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Effect of non-wine *Saccharomyces* yeasts and bottle aging on the release and generation of aromas in semi-synthetic Tempranillo wines

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ABSTRACT

Interest in the use of non-conventional yeasts in wine fermentation has been increased in the last years in the wine sector. The main objective of this manuscript was to explore the aromatic diversity produced by wild and non-wine strains of *S. cerevisiae*, *S. eubayanus*, *S. kudriavzevii*, and *S. uvarum* species in young and bottle-aged Tempranillo wines as well as evaluate their fermentation capacity and the yield on ethanol, glycerol, and organic acids, that can contribute to diminishing the effects of climate change on wines.

S. uvarum strain U1 showed the highest ability to release or *de novo* produce monoterpenes, such as geraniol and citronellol, whose values were 1.5 and 3.5-fold higher than those of the wine *S. cerevisiae* strain. We found that compared to the normal values for red wines, β-phenylethyl acetate was highly synthesized by U1 and E1 strains, achieving 1 mg/L. Additionally, after aging, wines of *S. eubayanus* strains contained the highest levels of this acetate. Malic acid was highly degraded by *S. kudriavzevii* yeasts, resulting in the highest yields of lactic acid (>5-fold) and ethyl lactate (>2.8-fold) in their wines. In aged wines, we observed that the modulating effects of yeast strain were very high in β-ionone. *S. uvarum* strains U1 and BMV58 produced an important aging attribute, ethyl isobutyrate, which was highly enhanced during the aging. Also, the agave *S. cerevisiae* strain develops an essential aroma after aging, reaching the highest ethyl leucate contents.

According to the results obtained, the use of wild non-wine strains of *S. cerevisiae* and strains of the cryotolerant species *S. eubayanus*, *S. kudriavzevii*, and *S. uvarum* in Tempranillo wine fermentation increase the aroma complexity. In addition, wines from *S. kudriavzevii* strains had twice additional glycerol, those from *S. uvarum* 4-fold more succinic acid, while wines from wild strains yielded 1% v/v less ethanol which may solve wine problems associated with climate change.

1. Introduction

Wine aroma is widely known to be one of the most relevant determinants of the overall wine quality (Charters and Pettigrew, 2007; San-Juan et al., 2011). Its composition consists of several volatile molecules at concentrations ranging from ng/L to mg/L. According to their origin, they can be divided into three main categories, the aroma of varietal, fermentative, and aging origin. The most abundant volatile compounds are from fermentative origin, such as ethyl esters, acetate esters, higher alcohols, and volatile fatty acids, mainly derived from nitrogen and carbon yeast metabolisms (Rollero et al., 2017). However, the varietal aroma is the most influential group in terms of the substantial aroma contribution of the odorants to wine. Among others, this group includes polyfunctional mercaptans, norisoprenoids, terpenoids, volatile phenols, and vanillin derivatives (Ferreira and López, 2019). Initially, most of these molecules do not contribute directly to wine aroma as they are presented at bound forms, such as a glycosylated, whereas only a small fraction is found as free aroma molecules (Ferreira and López, 2019; Hjelmeland and Ebeler, 2015; Liu et al., 2017). In general, aglycones are released from their non-volatile precursors by a

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slow process of acid hydrolysis that mainly occurs during the aging process (Alegre et al., 2020a; Ferreira and López, 2019). On the other hand, yeasts can release the aromatic aglycone from sugar moieties through endogenous enzymatic reactions (Ugliano, 2009). They can even generate some varietal aromas within their cells through the *de novo*-synthesis process (Gamero et al., 2011a, b).

Recent studies have determined the importance of yeast and bottle aging in the generation or release of this type of compounds from the grapes non-volatile aroma precursors, such as β-damascenone, linalool, rose oxide, α-terpineol, guaiacol, eugenol, methoxyeugenol, 2,6-dimehtoxyphenol, and vanillin derivates (Alegre et al., 2020a; Denat et al., 2021). These insights have established new aromas origin sub-categories and have emphasized the importance of yeast strain and aging time on the development of varietal and fermentative aromas.

Currently, wine researches have shown that the most widely used and adapted strains for wine fermentation have been set aside in favor of what are known as indigenous or wild yeasts. These wild *S. cerevisiae* or *S.* non-*cerevisiae* strains have been isolated from spontaneous fermentations or found in nature. The implementation of non-conventional yeasts in winemaking could lead to the discovery of new aromatic profiles as well as some improvements in wine quality (Querol et al., 2018). For instance, cryotolerant species, *S. kudriavzevii*, *S. uvarum*, and *S. eubayanus* have been characterized to produce wines with low alcohol levels, high glycerol content, and high concentrations of certain higher alcohols and their acetate esters, thus decreasing the effect of climate change on grapes (Minebois et al., 2020a; Stribny et al., 2015). Additionally, some studies revealed that some *S. cerevisiae* strains could differ in the final wine flavor profile due to their capacity to produce ethyl esters, higher alcohols, and volatile fatty acids from their nitrogen metabolism (Cordente et al., 2019). In general, when isolated from natural or non-wine environments, these yeasts usually show poor fermentative abilities due to some must and fermentation conditions: high fermentation temperature, pH, high sugar content, increased ethanol and SO_2 levels (Arroyo-López et al., 2010; Origone et al., 2017). However, through screening studies, some non-*cerevisiae* and non-wine *S. cerevisiae* strains have been selected for their optimal wine fermentation capacities (Pérez et al., 2021).

Tempranillo cv. (*Vitis vinifera* L) is one of the most important red grape varieties in Spain, especially in the region of La Rioja. Despite being a neutral grape variety, its wines have a distinctive aroma profile originated after yeast and bottle-aging action on varietal-specific odorless precursors (Hernández-Orte et al., 2008; López et al., 2004; Alegre et al., 2020b). In this context, given the impact of yeasts and bottle aging on aroma modulation, in this work, we aim to explore in-depth the variability and contribution of yeast strains not used previously in wine fermentations on the aroma of young and aged Tempranillo wines. We also evaluate these strains in terms of their fermentation capacity and their ability to provide oenological qualities that diminish the effects of climate change on wines.

2. Materials and methods

2.1. Semi-synthetic must composition

Must composition was prepared according to Hernández-Orte et al. (2006) with minor modifications: 210 g/L reducing sugars (105 g/L glucose + 105 g/L fructose); 4 g/L L-tartaric acid; 0.3 g/L citric acid; 3 g/L L-malic acid; 2 mg/L KH_2PO_4; 0.2 g/L $MgSO_4{\cdot}7H_2O$; 4.7 mg/L $MnCl_2{\cdot}4H_2O$; 1.35 mg/L $CuSO_4{\cdot}5H_2O$; 1.29 mg/L KIO_3; 0.22 mg/L $CoCl_2$; 1 mg/L H_3BO_3; 2 mg/L $ZnCl_2$; 0.155 g/L $CaCl_2{\cdot}2H_2O$; 0.19 mg/L $NaMoO_4{\cdot}2H_2O$; 0.3 g/L myo-inositol; 0.04 mg/L biotin; 1 mg/L thiamin hydrochloride; 1 mg/L pyridoxine hydrochloride; 1 mg/L nicotinic acid; 1 mg/L calcium pantothenate;1 mg/L *p*-aminobenzoic acid; 15 mg/L ergosterol; 0.05% Tween 80 in ethanol (v/v); 0.2 mg/L folic acid and 0.2 mg/L riboflavin.

The nitrogen compounds were defined by two fractions with a final nitrogen concentration (YAN) of 355 mg/L. The basal nitrogen content was composed of 0.22 g/L $(NH_4)_2HPO_4$; 14.34 mg/L tyrosine; 44.37 mg/L γ-aminobutyric acid (GABA); 14.43 mg/L isoleucine; 13.42 mg/L leucine; 58.51 mg/L alanine and 17.73 mg/L valine. The second was a specific Tempranillo amino acids fraction, composed by 86.52 mg/L aspartic acid; 85.24 mg/L glutamic acid; 60.08 mg/L serine; 6.47 mg/L glycine; 137.4 mg/L histidine; 72.27 mg/L threonine; 673.1 mg/L arginine; 302.3 mg/L proline, 25.2 mg/L methionine; 7.53 mg/L phenylalanine; 13.69 mg/L lysine and 177.3 mg/L glutamine.

Before the must was filtered by sterilization (0.2 μm), the pH was adjusted to 3.5, and 10 mL/L of phenolic and of aromatic fraction of Tempranillo grapes (PAF) was aseptically added.

The phenolic and aromatic non-volatile extract from Tempranillo grapes (PAF) was prepared at Zaragoza University (Spain) following the protocol described by Alegre et al. (2020a) and was conserved in ethanol. Before being introduced into the synthetic must, the alcohol content was entirely evaporated to dryness under vacuum using a rotary evaporator. Finally, the extract was reconstituted in the same volume with sterile water and added to the must immediately before the inoculation to avoid possible oxidation.

2.2. Yeast strains and fermentation conditions

Yeast strains belonging to the species *S. cerevisiae*, *S. uvarum*, *S. kudriavzevii*, and *S. eubayanus*, isolated from natural habitats and spontaneous fermentation, were used to conduct the alcoholic fermentation (Table 1).

A day before starting the fermentation, each strain was grown at 25 °C in 5 mL of GPY (2% glucose, 0.5% yeast extract, and 0.5% peptone, PanReac AppliChem, Spain), then each must was inoculated with these pre-cultures at an initial population of 1×10^6 cells/mL.

