectors are the ethyl ester aroma vector, integrated by 13 ethyl esters (De-la-Fuente *et al*, 2020) and the methoxyphenols vector, which integrates 10 odorants, most of them at sub-threshold levels. Table 2 gives the combined OAVs of the 17 aroma vectors, together with results from the two-way ANOVA to evaluate

the effect of yeast, ageing and their interaction on those aroma vectors. The exact composition of each vector and the complete ANOVA results are available in Supplementary Material (Tables S2 and S4, respectively).

2. The effect of accelerated ageing on aroma vectors

Twelve out of the 17 aroma vectors were significant for the ageing effect. These are marked with a T in Table S4 and are diacetyl, acetic acid, ethyl acetate, acetates, β-phenylethyl acetate, cinnamates, ionones, terpenes 1, ethyl esters, γ-lactones, methoxyphenols and TDN. Interestingly, 2 vectors (ethyl acetate and TDN) showed a significant interaction of yeast and ageing factors (marked with a *), suggesting that the effect of ageing on these vectors was dependent on the yeast that fermented the original wine. Results are also represented as boxplots in Figure 1. Ageing was not significant for acetaldehyde, higher alcohols, linear and branched fatty acids and β-damascenone, mostly in accordance with previous reports (Cassino *et al.*, 2019; Makhotkina and Kilmartin, 2012; Marais and Pool, 1980).

Regarding fermentative compounds, levels of diacetyl decreased by a factor of 2 on average during ageing, likely because of its reactivity towards amino acids (Bueno *et al.*, 2018; Pripis-Nicolau *et al.*, 2000), cysteine (Marchand *et al.*, 2000)
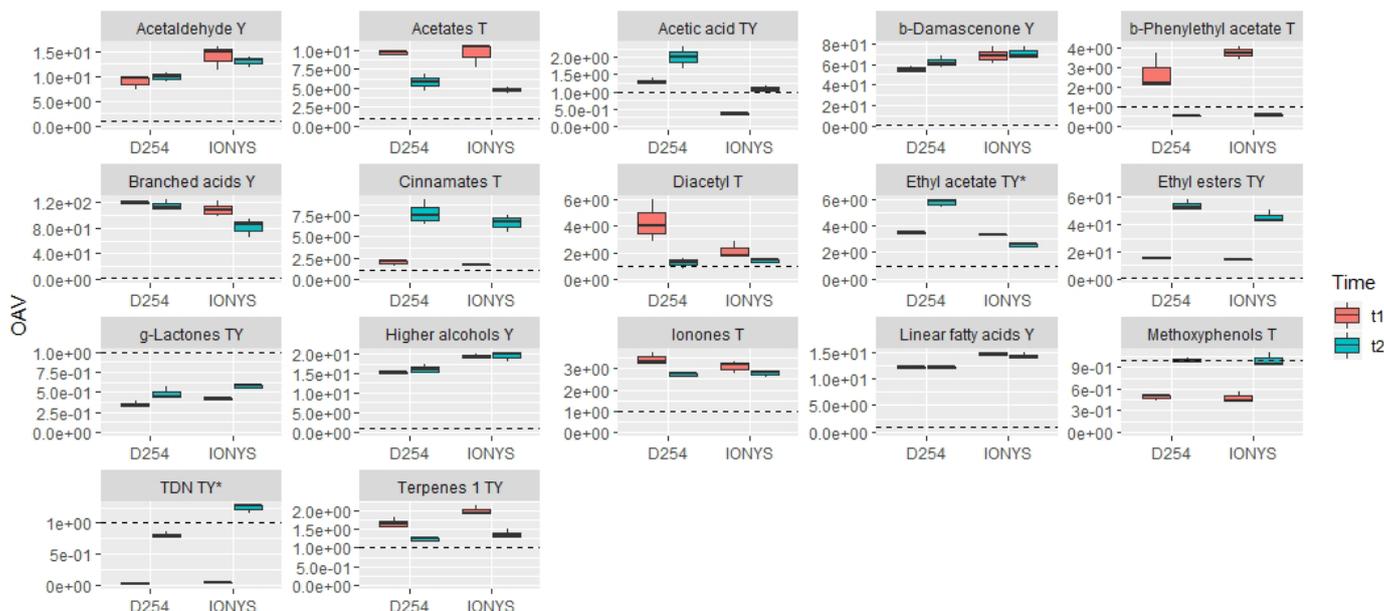
FIGURE 1. Boxplots representing the combined OAVs of each aroma vector for the four different samples produced in this study.