As fermenters, 100 mL sterile glass flasks with a stirrer magnet were used. Standard cartridges filled with previously conditioned LiChrolut-EN resin (Denat et al., 2021) were inserted at the airlock output. The silicone caps were pierced with fixed syringes to collect samples without

Table 1
Saccharomyces cerevisiae, *Saccharomyces eubayanus*, *Saccharomyces kudriavzevii*, and *Saccharomyces uvarum* strains were used in this study.

Species	Code used	AQ code[a]	Source of isolation	Geographic origin
S. cerevisiae	T73	AQ0029	Wine, Commercial (Lallemand), T73™	Spain
	C1	AQ2543	Cachaça fermentation	Brazil
	C2	AQ2493	Agave fermentation	Mexico
	C3	AQ2458	Wasp	Peru
S. eubayanus	E1	AQ2875	Oak (*N. pumili*)[b]	Chile
	E2	AQ2596	Tree seeds (*A. araucana*)[c]	Argentina
	E3	AQ2600	Tree bark (*A. araucana*)[c]	Argentina
S. kudriavzevii	K1	AQ2641	Monosporic derivative from oak (*Q. faginea*) isolate	Spain
	K2	AQ2148	Oak (*Q. faginea*)	Spain
	K3	AQ2619	Monosporic derivative from oak (*Q. faginea*) isolate	Spain
S. uvarum	BMV58	AQ1580	Wine, Commercial (Lallemand), Velluto BMV58™	Spain
	U1	AQ1124	Non fermented liquor (Mistela)	Spain
	U2	AQ1179	Cider fermentation	Ireland
S. cer × *S. uv*	VellEvol	AQ2868	Wine, Commercial (Lallemand); Velluto Evolution™	Spain

[a] Dr. Querol yeast collection at IATA (CSIC).
[b] Molecular Genetic Laboratory of Santiago de Chile University.
[c] North Patagonian Culture Collection, Neuquén, Argentina.

introducing oxygen or losing aroma.

For each strain, three fermentations were carried out in 50 mL of must containing PAF, and as controls, non-inoculated musts containing PAF, were included. Fermentations were carried out at 20 °C with agitation (100 rpm) while daily weight loss was monitored. Once the fermenter's weight was constant and reducing sugars contents were less than 2 g/L, the fermentation process was considered finished. By the mathematical model of non-linear regression, the fermentation curves of weight loss were adjusted using the reparametrized Gompertz equation proposed by Zwietering et al. (1990) for bacterial growth:

$$y = A^{*}exp\{ - exp[((\mu_{max}{}^{*}e/A)^{*}(\lambda - t)) + 1]\}$$

For the adjustment to the fermentation process, these kinetic parameters were adapted, being λ the time to the vigorous starts of fermentation, Vmax the maximum specific fermentation rate, and A, as the maximum weight lost reached. In addition, from these parameters, the time needed to reach 75% of fermentation was calculated (T75%).

2.3. Analysis of organic acids, sugars, and alcohols

After fermentation, samples were analyzed by HPLC (High-Performance Liquid Chromatography, Thermo Fisher Scientific, Waltham, MA, USA). The concentrations of the following metabolites were determined: glucose, fructose, ethanol, glycerol, succinic, and malic acid. The instrument was composed of a Hyper REZTM XP Carbohydrate H+ 8 μm column equipped with a Hyper RETZM XP Carbohydrate Guard and a refraction index detector for sugars and alcohols, and a UV detector for organic acids (Thermo Fisher Scientific). The analysis conditions were: 1.5 mM of H_2SO_4. 0.6 mL/min flux, a pressure of 30 bars, and oven temperature were held at 50 °C. Samples were previously filtered by a nylon filter (0.22 μm), and the concentrations of these compounds (g/L and % v/v for ethanol) were determined using standard calibration curves.

2.4. Determination of volatile aromatic compounds

2.4.1. Analysis of aromas volatilized during fermentation

Once fermentations were finished, each cartridge was removed from the airlock and immediately dried under vacuum. Then, the retained volatile compounds were extracted and collected by elution with 1.6 mL of dichloromethane solution containing 5% v/v of methanol. Next, 50 μL of each sample extract was mixed in one solution to concentrate under nitrogen flow.

This extract was analyzed by Gas-Chromatography-Olfactometry (GC-O) using a gas chromatograph (Thermo 8000 series) equipped with a Flame Ionization Detector (FID) and a sniffing port ODO-1 (SGE, Ringwood, Australia), which was attached to the column output by a flow splitter. The methodology used for GC-O was as described in San-Juan et al. (2010).

The sensory evaluations in the GC-O were realized by a panel of six experienced judges, two men, and four women. The aroma evaluation consisted of a 40 min chromatographic run, so judges were asked to do it in separate 20 min sessions to avoid fatigue and twice on different days. They were asked to describe the odor perceived, the intensity on a scale of 0 to 3 (being 0 not detected; 1 weak, hardly recognizable odor; 2 clear but no intense odor; 3 intense), and write down the detection time.

The data collected from the panel were analyzed using the model proposed by Dravnieks (1985), obtaining scores that correspond to the detection frequency of aromatic attributes and the average intensity of their maximum intensity. Then the compounds were identified by comparing the descriptors, the chromatographic retention index obtained in DB-WAX and DB-5 columns, and the MS spectra with those of pure reference compounds. The GC–MS conditions were as Denat et al. (2021) described.

2.4.2. Analysis of major aromas compounds in young wines samples

Immediately after fermentation, higher alcohols, volatile fatty acids, and major esters were extracted by liquid-liquid microextraction and quantified by GC-FID analysis using the protocol described by Ortega et al. (2001). The quantification of each compound (mg/L) was done in reference to the relative response factor obtained from the areas and concentrations of each standard compound dosed to a wine.

2.4.3. Analysis of trace aromas compounds in young and aged wines samples

Part of each finished wine was introduced in 18 mL vials with a non-metallic screw cap and treated in an anoxic chamber (Jacomex, Dagneux, France). Samples were introduced in vacuum plastic bags containing oxygen scavengers AnaeroGen (Thermo Scientific, USA). These bags containing 18 mL-vials with the free-O_2 wine samples were incubated at 50 °C for 5 weeks to simulate bottle aging. Those compounds found at concentrations ranging from 0.1 to 200 μg/L (minor or traces compounds) were analyzed in young and aged wines following the protocol described by López et al. (2002). The identification and quantification of the minor compounds were carried out by a gas chromatograph coupled to a mass spectrometer detector, GC–MS (Shimadzu QP2010, Quioto, Japan), and the concentrations were obtained in reference to the relative response factor obtained from the areas and concentrations of each standard compound dosed to a wine.

2.5. Statistical analysis

Every fermentation parameter and compound was expressed as the arithmetic means of three repetitions with their corresponding standard deviation. One-way ANOVA with Tukey test was applied considering significant differences at p-value < 0.05. Genesis software 1.7.7 (Graz University of Technology, Austria) was used to acquire the heat map, the hierarchical clustering using Euclidean distance, and the average linkage as agglomeration rule. Principal component analysis (PCA), as a multivariate methodology, was applied to obtain visualization in a reduced dimension of data and determine which variable contributes the most and how the samples are grouped around them. All statistical analyses and plots were obtained by the use of Infostat software, version 2011 (Grupo Infostat, Córdoba, Argentina), and GraphPad Prism version 8.0 (Graph-Pad Software, Inc., La Jolla, CA).

3. Results and discussion

Microfermentations were carried out by 14 different yeast strains of four *Saccharomyces* species (Table 1) in synthetic musts containing PAF (phenolic and aromatic fraction) extracted from Tempranillo grapes.

3.1. Fermentation performances

Lag phase, Vmax, and T75 resulting from the fermentation of each strain are plotted in Fig. 1. Most strains with fermentative background (dot-filled bars) showed a better adaptation to the must conditions at the beginning of the fermentation, resulting in shorter lag times. In contrast, most of the wild strains presented the longest lag times.

On the other hand, as the fermentation progressed, the natural *S. kudriavzevii* strains K1 and K2 as well as VellEvol and C1 displayed a better performance, achieving the 75% of the fermentation earlier and with the fastest Vmax. On the contrary, the other *S. kudriavzevii* strain, K3, had the opposite tendency; it started fermenting quite fast, but its T75% was the longest, and its Vmax was the slowest. Such different fermentation behaviors are probably due to different tolerances among yeast strains to factors encountered during fermentation, such as pH, temperature, hexose concentrations, and ethanol increments (Origone et al., 2017). However, the most relevant finding in this work was that the natural *S. kudriavzevii* K1 strain showed a fermentation capacity equal or even better than the wine strains VellEvol (*S. cerevisiae* ×

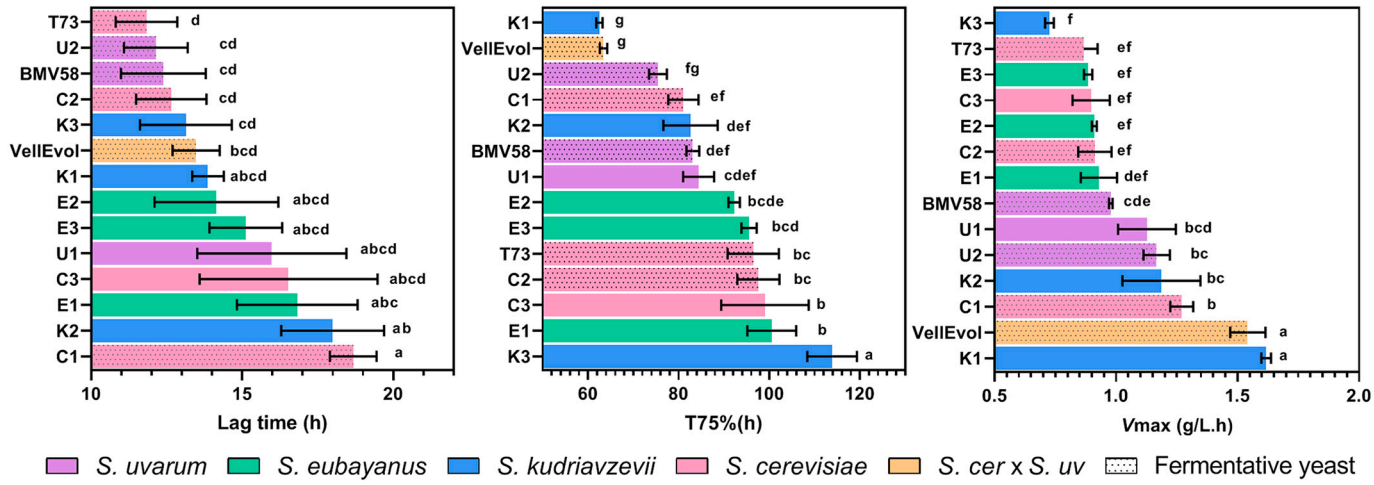

Fig. 1. Kinetic parameters (mean ± SD) of the alcoholic fermentation curves: lag phase, Vmax (maximal fermentation rate), and T75% (time to reach 75% of the fermentation) of the different strains represented in each bar, differentiating the species by color and the dotted filing indicates that they are of fermentative origin. ANOVA results are presented in the supplementary material (Table S1).

S. uvarum hybrid), T73 (*S. cerevisiae*), and BMV58 (*S. uvarum*).

3.2. Major fermentation metabolites

Relevant differences in organic acids and glycerol production have been observed (Fig. 2; Table S2). Malic acid contents ranged from 2.53 to 4.23 g/L, with the highest values for C2 and U2 strains (4.23 and 4.18 g/L, respectively) and lowest for the *S. kudriavzevii* strains (2.53 to 2.74 g/L), resulting in wines with 1.5 g/L below C2 and U1 contents. Considering that the original must contain 3 g/L malic acid, strain C2, U1, E2, T73, E1, BMV58, and C1 seem to have produced this acid through the reductive branch of the Krebs cycle (TCA), whereas *S. kudriavzevii* yeasts appear to have degraded the must's malic acid, as a different way to recycle redox cofactors (Redzepovic et al., 2003; Waterhouse et al., 2016). In line with this last statement, the degradation of malic acid by *S. kudriavzevii* strains could be related to the highest lactic acid synthesized by them, having statistically different values and up to five-fold higher than the rest of the yeasts (Fig. 2). In the case of succinic acid, *S. uvarum* BMV58 and the commercial hybrid (*S. cerevisiae* × *S. uvarum*) produced the highest levels (4.24 and 3.54 g/L, respectively). In contrast, *S. cerevisiae* strains produced the lowest levels of this acid, up to 3 g/L below those maxima values. In the case of glycerol, differences as high as 2–3.5 g/L were observed between strains from different sources: strains from fermentation environments reached the lowest values, whereas strains from natural environments had the highest values of this alcohol.

According to these results, we confirmed the ability of *S. uvarum* and *S. kudriavzevii* strains as well as the wild/natural *S. cerevisiae* strains to produce higher amounts of glycerol and organic acids as well as to reduce the ethanol yield (Minebois et al., 2020a, 2020b, 2021; Pérez et al., 2021; Querol et al., 2018). Differences among glycerol and organic acids synthesis (mainly succinic acid) have been related to different

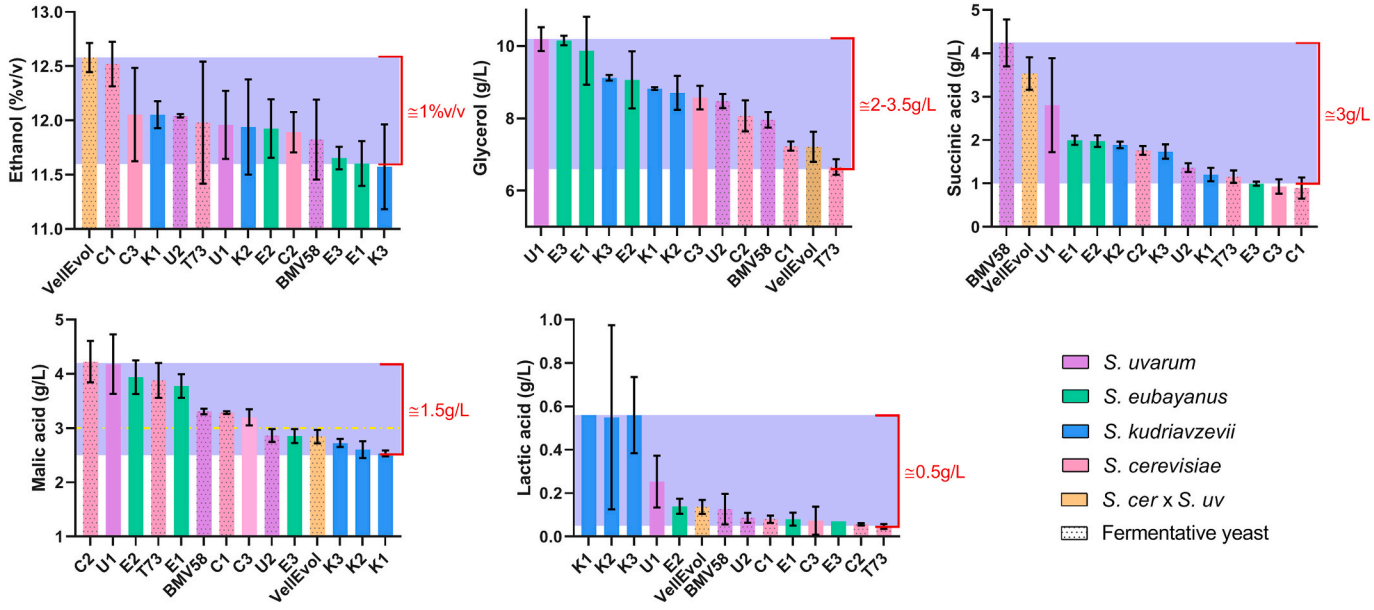

Fig. 2. Main metabolites (mean ± SD) determined in young wines fermented by the different strains: ethanol (% v/v), glycerol (g/L), malic acid (g/L), succinic acid (g/L) and lactic acid (g/L). The color of each bar indicates the species and dot-filling strains with fermentative origin. The differences between wines with maximum and minimum average values are indicated in red. The dashed yellow line indicates the original malic acid value in must. ANOVA results are presented in the supplementary material (Table S2). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

metabolic strategies used by the cryophilic non-*cerevisiae* and wild *S. cerevisiae* strains to maintain the NAD^+/NADH balance during alcoholic fermentation (Minebois et al., 2020a, 2020b, 2021; Oliveira et al., 2014). So, we confirm the applications of non-conventional yeasts to solve the current winemaking demands.

3.3. Aroma analysis

3.3.1. Volatiles lost during fermentation

The aromas volatilized during fermentation were retained into aroma trap systems containing LiChrolut-EN resins installed in the airlocks. The trapped aroma was further eluted with solvent. For olfactometric screening, aliquots from all fermentation extracts were mixed, concentrated by evaporation, and then analyzed by GC-Olfactometry using a sensory panel with six trained judges. This extract representing the "average aroma effluent" from all fermentations was composed of 11 identified odorants given in Table 2 and by some other weaker odorants with an MF < 30% not given in the table.

Odorants in Table 2 are ranked attending to their olfactometric score so that those in the first position are the most intense. Two central observations can be deduced from these data. First, all compounds in the table except o-cresol and 4-mercapto-4-methylpentan-2-one (4MMP) have a fermentative origin. Second, the order of relevance is quite different from that found in a similar experiment with the same semi-synthetic must using commercial strains (Denat et al., 2021). A most remarkable difference is the presence in this extract of 4MMP, a volatile thiol responsible for the "box tree" aroma that characterized Sauvignon Blanc wines (Tominaga et al., 1995). Polyfunctional mercaptans can be released from their non-odorous form by yeast action (Roland et al., 2011; Swiegers and Pretorius, 2007). However, using wine strains in the previous experiment, PFMs were not detected in volatile fraction released during fermentation, which strongly suggests that some of the non-conventional yeasts used in this experiment released it from its specific non-aromatic precursor since the early stages of fermentation. Also most remarkable is the absence in the list of the Strecker aldehyde isovaleraldehyde, which was the second most intense odorant emitted by commercial *Saccharomyces* yeasts, and together with isobutyraldehyde, a major component of the effluent of those fermentations (Denat et al., 2021).

3.3.2. Aroma composition in young wines

Among the major volatile compounds (22 volatile compounds found at concentrations >0.2 mg/L), 1-hexanol was the only compound that did not present differences between young wines. However, PCA analysis (Fig. 3) shows that differences between strains were not very large, explaining 38.2% of the total variance. Although all *S. eubayanus* strains were in the up-left part of the plot because of decanoic acid and butanol highest levels, this should not represent any sensory mismatch. Nevertheless, some differences and observations should be mentioned; first, the significant levels of decanoic acid reached by the three *S. eubayanus* strains, particularly E3 producing 3 mg/L. Secondly, decanoic acid, also known as capric acid, is a known toxic compound able to inhibit the growth of cells by altering their membranes (Lafon-Lafourcade et al., 1984; Viegas et al., 1989).