t1, wines recently fermented (in red); t2, wines after accelerated ageing (in blue). Significance of the factors yeast, ageing time and their interaction are indicated by Y, T and * respectively. The dotted horizontal line is set at y=1.

and polyphenols (Blanco-Vega *et al.*, 2011). On the contrary, levels of acetic acid increased significantly around 200 mg/L for both strains. The cause of these increases is not clear. As ageing took place under strict anoxia and at 50 °C, and other oxidation or spoilage markers, such as acetaldehyde or fatty and branched acids, respectively, did not show any increase, oxidative or microbiological origin does not seem likely. This implies that its origin could be the hydrolysis of acetates. Certainly, isoamyl and β-phenylethyl acetates strongly decreased during ageing, but their amounts are too low to justify the observed increase in acetic acid, which suggests the existence of an acetate not quantified in the present experiment. On the other hand, the ethyl esters aroma vector strongly increased (factor > 3) during ageing. The increase is nearly entirely attributed to the slow esterification with ethanol of branched acids, 2-methylbutyric, isobutyric and isovaleric acids (Table S3), to yield the corresponding aroma-powerful ethyl esters whose concentration increases by a factor 4. Ethyl esters of short-chain fatty acids (ethyl propanoate, ethyl butyrate), esters of organic acids (ethyl succinate, lactate) and ethyl leucate also increased around factor 1.5; while esters of long-chain fatty

acids (ethyl octanoate and hexanoate) slightly decreased. Similar evolutions have already been observed during bottle ageing (Cassino *et al.*, 2019; Díaz-Maroto *et al.*, 2005; Marais and Pool, 1980).

Leaving aside β-damascenone, varietal aroma vectors deeply change during ageing. Ionones and terpenes, which are labile aroma compounds, decreased, while ethyl cinnamates, γ-lactones, methoxyphenols and TDN, increase, mostly in agreement with previous reports (Denat *et al.*, 2021; N. Loscos *et al.*, 2010; Oliveira and Ferreira, 2019). The vector called cinnamates is formed by the ethyl ester of cinnamic acid— ethyl cinnamate—and by ethyl dihydrocinnamate. The former odorant, whose concentration increased, has a characteristic sweet and flowery note, counterbalancing the loss of floral terpenols, particularly of the most powerful geraniol and linalool, whose levels strongly decrease during ageing. The increase of TDN with its kerosene note may have some sensory relevance, as well as the increase of the vector formed by methoxyphenols, with spicy and toasted notes. As aforementioned, this complex vector is composed of 10 subthreshold odorants (Table S3) and its increase is due to strong increases with ageing of 4-vinylguaiacol, 4-vinylphenol,
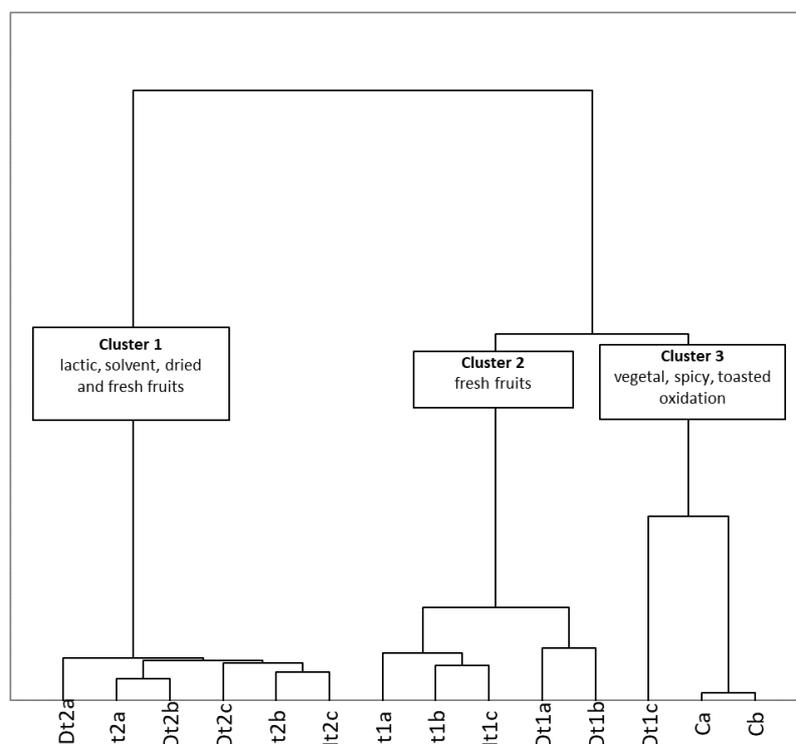
FIGURE 2. Dendrogram obtained from the data generated with the sorting task

Samples are encoded as follows: I, IONYS; D, D254; t1, wines recently fermented; t2, wines after accelerated aging; C, commercial wine; a-b-c, replicates..

guaiacol and methoxyeugenol (4-allyl-2,6-dimethoxyphenol), likely as the consequence of the hydrolysis of glycosidic precursors (Ferreira and Lopez, 2019).