S. kudriavzevii strains contained the highest levels of ethyl lactate, which corresponds with the highest lactic acid production observed. As it is well documented in other works (Minebois et al., 2020a, 2020b; Pérez et al., 2021; Stribny et al., 2015), the species *S. uvarum* (particularly, U1 and BMV58 strains), followed by the *S. eubayanus* (particularly, E1 and E2 strains), were characterized by a high production of β-phenylethanol.

Since the esters are not particularly abundant in red wines, and they are very easily vaporized when fermentation is carried out in little volumes, it should be noted that some positive traits were found among the fruity ethyl esters. Namely, ethyl octanoate was only detected in BMV58 strain, the *S. cerevisiae* C1 produced ethyl decanoate highest levels, and ethyl propanoate was 8 and 4-fold higher in E1 and U1 (0.22 mg/L and 0.12 mg/L, respectively) than in the rest of the strains (0.03 mg/L). The latter result is relevant because ethyl propanoate, not exceeding its perception threshold, can act together with other esters as a "mature fruit" aroma enhancer (Puertas et al., 2018). Finally, K1 strain synthesized diacetyl at higher levels. This compound may give a butter or lactic character to young wine (Ugliano and Henschke, 2009), but it is very reactive towards polyphenols so that it will not survive aging.

The aromatic diversity of trace compounds in young wines introduced by the yeast is summarized in the heatmap shown in Fig. 4. Strains can be classified into three main groups. Cluster A contained the yeasts E1, U1, C1, and T73 and is characterized by major levels of C_{13}-norisoprenoids, monoterpenes, vanillin derivatives, acetates, and ethyl leucate. Strains in cluster C were C3, BMV58, E2, K2, and VellEvol had higher levels of varietal volatile phenols, while strains in cluster B seem to be more neutral.

Regarding this group of aroma compounds, measured levels of all of them also fell within the normal range of these compounds in red wines; however, the most significant group of yeasts was cluster A. Within this cluster, the single compound found at sensory-relevant levels and slightly above usual red wine contents (Ferreira et al., 2000) was phenylethyl acetate, found in E1 and U1 close to 1 mg/L (Table S4). Besides the monoterpenes were found at concentrations under their OTs (odor thresholds), it is interesting that *S. uvarum* U1 achieved the maximal levels of β-citronellol and geraniol. This characteristic is attributed to the ability of *S. uvarum* strains to release geraniol from the odorless precursors (Gamero et al., 2011a, 2011b) whereas, some studies have determined that β-citronellol is completely synthesized from geraniol by yeasts (Fernández-González and Di Stefano, 2004; King and Dickinson, 2000; Takoi et al., 2014).

Since Tempranillo is a neutral variety, yeast's role in releasing its aromas in young wines is considered of great significance (Ferreira and López, 2019). Based on the most relevant major and minor aroma compounds found in young wines, we observed that the wild strains of *Saccharomyces* species could improve the aroma profile of Tempranillo. According to the obtained, E1 strain was characterized by ethyl propanoate, lactones, and acetates; C1 strain by vanillin derivatives and ethyl decanoate; K3 strain by fruity branched ethyl esters and β-ionone and U1 by monoterpenes.

3.3.3. Trace aroma compounds in aging wines

Accelerated anoxic aging (5 weeks at 50 °C) was applied only with the ten strains that presented a better fermentation capability and to the unfermented control must. As expected, aging involved an intense

Table 2
Odorants identified in a mix of volatiles released during all fermentations that were trapped into the LiChrolut-EN cartridges placed in each airlock. Determination of the retentions indexes (RI) in DB-WAX and DB-5 columns, olfatometrics scores (MF%), and odor descriptors.

RI_{DB-WAX}	RI_{DB-5}	Compound	Odor description*	MF (%)
1224	<900	Isoamyl alcohol	*Glue, cheese*	73
1932	1115	β-Phenylethanol	*Roses, flowery*	68
953	<900	Ethyl isobutyrate	*Strawberry cream*	65
1244	988	Ethyl hexanoate	*Fruity, flowery*	61
1037	<900	Ethyl butyrate	*Strawberry*	59
2030		o-Cresol	*Metallic*	58
1388		4-Mercapto-4-methylpentan-2-one	*Tropical fruit, sweat*	50
1515	1193	Ethyl octanoate	*Plastic, woody, green*	50
974	<900	Isopropyl acetate	*Strawberry, fruity*	41
1129	<900	Isoamyl acetate	*Banana, nail polish*	37
1832	1263	β-Phenylethyl acetate	*Roses, flowery*	30

* Odor descriptors correspond to those identified in the mix-sample by the panel that performed the GC-O test.

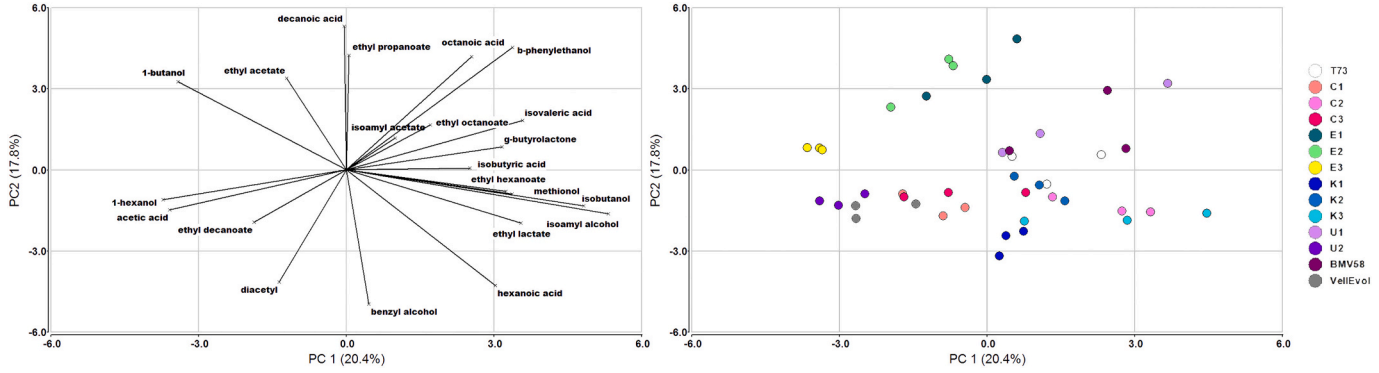

Fig. 3. Principal component analysis (PCA) applied on the major aroma compounds (>0.2 mg/L) determined in young wines fermented by the different strains where each replicate is represented. ANOVA results are presented in the supplementary material (Table S3).

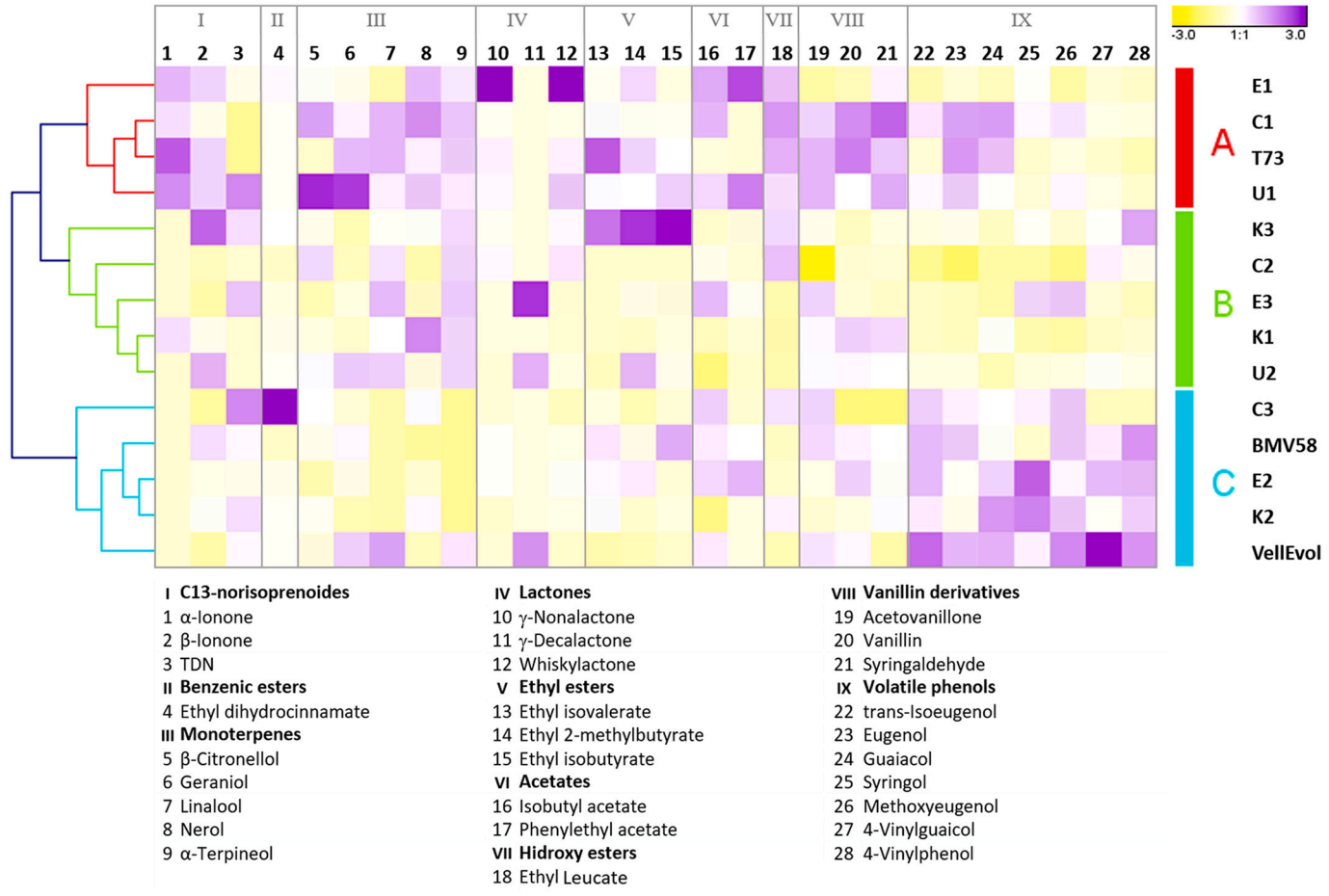

Fig. 4. For each aroma compound determined in the young wines fermented by 14 yeast strains, data were normalized by the average value of all the strains and were represented by a color scale being in yellow the lowest values and violet the highest values of each compound. Using these aroma data, hierarchical grouping was applied to obtain a dendrogram representing the separation of the yeasts according to their similarities in the aroma profiles. The values of each concentration and the ANOVA results are presented in Supplementary Material Table S4. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

aroma formation, and many aroma compounds reached sensory-relevant concentrations, particularly true for two norisoprenoids and the ethyl esters of branched acids.