In summary, ageing deeply changes the aroma profiles of wine in our accelerated ageing conditions. The most powerful effects are the strong decrease in the characteristic fruity and flowery notes of acetates and terpenes, which are replaced by the subtler flowery notes of ethyl dihydrocinnamate and by the fruity notes of ethyl esters, and the spicy, toasty and empyreumatic character developed with time as the consequence of the increases in methoxyphenols and TDN.

3. The effect of accelerated ageing on sensory properties: sorting task

In the non-verbal classification, control duplicate samples were clustered close together, confirming that the classification task was solid. The 14 samples (the 12 wines plus the two controls) led to 3 main clusters as can be seen in Figure 2.

It can be observed that samples were clearly differentiated according to ageing time, which was the dominant factor in the sensory characteristics of the samples. Aged wines were grouped in cluster 1 and were mainly characterised by the descriptors "lactic", "solvent", "dried fruits" and "fresh fruits". The effect of yeast strain was not clearly recognised in this set of wines, suggesting that age was a too-strong dominant or salient factor. It is known that in these types of tasks, panellists tend to sort samples according to the most salient differences (Moussaoui and Varela, 2010). On the contrary, in recently fermented wines the effect of strain is secondary, but it can be glimpsed. Those wines were classified together in cluster 2, with a mismatch in Dt1c, and were described as "fresh fruits". Cluster 3 includes the mismatched replicate of D254 and the duplicates of the commercial young wine and is mainly described as "vegetal" and "spicy". Both series of samples were further analysed separately to evaluate more precisely the effect of the strain.

TABLE 2. Average sum of OAVs for each aroma vector (average ± standard deviation) in wines recently fermented by two *S. cerevisiae* yeasts and after accelerated aging.

	Young wines		Aged wines	
	D254	IONYS	D254	IONYS
acetic acid TY	1.30 ± 0.08	0.35 ± 0.03	2.0 ± 0.3	1.1 ± 0.1
ethyl acetate TY*	3.46 ± 0.04	3.30 ± 0.05	5.7 ± 0.3	2.5 ± 0.2
higher alcohols Y	15.1 ± 0.1	19.5 ± 0.6	16 ± 1	20 ± 1
cinnamates T	1.9 ± 0.4	1.7 ± 0.1	8 ± 2	7 ± 1
ionones T	3.4 ± 0.3	3.1 ± 0.3	2.7 ± 0.1	2.8 ± 0.2
β-phenylethyl acetate T	2.7 ± 0.9	3.7 ± 0.3	0.54 ± 0.01	0.55 ± 0.04
terpenes 1 TY	1.7 ± 0.1	2.0 ± 0.1	1.23 ± 0.05	1.4 ± 0.1
acetates T	9.7 ± 0.4	10 ± 2	6 ± 1	4.8 ± 0.4
β-damascenone Y	55 ± 3	69 ± 8	62 ± 6	71 ± 6
ethyl esters TY	15.5 ± 0.4	14.0 ± 0.3	54 ± 4	45 ± 5
ɣ-lactones TY	0.34 ± 0.04	0.42 ± 0.02	0.48 ± 0.08	0.57 ± 0.03
branched acids Y	119 ± 4	109 ± 12	115 ± 9	82 ± 15
diacetyl T	4 ± 2	2.1 ± 0.6	1.2 ± 0.4	1.4 ± 0.2
linear fatty acids Y	12.2 ± 0.2	14.7 ± 0.3	12.0 ± 0.2	14.2 ± 0.7
methoxyphenols T	0.49 ± 0.04	0.48 ± 0.07	1.00 ± 0.04	1.0 ± 0.1
TDN TY*	0.04 ± 0.01	0.037 ± 0.004	0.81 ± 0.06	1.25 ± 0.08
acetaldehyde Y	9 ± 1	14 ± 2	10 ± 1	13 ± 1

Significance of the effect of the factors aging (young or aged), yeast (IONYS or D254) and their interaction is indicated by T, Y and * respectively (pvalue < 0.05).

4. Effect of yeasts on aroma vectors

The strain of yeast exerted a significant effect on 11 out of the 17 aroma vectors. Seven out of 10 fermentative vectors and 4 out of 7 varietal vectors were affected, as can be seen in Table 2. Diacetyl and the two acetate vectors were the single vectors not affected among fermentative compounds, while cinnamates, ionones and methoxyphenols were the varietals not significantly affected by the strain of yeast. Detailed results of the ANOVA study are given in Table S4.