The two C_{13}-norisoprenoids, β-ionone, and β-damascenone reach maxima levels close to 1 ppb and 16 ppb, respectively (Table 3). These two aroma compounds play the most relevant role in red wine aroma. β-Ionone is a powerful aroma usually found in red wines providing floral, red, or dark berry aroma notes (Tomasino and Bolman, 2021), and β-damascenone is a potent aroma enhancer and modulator (Pineau et al., 2007). At low concentrations, this compound contributes to fresh fruity notes, and at higher levels, it can increase the ripen character of the fruit (San-Juan et al., 2011). Its effects are strongly dependent on the sensory context (Tomasino and Bolman, 2021). The levels of both compounds found in the present work are exceptionally high (San-Juan et al., 2012). The effects of the yeast strain were very high in the case of β-ionone, whose levels ranged from 0.13 in VellEv to 0.86 in T73, E1,

Table 3
Concentration (μg/L) and odor threshold of trace aroma compounds detected in wines and must containing PAF after aging.

	Must	T73	C1	C2	E1	E2	K1	K2	U1	BMV58	VellEv	OT (μg/L)
C_{13}-norisoprenoids												
α-Ionone	0.15 ± 0.03^{ab}	0.13 ± 0.01^{b}	0.13 ± 0.04^{b}	0 ± 0^{c}	0.15 ± 0.01^{ab}	0.18 ± 0.02^{a}	0 ± 0^{c}	0.14 ± 0.01^{ab}	0 ± 0^{c}	0 ± 0^{c}	0 ± 0^{c}	2.6[III]
β-Ionone	**0.33 ± 0.01^{bcd}**	**0.86 ± 0.07^{a}**	**0.43 ± 0.21^{bc}**	**0.18 ± 0.05^{cd}**	**0.86 ± 0.11^{a}**	**0.33 ± 0.02^{bcd}**	**0.34 ± 0.04^{bcd}**	**0.31 ± 0.01^{bcd}**	**0.86 ± 0.08^{a}**	**0.59 ± 0.2^{ab}**	**0.13 ± 0.03^{d}**	0.09[IV]
TDN	**18.29 ± 4.67^{b}**	**34.1 ± 7.17^{a}**	**25.37 ± 3.75^{ab}**	**35.96 ± 5.62^{a}**	**29.21 ± 4.05^{ab}**	**22.49 ± 2.33^{ab}**	**28.11 ± 5.26^{ab}**	**23.72 ± 1.14^{ab}**	**33.28 ± 5.92^{a}**	**26.41 ± 5.35^{ab}**	**23.49 ± 2.68^{ab}**	2[VII]
β-Damascenone	**11.2 ± 1.36^{b}**	**14.46 ± 0.99^{ab}**	**13.88 ± 0.71^{ab}**	**13.69 ± 1.43^{ab}**	**14.98 ± 0.91^{ab}**	**13.12 ± 0.93^{ab}**	**14.33 ± 0.25^{ab}**	**15.64 ± 0.69^{a}**	**11.96 ± 2.1^{ab}**	**13.47 ± 3.06^{ab}**	**13.36 ± 1.31^{ab}**	0.05[VI]
Vitispirane A and B*	0.4 ± 0.05^{abc}	0.49 ± 0.04^{ab}	0.41 ± 0.04^{abc}	0.51 ± 0.06^{a}	0.46 ± 0.05^{abc}	0.38 ± 0.03^{bc}	0.4 ± 0.04^{abc}	0.37 ± 0.02^{c}	0.46 ± 0.05^{abc}	0.43 ± 0.03^{abc}	0.39 ± 0.04^{bc}	
Riesling acetal*	0.23 ± 0.01^{a}	0.23 ± 0.02^{a}	0.22 ± 0.01^{a}	0.22 ± 0.02^{a}	0.23 ± 0.01^{a}	0.2 ± 0.0049^{a}	0.22 ± 0.01^{a}	0.21 ± 0.01^{a}	0.22 ± 0.02^{a}	0.21 ± 0.01^{a}	0.22 ± 0.02^{a}	
Monoterpenes												
β-Citronellol	0.17 ± 0.02^{d}	1.01 ± 0.07^{abc}	1.34 ± 0.06^{ab}	0.44 ± 0.09^{cd}	0.93 ± 0.37^{abcd}	0.23 ± 0.06^{cd}	0.79 ± 0.18^{abcd}	0.69 ± 0.11^{bcd}	1.54 ± 0.67^{a}	0.88 ± 0.4^{abcd}	0.54 ± 0.11^{cd}	40[IX]
Geraniol	0 ± 0^{d}	1.28 ± 0.38^{a}	1.05 ± 0.19^{abc}	0.52 ± 0.12^{bcd}	0.76 ± 0.06^{abc}	0.44 ± 0.24^{cd}	0.8 ± 0.06^{abc}	0.7 ± 0.09^{abc}	1.13 ± 0.43^{ab}	0.75 ± 0.08^{abc}	0.71 ± 0.17^{abc}	30[VI]
Linalool	0 ± 0^{b}	2.06 ± 0.12^{a}	0 ± 0^{b}	0 ± 0^{b}	1.83 ± 0.22^{a}	0 ± 0^{b}	1.61 ± 0.42^{a}	0 ± 0^{b}	1.87 ± 0.38^{a}	1.69 ± 0.14^{a}	0 ± 0^{b}	25.2[IV]
Linalool oxide	3.81 ± 0.39^{ab}	4.14 ± 0.4^{a}	3.49 ± 0.52^{ab}	4.14 ± 0.48^{a}	3.72 ± 0.41^{ab}	2.79 ± 0.22^{b}	3.14 ± 0.43^{ab}	2.68 ± 0.24^{b}	3.86 ± 0.42^{ab}	3.45 ± 0.56^{ab}	3.12 ± 0.4^{ab}	4000(*c*)/4000(*t*)[X]
α-Terpineol	4.83 ± 0.74^{d}	11.2 ± 1.04^{ab}	10.55 ± 0.88^{abc}	8.76 ± 2.25^{bcd}	10.36 ± 1.04^{abc}	7.69 ± 0.3^{bcd}	8.95 ± 0.31^{abcd}	6.68 ± 0.33^{cd}	13.15 ± 3.38^{a}	9.14 ± 0.76^{abcd}	10.39 ± 2^{abc}	250[IV]
Lactones												
γ-Nonalactone	0.88 ± 0.44^{b}	2.7 ± 0.3^{a}	2.37 ± 0.11^{a}	2.42 ± 0.41^{a}	2.46 ± 0.15^{a}	2.23 ± 0.05^{a}	2.14 ± 0.09^{a}	2.38 ± 0.25^{a}	2.53 ± 0.24^{a}	2.49 ± 0.26^{a}	2.07 ± 0.09^{a}	30[XI]
γ-Decalactone	0 ± 0^{e}	0.74 ± 0.03^{d}	0.97 ± 0.11^{bcd}	1.03 ± 0.07^{abcd}	1.68 ± 0.55^{a}	1.07 ± 0.1^{abcd}	1.5 ± 0.16^{abc}	1.25 ± 0.26^{abcd}	1.64 ± 0.39^{ab}	1.08 ± 0.12^{abcd}	0.83 ± 0.1^{cd}	88[II]
Whisky lactone	0 ± 0^{f}	5.7 ± 0.69^{bcd}	3.9 ± 0.27^{cde}	5.74 ± 0.77^{bc}	12.6 ± 1.88^{a}	4.07 ± 0.11^{cde}	3.3 ± 0.14^{de}	3.82 ± 0.08^{cde}	7.13 ± 1.47^{b}	3.85 ± 0.74^{cde}	2.9 ± 0.27^{e}	790 (*t*)/67 (*c*)[XII]
Massoia lactone	**1.69 ± 0.04^{b}**	**1.51 ± 0.25^{bc}**	**1.3 ± 0.17^{bc}**	**1.69 ± 0.22^{b}**	**2.37 ± 0.28^{a}**	**1.4 ± 0.13^{bc}**	**1.17 ± 0.11^{bc}**	**1.28 ± 0.16^{bc}**	**1.42 ± 0.33^{bc}**	**1.29 ± 0.19^{bc}**	1.07 ± 0.08^{c}	11[XIV]XV
Ethyl esters												
Ethyl isovalerate	0 ± 0^{d}	**72.96 ± 9.41^{a}**	**32.19 ± 8.14^{bc}**	**22.46 ± 6.39^{bcd}**	**25.65 ± 8.47^{bcd}**	**28.31 ± 6.31^{bcd}**	**26.82 ± 3.36^{bcd}**	**35.9 ± 1.79^{bc}**	**44.8 ± 23.45^{ab}**	**42.12 ± 13.74^{b}**	**8.74 ± 2.09^{cd}**	3[IV]
Ethyl 2-methylbutyrate	0 ± 0^{d}	**75.42 ± 13.53^{a}**	**34.28 ± 6.66^{bc}**	**27.83 ± 6.48^{bc}**	**48.65 ± 14.07^{ab}**	**50.88 ± 3.98^{ab}**	**27.69 ± 3.14^{bc}**	**31.05 ± 1.77^{bc}**	**50.77 ± 17.3^{ab}**	**42.56 ± 12.41^{b}**	10.52 ± 2.49^{cd}	18[IV]
Ethyl isobutyrate	0 ± 0^{c}	**283.34 ± 39.19^{b}**	**202.76 ± 50.23^{bc}**	**130.01 ± 45.35^{bc}**	**146.69 ± 34.38^{bc}**	**140.72 ± 3.48^{bc}**	**254.33 ± 29.12^{b}**	**205.59 ± 38.65^{bc}**	**556.57 ± 153.05^{a}**	**659.67 ± 201.42^{a}**	**96.05 ± 28.87^{bc}**	15[IV]
Ethyl D/L-leucate	0 ± 0^{c}	**90.71 ± 40.88^{ab}**	**61.4 ± 11.43^{bc}**	**160.09 ± 65.1^{a}**	45.46 ± 6.55^{bc}	40.53 ± 4.06^{bc}	34.88 ± 4.87^{bc}	**73.97 ± 9.89^{bc}**	**69.37 ± 26.42^{bc}**	53.21 ± 18.15^{bc}	34.46 ± 8.15^{bc}	900 (D)/300 (L)[XIII]
Acetates												
β-Phenylethyl acetate	0 ± 0^{c}	**28.95 ± 7.67^{bc}**	**27.38 ± 4.21^{bc}**	24.44 ± 0.46^{bc}	**162.05 ± 21.83^{a}**	**167.11 ± 55.35^{a}**	**33.71 ± 3.46^{bc}**	**42.29 ± 1.73^{bc}**	**78.22 ± 8.85^{b}**	**75.12 ± 4.59^{b}**	**41.42 ± 7.57^{bc}**	250[VI]
Isobutyl acetate	0 ± 0^{f}	23.22 ± 1.2^{abc}	27.18 ± 6.08^{ab}	20.08 ± 7.72^{abcd}	8.77 ± 1.5^{def}	16.37 ± 2.06^{bcde}	20.22 ± 4.65^{abcd}	4.95 ± 0.52^{ef}	10.6 ± 1.19^{cdef}	32.77 ± 10.82^{a}	18.54 ± 0.69^{bcde}	1605[V]
Vanillin derivatives												
Acetovanillone	2.98 ± 1.04^{c}	24.5 ± 2.09^{ab}	26.54 ± 2.21^{a}	20.18 ± 1.46^{b}	23.71 ± 1.44^{ab}	25.75 ± 0.43^{a}	24.97 ± 0.27^{ab}	25.21 ± 3.97^{ab}	25.73 ± 1.55^{a}	24.77 ± 0.96^{ab}	22.04 ± 0.61^{ab}	1000[I]
Vanillin	5.87 ± 0.55^{d}	12.62 ± 1.36^{ab}	14.11 ± 3.32^{a}	8.24 ± 0.69^{bcd}	8.6 ± 1.21^{bcd}	11.02 ± 0.01^{abc}	9.03 ± 1.35^{bcd}	7.53 ± 2.28^{cd}	10.88 ± 1.59^{abc}	8.85 ± 0.68^{bcd}	10.93 ± 0.28^{abc}	200[VI]
Syringaldehyde	145.57 ± 11.09^{ab}	94.93 ± 29.19^{b}	110.53 ± 4.45^{b}	205.07 ± 51.43^{a}	60.21 ± 12.71^{b}	103.67 ± 8.87^{b}	113.15 ± 1.97^{ab}	140.13 ± 58.5^{ab}	139.25 ± 3.42^{ab}	124.97 ± 27.59^{ab}	119.93 ± 53.4^{ab}	50,000[I]
Volatile phenols												
trans-Isoeugenol	0 ± 0^{b}	0.41 ± 0.08^{a}	0.5 ± 0.09^{a}	0.34 ± 0.04^{a}	0.34 ± 0.09^{a}	0.39 ± 0.07^{a}	0.42 ± 0.04^{a}	0.36 ± 0.07^{a}	0.43 ± 0.09^{a}	0.45 ± 0.03^{a}	0.4 ± 0.06^{a}	6[I]
Eugenol	0 ± 0^{d}	0 ± 0^{d}	0.51 ± 0.02^{a}	0 ± 0^{d}	0 ± 0^{d}	0 ± 0^{d}	0.33 ± 0.03^{c}	0 ± 0^{d}	0.42 ± 0.04^{b}	0 ± 0^{d}	0.4 ± 0.04^{bc}	6[IV]
Guaiacol	**5.04 ± 0.6^{a}**	**3.47 ± 0.36^{a}**	**5.15 ± 0.87^{a}**	**3.26 ± 0.81^{a}**	**3.86 ± 1.41^{a}**	**3.04 ± 0.58^{a}**	**4.2 ± 0.43^{a}**	**3.4 ± 0.75^{a}**	**5.08 ± 1.12^{a}**	**3.06 ± 0.25^{a}**	**4.82 ± 0.9^{a}**	9.5[IV]
Syringol	**91.86 ± 9.85^{a}**	43.53 ± 9.41^{b}	55.35 ± 4.28^{b}	44.57 ± 7.01^{b}	44.77 ± 21.71^{b}	53.79 ± 12.5^{b}	39.1 ± 4.26^{b}	33.04 ± 8.25^{b}	**57.85 ± 15.8^{b}**	52.57 ± 4.54^{b}	43.77 ± 5.24^{b}	570[VIII]
Methoxyeugenol	1.78 ± 0.23^{d}	3.79 ± 0.64^{ab}	3.88 ± 0.14^{a}	2.86 ± 0.37^{bc}	2.97 ± 0.12^{abc}	2.78 ± 0.28^{c}	2.91 ± 0.33^{abc}	2.46 ± 0.23^{cd}	3.36 ± 0.16^{abc}	3.23 ± 0.51^{abc}	2.77 ± 0.31^{c}	1200[I]
4-Vinylguaicol	22.18 ± 5.55^{c}	24.13 ± 2.34^{c}	28.46 ± 4.87^{bc}	19.07 ± 4.06^{c}	19.8 ± 2.37^{c}	23.3 ± 0.1^{c}	49.89 ± 2.77^{a}	40.06 ± 11.71^{ab}	23.84 ± 1.66^{c}	26.33 ± 6.2^{bc}	23.38 ± 0.62^{c}	1100[IV]
4-Vinylphenol	**119.56 ± 23.94^{ab}**	**120.66 ± 25.79^{ab}**	**124.16 ± 17.78^{ab}**	**98.22 ± 14.74^{ab}**	**87.75 ± 9.59^{b}**	**114.59 ± 9.94^{ab}**	**151.35 ± 7.88^{a}**	**117.36 ± 41.92^{ab}**	**106.31 ± 6.21^{ab}**	**110.56 ± 17.39^{ab}**	**108.67 ± 6.15^{ab}**	180[III]

Data are expressed as mean value ± SD (n = 3). Different letters in the same row indicate significant differences according to HSD Tukey test ($p < 0.05$). Each compound whose concentration exceeded its threshold of perception about OAV > 0.1, marked in bold type.

[*] Data expressed as relative area.

[I] Escudero et al., 2007.
[II] Etievant, 1991.
[III] Boidron et al., 1988.
[IV] Ferreira et al., 2000.
[V] Ferreira et al., 2002.
[VI] Guth, 1997.
[VII] Sacks et al., 2012.
[VIII] López et al., 2002.
[IX] Ohloff, 1978.
[X] Ribéreau-Gayon et al., 1975.
[XI] Nakamura et al., 1988.
[XII] Otsuka et al., 1974.
[XIII] Falcao et al., 2012.
[XIV] Pons et al., 2017.
[XV] Simpson, 1978.

and U1, and moderate in the case of β-damascenone (Table 3). Levels between yeasts were not significantly different, as all yeasts produced levels of this compound above those found in the unfermented control. This result confirms that yeast cannot produce this compound but can accelerate its release, as recently reported (Denat et al., 2021).

Levels of ethyl esters of branched acids are also relatively high compared to commercial *S. cerevisiae* strains, such as the levels of ethyl leucate (Denat et al., 2021). In any case, levels of these families of esters increase with aging because they are slowly formed by esterification with ethanol of their corresponding acids (Díaz-Maroto et al., 2005). Differences between maxima and minima levels reached were close to 9, 7.5, 7.0, and 5-fold in ethyl isovalerate, 2-methylbutyrate, isobutyrate, and leucate, respectively, depending on the strain in all cases (Table 3). Ethyl isovalerate and ethyl 2-methylbutyrate were maximal in T73, followed by U1 and BMV58, with the highest ethyl isobutyrate concentrations. In contrast, the maxima levels of ethyl leucate were found in C2. Some authors have suggested, generally working with elementary models, that some of the individual ethyl esters could play a role in the specific aroma nuances of wines (Lytra et al., 2012). However, it has been recently demonstrated that 14 different fruity ethyl esters (including the ethyl esters of linear fatty acids) qualitatively integrate within a unique fruity descriptor (De-la-Fuente-Blanco et al., 2020), and the intensity is the addition of the individual aromas (Ferreira et al., 2021). Therefore, the aroma of these compounds is the nuclear part of the fruity perception in red wines, and the results presented here reveal the most notable differences between yeast strains.