The relative weight of each one of the aroma vectors on the differentiation between strains can be seen with the help of Figure 3. That figure shows, for young and aged wines, the ratios between the highest average-OAV and the lowest average-OAV for each pair of samples fermented with the two strains. The most noticeable observation is that most differences are of little magnitude. In fact, such ratio is significant and above 1.5 only for acetic acid and acetaldehyde in young wines (diacetyl was too variable), while only acetic acid, ethyl acetate and TDN were above this value in aged wines. It should be noted, however, that these most discriminant aromatic vectors are not likely to have a major sensory impact given their actual levels in the present set of wines (see Table S3). The higher levels of acetic acid measured in D254 samples (Table 2), will surely make the fruity character of this sample decrease, as it has been previously reported for levels above 0.5 g/L (San Juan *et al* 2011), but levels are not enough to perceive the specific acetic acid character. The effects of acetaldehyde, present at 4.5 and 7 mg/L will be most likely significant but subtle, as recently suggested (Arias-Pérez *et al*., 2021). As for ethyl acetate, its maximum levels are below 70 mg/L, while it has been reported that its nail polish remover notes only become perceptible above 80 mg/L (Plata *et al*., 2003). Finally, TDN levels are above reported thresholds in white wine, but yet, are very low and it can be anticipated that effects in red wine will be less obvious. The other seven aroma vectors introducing significant differences differ by factors smaller than 1.5, as can be seen in Figure 3, which suggests that the sensory effects introduced by yeast are subtle and are the consequence of little variations in many aroma vectors.

5. Effect of yeasts on sensory properties: descriptive analysis

Results from the descriptive analysis were processed by GPA and HCA, as summarised in Figure 4. The clusters found in the HCA are within the dotted circles which, as can be seen, contain samples made with a single strain. This confirms that in both young and aged wines, the sensory effects of variety were clearly recognised. As the ANOVA study revealed, young wines fermented by D254 were characterised by black fruits, while those fermented by IONYS were characterised by white fruits. Aged samples made with D254 were characterised by fresh fruits and fruity, while those made with IONYS are described by the terms "lactic", "fruits in syrup" and "white fruits". As observed in the sorting task, the replicates Dt1c and Dt2a were more dissimilar.

The relationships between the chemical and sensory spaces are summarised in the PCA plots provided in Figures 5a and b, for recently fermented and aged samples, respectively. The first relevant observation is that in both plots some descriptors are in areas without aroma vectors. This is particularly obvious in plot 5b for descriptors green, metallic and lactic, but also in plot 5a for the descriptor vegetal. This result is not surprising and has a double origin. First, it has been shown that some vegetal character seems to be part of any wine aroma reconstitution not having any marked sensory descriptor (Ferreira *et al*., 2016) and, second; vegetal, green and metallic characters may be also related to the presence of up to 15 aldehydes (unsaturated, saturated and Strecker aldehydes) at sub and peri-threshold levels which were not quantified here (Arias-Pérez *et al*., 2021).

A second relevant observation is that the two sensory descriptors projected on the centre of both plots, alcoholic in 5a and dried fruits in 5b, have been also reported to be common characters to all wine-like aroma reconstitutions (Alegre *et al*., 2020; Ferreira *et al*., 2016). This implies that they are not discriminant which explains their position in the plots.