To summarize, when aging was applied to young wines, a synergic effect with some non-conventional yeasts was observed, favoring the release and generation of compounds of relevant aroma impact and importance for the aged wines. Some of the red-fruit smelling compounds were ethyl leucate and ethyl isobutyrate, which were found at high levels in C2 and U1 strain, respectively. While another important compound, such as β-ionone, with floral-fruity notes, was highly increased by U1 and E1 yeasts.

4. Conclusion

In this study, *S. cerevisiae* and *S.* non-*cerevisiae* strains isolated from wild and fermentative sources were used to ferment synthetic must with the addition of odorless aroma precursors and phenolic compounds extracted from Tempranillo grapes. Then, accelerated anoxic aging was applied to the young wines. The main objective was to determine the aromatic diversity introduced by using non-wine strains in young and aged Tempranillo wines.

Considering the aromas volatilized during fermentation, the presence of 4MMP showed that the non-conventional yeasts used could have a greater capacity to release these compounds than the commercial strains.

The young wines obtained with non-wine strains as U1 (*S. uvarum*), E1 (*S. eubayanus*), C3, C1 (*S. cerevisiae*), and K3 (*S. kudriavzevii*) were characterized by the highest levels of fruity ethyl esters, acetates, monoterpenes, and C_{13}-norisoprenoids. *S. uvarum* strains were also characterized by the ability to release or *de novo*-produced geraniol and citronellol, as well as by the highest production of β-phenylethyl acetate, as has been previously described by other authors. In addition, *S. eubayanus* strains can release high levels of this acetate in young and aged wines. Due to their non-fermentative origin, these yeasts showed lower fermentative capacity compared to the wine *S. cerevisiae* strain. However, they were able to complete the fermentation process, and the resulting wines reached enological parameters of interest in the wine industry (lower ethanol level, higher organic acid, and glycerol content).

Several compounds were highly enhanced during aging by the yeast action. T73, U1, and BMV58 strains exhibited important aging compounds: ethyl isobutyrate, ethyl 2-methylbutyrate, and ethyl isovalerate. E1, T73, and U1 strains were shown to accelerate the release of β-ionone, reaching the highest levels. In addition, aging allowed some yeast that had not been prominent in the young wines to develop relevant aromas after aging, as the case of the highest production of ethyl leucate determined in C2 aged wine.

Therefore, according to the results obtained in this study and considering the current consumer preferences for more complex aromatic profiles, wild non-wine and cryotolerant strains would be recommended for the wine industry.

CRediT authorship contribution statement

Dolores Pérez: Investigation, Formal analysis, Writing – original draft. **Marie Denat:** Investigation, Formal analysis. **José María Heras:** Conceptualization, Methodology. **José Manuel Guillamón:** Conceptualization, Methodology. **Vicente Ferreira:** Conceptualization, Methodology. **Amparo Querol:** Conceptualization, Methodology, Writing – original draft, Funding acquisition.

Declaration of competing interest

All the authors have seen and approved the present manuscript, they have contributed significantly to different parts of the work, and this manuscript has not been published elsewhere and is not being considered for publication in any other journal.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijfoodmicro.2022.109554.

References

Alegre, Y., Arias-Pérez, I., Hernández-Orte, P., Ferreira, V., 2020a. Development of a new strategy for studying the aroma potential of winemaking grapes through the accelerated hydrolysis of phenolic and aromatic fractions (PAFs). Food Res. Int. 127, 108728 https://doi.org/10.1016/j.foodres.2019.108728.

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

Arroyo-López, F.N., Salvadó, Z., Tronchoni, J., Guillamón, J.M., Barrio, E., Querol, A., 2010. Susceptibility and resistance to ethanol in Saccharomyces strain isolated from wild and fermentative environments. Yeast (September), 191–198. https://doi.org/10.1002/yea.

Boidron, J.N., Chatonnet, P., Pons, M., 1988. Influence of wood on some aromatic substances in wines. Connaiss Vigne Vin 22 (4), 275–294.

Charters, S., Pettigrew, S., 2007. The dimensions of wine quality. Food Qual. Prefer. 18 (7), 997–1007. https://doi.org/10.1016/j.foodqual.2007.04.003.

Cordente, A.G., Schmidt, S., Beltran, G., Torija, M.J., Curtin, C.D., 2019. Harnessing yeast metabolism of aromatic amino acids for fermented beverage bioflavouring and bioproduction. Appl. Microbiol. Biotechnol. 103 (11), 4325–4336. https://doi.org/10.1007/s00253-019-09840-w.

Dravnieks, A., 1985. Atlas of odor character profiles. ASTM, Philadelphia.

De-la-Fuente-Blanco, A., Sáenz-Navajas, M.P., Valentin, D., Ferreira, V., 2020. Fourteen ethyl esters of wine can be replaced by simpler ester vectors without compromising quality but at the expense of increasing aroma concentration. Food Chemistry 307 (April 2019), 125553. https://doi.org/10.1016/j.foodchem.2019.125553.

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 9 (December 2020). 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. J. Agric. Food Chem. 53 (9), 3503–3509. https://doi.org/10.1021/jf048157o.

Escudero, A., Campo, E., Fariña, L, Cacho, J., Ferreira, V., 2007. Analytical characterization of the aroma of five premium red wines. Insights into the role of odor families and the concept of fruitiness of wines. J. Agric. Food Chem. 55 (11), 4501–4510. https://doi.org/10.1021/jf0636418.

Etievant, P.X., 1991. Volatile compounds of food and beverages. Wine. Marcel Dekker, New York.

Falcao, L.D., Lytra, G., Darriet, P., Barbe, J.C., 2012. Identification of ethyl 2-hydroxy-4-methylpentanoate in red wines, a compound involved in blackberry aroma. Food Chem. 132 (1), 230–236. https://doi.org/10.1016/j.foodchem.2011.10.061.

Fernández-González, M., Di Stefano, R., 2004. Fractionation of glycoside aroma precursors in neutral grapes. Hydrolysis and conversion by Saccharomyces cerevisiae. LWT Food Sci. Technol. 37 (4), 467–473. https://doi.org/10.1016/j.lwt.2003.11.003.

Ferreira, V., López, R., Cacho, J.F., 2000. Quantitative determination of the odorants of young red wines from different grape varieties. J. Sci. Food Agric. 80 (12), 1659–1667.

Ferreira, V., Ortín, N., Escudero, A., López, R., Cacho, J., 2002. Chemical characterization of the aroma of grenache rosé wines: aroma extract dilution analysis, quantitative determination, and sensory reconstitution studies. J. Agric. Food Chem. 50 (14), 4048–4054. https://doi.org/10.1021/jf0115645.

Ferreira, V., López, R., 2019. The actual and potential aroma of winemaking grapes. Biomolecules 9 (12). https://doi.org/10.3390/biom9120818.

Ferreira, V., Sáenz-Navajas, M.P., de-la-Fuente-Blanco, A., 2021. Odorants to interpret complex aroma systems. Application to model wine aroma. Foods 10 (1627), 1–19. https://doi.org/10.3390/foods10071627.

Gamero, A., Hernández-Orte, P., Querol, A., Ferreira, V., 2011a. Effect of aromatic precursor addition to wine fermentations carried out with different saccharomyces species and their hybrids. Int. J. Food Microbiol. 147 (1), 33–44. https://doi.org/10.1016/j.ijfoodmicro.2011.02.035.

Gamero, A., Manzanares, P., Querol, A., Belloch, C., 2011b. Monoterpene alcohols release and bioconversion by saccharomyces species and hybrids. Int. J. Food Microbiol. 145 (1), 92–97. https://doi.org/10.1016/j.ijfoodmicro.2010.11.034.

Guth, H, 1997. Quantitation and sensory studies of character impact odorants of different white wine varieties. J. Agric. Food Chem. 45 (8), 3027–3032.

Hernández-Orte, P., Bely, M., Cacho, J., Ferreira, V., 2006. Impact of ammonium additions on volatile acidity, ethanol, and aromatic compound production by different Saccharomyces cerevisiae strains during fermentation in controlled synthetic media. Aust. J. Grape Wine Res. 12 (2), 150–160.

Hernández-Orte, P., Cersosimo, M., Loscos, N., Cacho, J., García-Moruno, E., Ferreira, V., 2008. The development of varietal aroma from non-floral grapes by yeasts of different genera. Food Chem. 107 (3), 1064–1077. https://doi.org/10.1016/j.foodchem.2007.09.032.

Hjelmeland, A.K., Ebeler, S.E., 2015. Glycosidically bound volatile aroma compounds in grapes and wine: a review. Am. J. Enol. Vitic. 66 (1), 1–11. https://doi.org/10.5344/ajev.2014.14104.

King, A., Dickinson, J.R., 2000. Biotransformation of monoterpene alcohols by Saccharomyces cerevisiae,Torulaspora delbrueckii and Kluyveromyces lactis. Yeast 16 (6), 499–506. https://doi.org/10.1002/(SICI)1097-0061(200004)16:6<499::AID-YEA548>3.0.CO;2-E.

Lafon-Lafourcade, S., Geneix, C., Ribereau-Gayon, P., 1984. Inhibition of alcoholic fermentation of grape must by fatty acids produced by yeasts and their elimination by yeast ghosts. Appl. Environ. Microbiol. 47 (6), 1246–1249. https://doi.org/10.1128/aem.47.6.1246-1249.1984.