Third, in both plots, the terms black fruit (5a) and fruity (5b), are at the opposite side of compoted and white fruits and, in both cases, the former terms are related to ethyl esters, acetic acid, cinnamates and ethyl acetate, and the latter terms are related to linear fatty acids, β-damascenone, acetaldehyde, higher alcohols and lactones. The similarity between both representations strongly suggests that these ratios are key determinants of the type of fruity descriptors perceived in these Tempranillo wines. It is noteworthy that this seems to happen even if
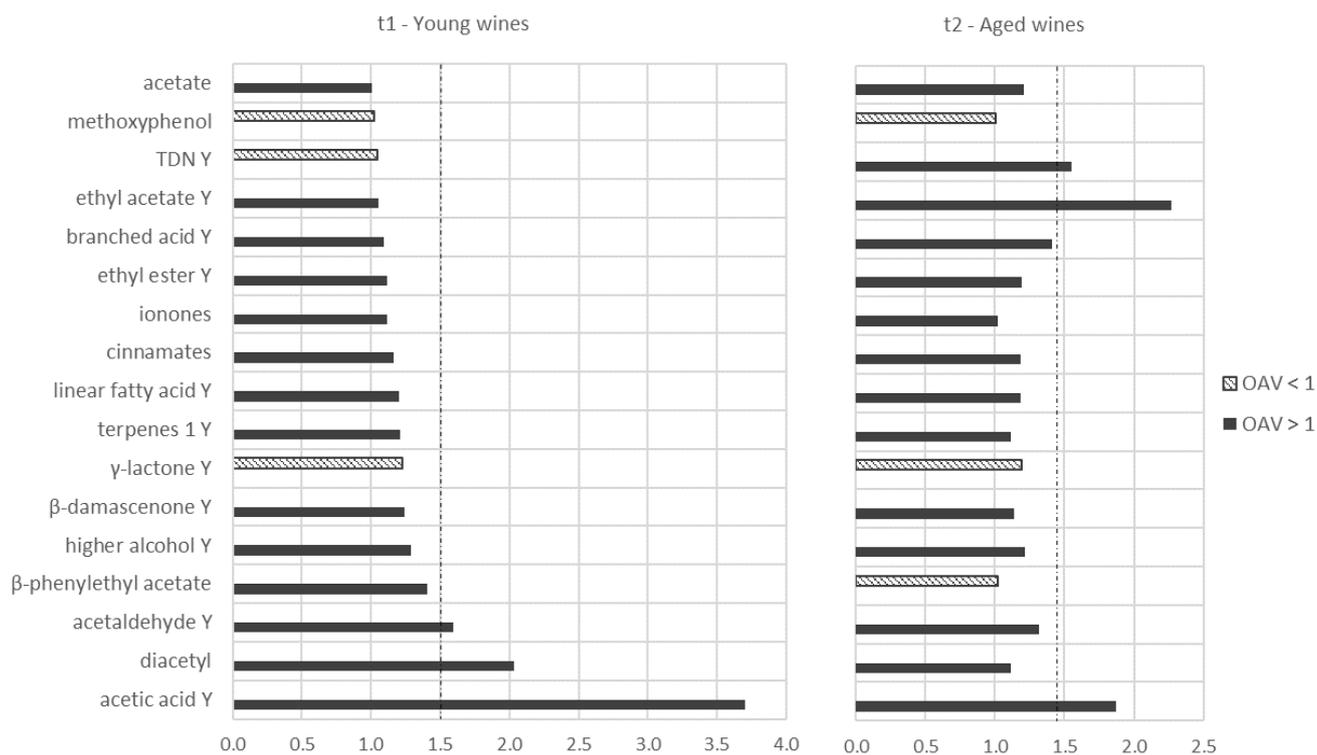
FIGURE 3. Representation of the ratio OAVmax/OAVmin for wines recently fermented (on the left) and aged wines (on the right).

Aroma vectors with OAV > 1 are coloured in black and the ones with OAV < 1 are hatched. The dotted line placed at 1.5 was placed to highlight the most discriminant vectors.
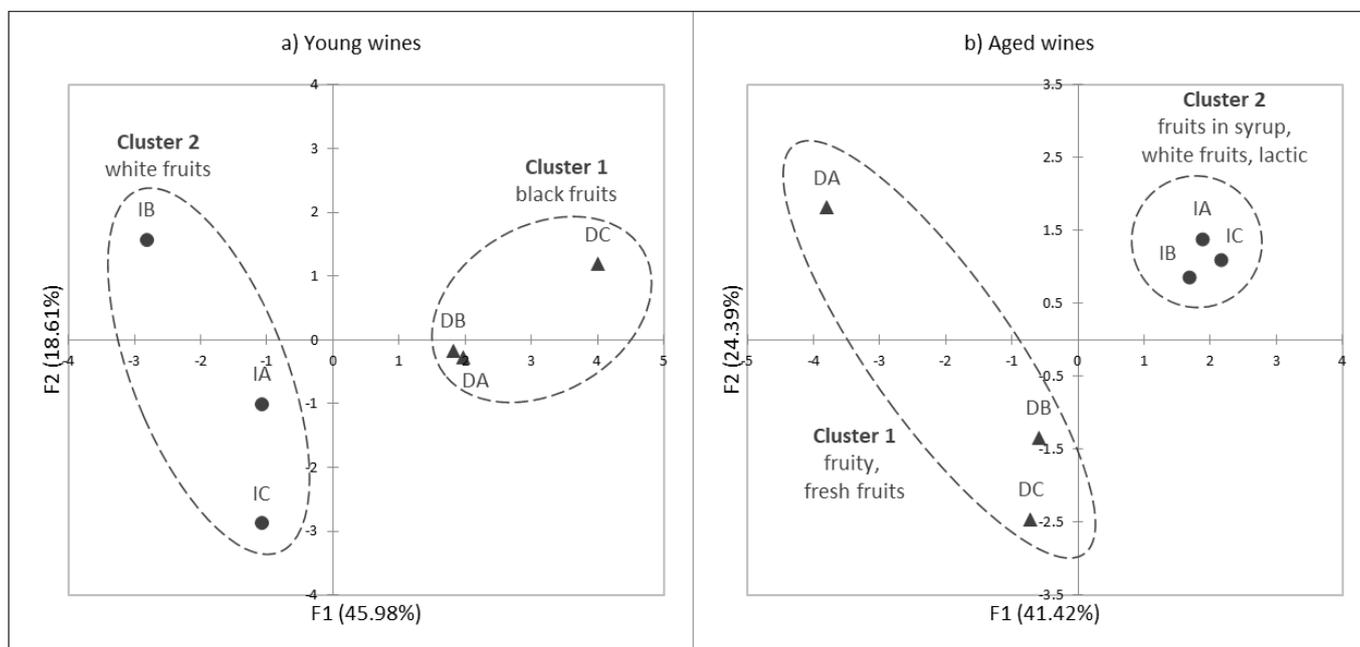
FIGURE 4. Graphical representation of the two first dimensions obtained by GPA from flash profile.

On the left, samples recently fermented; on the right, samples after accelerated aging. Dotted circles indicate the clusters generated in the HCA study and the associated descriptors. Samples are codified as follows: I, IONYS; D, D254; A-B-C, replicates.

FIGURE 5. PCA of the young (5a) and aged (5b) wines are represented.

Aroma vectors, as principal variables, are represented in grey. Descriptors, as supplementary variables, are represented in italic black.

the composition of the fruity vector completely changes as a result of ageing.

Finally, in both representations, it can be seen that the leather character could be related to the presence of methoxyphenols.

CONCLUSION

Both, ageing time and the strain of yeast used in fermentation, introduce deep changes in the chemical and sensory aroma profiles of Tempranillo red wines. Out of the 17 aroma vectors in which the odorants of the wines were classified, ageing affected to 12 and yeast strain to 11, respectively. From the sensory point of view, ageing was clearly dominant, since judges used age as the first criterion to classify samples. This is because during ageing and in our conditions of accelerated ageing, the characteristic fruity and flowery notes of acetates and terpenols were replaced by the less explicit fruity and sweet-flowery notes of ethyl esters and cinnamates, respectively. Levels of methoxyphenols and TDN also increased. Nevertheless, the effects of the yeast strain were evident and were consistently identified through ageing, both from the sensory and chemical points of view. Chemically, wines made with D254 contained consistently higher levels of ethyl esters, acetic acid, cinnamates and ethyl acetate and lower levels of linear fatty acids, β-damascenone, acetaldehyde, higher alcohols and lactones than those made with IONYS. The first profile was related to black and fresh fruit notes, while the second to white and compote fruits.

This highlights that, by introducing quantitatively small but systematic changes in many aroma vectors, both fermentative and varietal, the yeast strain can consistently modulate wine aroma throughout its shelf life.

The authors declare that no conflict of interest exists.

REFERENCES

Abric, J.-C. (2005). La recherche du noyau central et de la zone muette des représentations sociales. In ERES (Ed.), Méthodes d'étude des représentations sociales (pp. 59–80). https://doi.org/10.3917/eres.abric.2003.01.0059

Alegre, Y., Sáenz-Navajas, M. P., Hernández-Orte, P. & Ferreira, V. (2020). Sensory, olfactometric and chemical characterization of the aroma potential of Garnacha and Tempranillo winemaking grapes. Food Chemistry, 331, 127207. https://doi.org/10.1016/j.foodchem.2020.127207

Antalick, G., Perello, M. C. & de Revel, G. (2014). Esters in wines: New insight through the establishment of a database of french wines. American Journal of Enology and Viticulture, 65(3), 293–304. https://doi.org/10.5344/ajev.2014.13133

Arias-Pérez, I., Sáenz-Navajas, M. P., De-la-Fuente-Blanco, A., Ferreira, V. & Escudero, A. (2021). Insights on the role of acetaldehyde and other aldehydes in the odour and tactile nasal perception of red wine. Food Chemistry, 361, 130081. https://doi.org/10.1016/j.foodchem.2021.130081

Blanco-Vega, D., López-Bellido, F. J., Alía-Robledo, J. M. & Hermosín-Gutiérrez, I. (2011). HPLC-DAD-ESI-MS/MS characterization of pyranoanthocyanins pigments formed in model wine. Journal of Agricultural and Food Chemistry, 59(17), 9523–9531. https://doi.org/10.1021/jf201546j