Liu, J., Zhu, X.L., Ullah, N., Tao, Y.S., 2017. Aroma glycosides in grapes and wine. J. Food Sci. 82 (2), 248–259. https://doi.org/10.1111/1750-3841.13598.

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. J. Chromatogr. A 966 (1–2), 167–177. https://doi.org/10.1016/S0021-9673(02)00696-9.

López, R., Ezpeleta, E., Sánchez, I., Cacho, J., Ferreira, V., 2004. Analysis of the aroma intensities of volatile compounds released from mild acid hydrolysates of odourless precursors extracted from tempranillo and grenache grapes using gas chromatography-olfactometry. Food Chem. 88 (1), 95–103. https://doi.org/10.1016/j.foodchem.2004.01.025.

Lytra, G., Tempere, S., De Revel, G., Barbe, J.C., 2012. Distribution and organoleptic impact of ethyl 2-hydroxy-4-methylpentanoate enantiomers in wine. J. Agric. Food Chem. 60 (6), 1503–1509. https://doi.org/10.1021/jf204378u.

Minebois, R., Pérez-Torrado, R., Querol, A., 2020a. A time course metabolism comparison among Saccharomyces cerevisiae, S. uvarum and S. kudriavzevii species in wine fermentation. Food Microbiol. 90, 1–14. https://doi.org/10.1016/j.fm.2020.103484.

Minebois, R., Pérez-Torrado, R., Querol, A., 2020b. Metabolome segregation of four strains of Saccharomyces cerevisiae, Saccharomyces uvarum and saccharomyces kudriavzevii conducted under low temperature oenological conditions. Environ. Microbiol. 22 (9), 3700–3721. https://doi.org/10.1111/1462-2920.15135.

Minebois, R., Lairón-Peris, M., Barrio, E., Pérez-Torrado, R., Querol, A., 2021. Metabolic differences between a wild and a wine strain of Saccharomyces cerevisiae during fermentation unveiled by multi-omic analysis. Environ. Microbiol. 23, 3059–3076. https://doi.org/10.1111/1462-2920.15523.

Nakamura, S.E., Crowell, E.A., Ough, C.S., Totsuka, A., 1988. Quantitative analysis of γ-nonalactone in wines and its threshold determination. J. Food Sci. 53, 1243–1244.

Ohloff, G., 1978. Importance of minor components in flavors and fragances. Perfum. Flavor 3 (11), 11–22.

Oliveira, B.M., Barrio, E., Querol, A., Pérez-Torrado, R., 2014. Enhanced enzymatic activity of glycerol-3-phosphate dehydrogenase from the cryophilic saccharomyces kudriavzevii. PLoS ONE 9 (1). https://doi.org/10.1371/journal.pone.0087290.

Origone, A.C., del Mónaco, S.M., Ávila, J.R., González Flores, M., Rodríguez, M.E., Lopes, C.A., 2017. Tolerance to winemaking stress conditions of patagonian strains of Saccharomyces eubayanus and Saccharomyces uvarum. J. Appl. Microbiol. 123 (2), 450–463. https://doi.org/10.1111/jam.13495.

Ortega, C., López, R., Cacho, J., Ferreira, V., 2001. Fast analysis of important wine volatile compounds - development and validation of a new method based on gas chromatographic-flame ionisation detection analysis of dichloromethane microextracts. J. Chromatogr. A 923 (1–2), 205–214. https://doi.org/10.1016/S0021-9673(01)00972-4.

Otsuka, K., Zenibayashi, Y., Itoh, M., Totsuka, A., 1974. Presence and Significance of two diastereomers of β-methyl-γ-octalactone in aged distilled liquors. Agric. Biol. Chem. 38, 485–490.

Pérez, D., Jaehde, I., Guillamón, J.M., Heras, J.M., Querol, A., 2021. Screening of Saccharomyces strains for the capacity to produce desirable fermentative compounds under the influence of different nitrogen sources in synthetic wine fermentations. Food Microbiology 97 (September 2020), 103763. https://doi.org/10.1016/j.fm.2021.103763.

Pineau, B., Barbe, J.C., Van Leeuwen, C., Dubourdieu, D., 2007. Which impact for β-damascenone on red wines aroma? J. Agric. Food Chem. 55 (10), 4103–4108. https://doi.org/10.1021/jf070120r.

Pons, A., Allamy, L., Lavigne, V., Dubourdieu, D., Darriet, P., 2017. Study of the contribution of massoia lactone to the aroma of merlot and cabernet sauvignon musts and wines. Food Chem. 232, 229–236. https://doi.org/10.1016/j.foodchem.2017.03.151.

Puertas, B., Jimenez-Hierro, M.J., Cantos-Villar, E., Marrufo-Curtido, A., Carbú, M., Cuevas, F.J., Ruiz-Moreno, M.J., 2018. The influence of yeast on chemical composition and sensory properties of dry white wines. Food Chemistry 253 (June 2017), 227–235. https://doi.org/10.1016/j.foodchem.2018.01.039.

Querol, A., Pérez-Torrado, R., Alonso-del-Real, J., Minebois, R., Stribny, J., Oliveira, B. M., Barrio, E., 2018. New trends in the uses of yeasts in oenology. In: Advances in Food and Nutrition Research, , 1st ed.85, pp. 177–210. https://doi.org/10.1016/bs.afnr.2018.03.002.

Redzepovic, S., Orlic, S., Majdak, A., Kozina, B., Volschenk, H., Viljoen-Bloom, M., 2003. Differential malic acid degradation by selected strains of Saccharomyces during alcoholic fermentation. Int. J. Food Microbiol. 83 (1), 49–61. https://doi.org/10.1016/S0168-1605(02)00320-3.

Ribéreau-Gayon, P., Boidron, J.N., Terrier, A., 1975. Aroma of Muscat Grape Varieties. J. Agric. Food Chem. 23 (6), 1042–1047.

Roland, A., Schneider, R., Razungles, A., Cavelier, F., 2011. Varietal thiols in wine: discovery, analysis and applications. Chem. Rev. 111 (11), 7355–7376. https://doi.org/10.1021/cr100205b.

Rollero, S., Mouret, J.R., Bloem, A., Sanchez, I., Ortiz-Julien, A., Sablayrolles, J.M., Camarasa, C., 2017. Quantitative 13C-isotope labelling-based analysis to elucidate the influence of environmental parameters on the production of fermentative aromas during wine fermentation. Microb. Biotechnol. 10 (6), 1649–1662. https://doi.org/10.1111/1751-7915.12749.

Sacks, G.L., Gates, M.J., Ferry, F.X., Lavin, E.H., Kurtz, A.J., Acree, T.E., 2012. Sensory threshold of 1,1,6-trimethyl-1,2-dihydronaphthalene (TDN) and concentrations in young riesling and non-riesling wines. J. Agric. Food Chem. 60 (12), 2998–3004. https://doi.org/10.1021/jf205203b.

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. https://doi.org/10.1016/j.foodchem.2010.03.129.

San-Juan, F., Ferreira, V., Cacho, J., 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. J. Agric. Food Chem. 59 (14), 7916–7924. https://doi.org/10.1021/jf1048657.

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. J. Agric. Food Chem. 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. Vitis 17, 274–287.

Stribny, J., Gamero, A., Pérez-Torrado, R., Querol, A., 2015. Saccharomyces kudriavzevii and Saccharomyces uvarum differ from Saccharomyces cerevisiae during the production of aroma-active higher alcohols and acetate esters using their amino acidic precursors. Int. J. Food Microbiol. 205, 41–46. https://doi.org/10.1016/j.ijfoodmicro.2015.04.003.

Swiegers, J.H., Pretorius, I.S., 2007. Modulation of volatile sulfur compounds by wine yeast. Appl. Microbiol. Biotechnol. 74 (5), 954–960. https://doi.org/10.1007/s00253-006-0828-1.

Takoi, K., Itoga, Y., Takayanagi, J., Kosugi, T., Shioi, T., Nakamura, T., Watari, J., 2014. Screening of geraniol-rich flavor hop and interesting behavior of β-citronellol during fermentation under various hop-addition timings. J. Am. Soc. Brew. Chem. 72 (1), 22–29. https://doi.org/10.1094/ASBCJ-2014-0116-01.

Tomasino, E., Bolman, S., 2021. The potential effect of β-ionone and β-damascenone on sensory perception of pinot noir wine aroma. Molecules 26 (5). https://doi.org/10.3390/molecules26051288.

Tominaga, T., Masneuf, I., Dubourdieu, D., 1995. Mise en évidence d'un s-conjugué de la cystéine, précurseur d'arôme du sauvignon. J. Int. Sci. de La Vigne Du Vin 29 (4), 227–232.

Ugliano, M., 2009. Enzymes in winemaking. In: Wine Chemistry and Biochemistry. Springer, New York, pp. 103–126.

Ugliano, M., Henschke, P.A., 2009. Yeast and wine flavour. In: Wine Chemistry and Biochemistry. Springer, New York, pp. 313–392.

Viegas, C.A., Rosa, M.F., Correia, I.S., Novais, J.M., 1989. Inhibition of yeast growth by octanoic and decanoic acids produced during ethanolic fermentation. Appl. Environ. Microbiol. 55 (1), 21–28. https://doi.org/10.1128/aem.55.1.21-28.1989.

Waterhouse, A.L., Sacks, G.L., Jeffery, D.W., 2016. Understanding wine chemistry. John Wiley & Sons.

Zwietering, M.H., Jongenburger, I., Rombouts, F.M., Van't Riet, K., 1990. Modeling of the bacterial growth curve. Appl. Environ. Microbiol. 56 (6), 1875–1881 https://doi.org/0099-2240/90/061875-07$02.00/0.