Bueno, M., Marrufo-Curtido, A., Carrascón, V., Fernández-Zurbano, P., Escudero, A. & Ferreira, V. (2018). Formation and Accumulation of Acetaldehyde and Strecker Aldehydes during Red Wine Oxidation. Frontiers in Chemistry, 6(February). https://doi.org/10.3389/fchem.2018.00020

Cassino, C., Tsolakis, C., Bonello, F., Gianotti, V. & Osella, D. (2019). Wine evolution during bottle aging, studied by 1H NMR spectroscopy and multivariate statistical analysis. Food Research International, 116(August 2018), 566–577. https://doi.org/10.1016/j.foodres.2018.08.075

De-la-Fuente-Blanco, A., Sáenz-Navajas, M. P. & Ferreira, V. (2020). Wine aroma vectors and sensory attributed. In Managing Wine Quality, Second Edition (A. Reynold, pp. 1–20). Woodhead Publishing (Elsevier).

Denat, M., Pérez, D., Heras, J. M., Querol, A. & Ferreira, V. (2021). The effects of Saccharomyces cerevisiae strains carrying alcoholic fermentation on the fermentative and varietal aroma profiles of young and aged Tempranillo wines. Food Chemistry: X, 9(March 2020), 100116. https://doi.org/10.1016/j.fochx.2021.100116

Díaz-Maroto, M. C., Schneider, R. & Baumes, R. (2005). Formation pathways of ethyl esters of branched short-chain fatty acids during wine aging. Journal of

Agricultural and Food Chemistry, 53(9), 3503–3509. https://doi.org/10.1021/jf048157o

Ferreira, V., De-la-Fuente-Blanco, A. & Sáenz-Navajas, M.-P. (2021). Wine aroma vectors and sensory attributes. In A. Reynolds (Ed.), Managing Wine Quality: Volume I: Viticulture and wine quality (2nd ed.). Elsevier (Woodhead publishing).

Ferreira, V. & Lopez, R. (2019). The Actual and Potential Aroma of Winemaking Grapes. Biomolecules, 1–36. https://doi.org/10.3390/biom9120818

Ferreira, V., Sáenz-Navajas, M. P., Campo, E., Herrero, P., de la Fuente, A. & Fernández-Zurbano, P. (2016). Sensory interactions between six common aroma vectors explain four main red wine aroma nuances. Food Chemistry, 199, 447–456. https://doi.org/10.1016/j.foodchem.2015.12.048

Francis, I. L., Sefton, M. A. & Williams, P. J. (1994). The Sensory Effects of Pre-or Post-Fermentation Thermal Processing on Chardonnay and Semillon Wines. American Journal of Enology and Viticulture, 45(2), 243–251.

International Organisation of Vine and Wine (2019). Compendium of international methods of wine and must analysis.

King, E. S., Francis, L., Swiegers, J. H. & Curtin, C. (2011). Yeast strain-derived sensory differences retained in sauvignon blanc wines after extended bottle storage. American Journal of Enology and Viticulture, 62(3), 366–370. https://doi.org/10.5344/ajev.2011.10079

López, R., Aznar, M., Cacho, J. & 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. https://doi.org/10.1016/S0021-9673(02)00696-9

Loscos, N., Hernández-Orte, P., Cacho, J. & 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(1), 205–216. https://doi.org/10.1016/j.foodchem.2009.10.008

Loscos, Natalia, Hernandez-Orte, P., Cacho, J. & Ferreira, V. (2007). Release and Formation of Varietal Aroma Compounds during Alcoholic Fermentation from Nonfloral Grape Odorless Flavor Precursors Fractions. Journal of Agricultural and Food Chemistry, 55(16), 6674–6684. https://doi.org/10.1021/jf0702343

Makhotkina, O. & Kilmartin, P. A. (2012). Hydrolysis and formation of volatile esters in New Zealand Sauvignon blanc wine. Food Chemistry, 135(2), 486–493. https://doi.org/10.1016/j.foodchem.2012.05.034

Marais, J. & Pool, H. J. (1980). Effect of storage time and temperature on the volatile composition and quality of dry white table wines. Vitis, 19(2), 151–164.

Marchand, S., De Revel, G. & Bertrand, A. (2000). Approaches to wine aroma: Release of aroma compounds from reactions between cysteine and carbonyl compounds in wine. Journal of Agricultural and Food Chemistry, 48(10), 4890–4895. https://doi.org/10.1021/jf000149u

Molina, A. M., Guadalupe, V., Varela, C., Swiegers, J. H., Pretorius, I. S. & Agosin, E. (2009). Differential synthesis of fermentative aroma compounds of two related commercial wine yeast strains. Food Chemistry, 117(2), 189–195. https://doi.org/10.1016/j.foodchem.2009.03.116

Moussaoui, K. A. & Varela, P. (2010). Exploring consumer product profiling techniques and their linkage to a quantitative descriptive analysis. Food Quality and Preference, 21(8), 1088–1099. https://doi.org/10.1016/j.foodqual.2010.09.005

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), 164. https://doi.org/10.3390/microorganisms7060164

Ortega, C., López, R., Cacho, J. & Ferreira, V. (2001). Fast analysis of important wine volatile compounds. Journal of Chromatography A, 923(1–2), 205–214. https://doi.org/10.1016/s0021-9673(01)00972-4

Pérez, D., Capaldi, C., Mercado, L., Malizia, A. & Santiago Sari, Y. (2018). Efecto combinado de cepa de levadura y Terroir en vinos Malbec de Mendoza. E3S Web of Conferences, 50, 02005. https://doi.org/10.1051/e3sconf/20185002005

Plata, C., Millán, C., Mauricio, J. C. & Ortega, J. M. (2003). Formation of ethyl acetate and isoamyl acetate by various species of wine yeasts. Food Microbiology, 20(2), 217–224. https://doi.org/10.1016/S0740-0020(02)00101-6

Pretorius, I. S. & Bauer, F. F. (2002). Meeting the consumer challenge through genetically customized wine-yeast strains. Trends in Biotechnology, 20(10), 426–432. https://doi.org/10.1016/S0167-7799(02)02049-8

Pripis-Nicolau, L., De Revel, G., Bertrand, A. & Maujean, A. (2000). Formation of flavor components by the reaction of amino acid and carbonyl compounds in mild conditions. Journal of Agricultural and Food Chemistry, 48(9), 3761–3766. https://doi.org/10.1021/jf991024w

Ramey, D. D. & Ough, C. S. (1980). Volatile Ester Hydrolysis or Formation during Storage of Model Solutions and Wines. J. Agric. Food Chem., 28, 928–934. https://www.rameywine.com/wp-content/uploads/ester_hydrolysis.pdf. https://doi.org/10.1021/jf60231a021

Rapp, A., Güntert, M. & Ullemeyer, H. (1985). Über Veränderungen der Aromastoffe während der Flaschenlagerung von Weißweinen der Rebsorte Riesling. Zeitschrift Für Lebensmittel-Untersuchung Und -Forschung, 180(2), 109–116. https://doi.org/10.1007/BF01042633

Rollero, S., Bloem, A., Ortiz-Julien, A., Camarasa, C. & Divol, B. (2018). Fermentation performances and aroma production of non-conventional wine yeasts are influenced by nitrogen preferences. FEMS Yeast Research, 18(5), 1–11. https://doi.org/10.1093/femsyr/foy055

San Juan, F., Cacho, J., Ferreira, V. & Escudero, A. (2012). Aroma chemical composition of red wines from different price categories and its relationship to quality. Journal of Agricultural and Food Chemistry, 60(20), 5045–5056. https://doi.org/10.1021/jf2050685

Simpson, R. F. (1978). Aroma and compositional changes in wine with oxidation storage and ageing. The Australian Wine Research Institute, 17, 274–287.

Simpson, R. & Miller, G. (1983). Aroma composition of aged Riesling wine. Vitis, 22(1), 51–63.

Singleton, V. L., Ough, C. S. & Amerine, M. A. (1964). Chemical and Sensory Effects of Heating Wines under different Gases. Am J Enol Vitic., 15, 134–145.

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(March), 1–13. https://doi.org/10.3389/fchem.2018.00066

Swiegers, J. H., Bartowsky, E. J., Henschke, P. A. & Pretorius, I. S. (2005). Yeast and bacterial modulation of wine aroma and flavour. Australian Journal of Grape and Wine Research, 11(2), 139–173. https://doi.org/10.1111/j.1755-0238.2005.tb00285.x

Vela, E., Hernández-Orte, P., Franco-Luesma, E. & Ferreira, V. (2017). The effects of copper fining on the wine content in sulfur off-odors and on their evolution during accelerated anoxic storage. Food Chemistry, 231, 212–221. https://doi.org/10.1016/j.foodchem.2017.03.125

Waterhouse, A. L., Sacks, G. L. & Jeffery, D. W. (2016). Understanding Wine Chemistry. John Wiley & Sons, Ltd. https://doi.org/10.1002/9781118730720.