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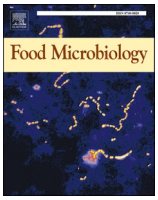

Modulation of aroma and chemical composition of Albariño semi-synthetic wines by non-wine *Saccharomyces* yeasts and bottle aging

Dolores Pérez [a,b,d], Marie Denat [c], Romain Minebois [d], José María Heras [a], José Manuel Guillamón [d], Vicente Ferreira [c], Amparo Querol [d,*]

[a] *Lallemand Bio S.L., 08028, Barcelona, Spain*
[b] *Estación Experimental Agropecuaria Mendoza (EEA), Instituto Nacional de Tecnología Agropecuaria (INTA), 5507, Luján de Cuyo, Mendoza, Argentina*
[c] *Laboratory for Aroma Analysis and Enology (LAAE), Department of Analytical Chemistry, Universidad de Zaragoza, Instituto Agroalimentario de Aragón (IA2) (UNIZAR-CITA), C/Pedro Cerbuna 12, 50009, Zaragoza, Spain*
[d] *Departamento de Biotecnología de Los Alimentos, Grupo de Biología de Sistemas en Levaduras de Interés Biotecnológico, Instituto de Agroquímica y Tecnología de Los Alimentos (IATA)-CSIC, 46980, Valencia, Spain*

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ABSTRACT

Saccharomyces yeasts from different origins and species fermented in a semi-synthetic must containing aroma precursor of cv. Albariño and polyfunctional mercaptans precursors. The resulting wines were subjected to accelerate anoxic aging. Afterward, aroma profiles were analyzed by distinct gas chromatography methodologies.

Cryotolerant strains showed better fermentation performances with significant differences in volatile and non-volatile fermentation products than *Saccharomyces cerevisiae* (*S. cerevisiae*). We suggested that the highest levels γ-butyrolactone and diethyl succinate in *Saccharomyces uvarum* (*S. uvarum*) strains, together with their substantial succinic acid yields, could be related to greater flux through the GABA shunt. These strains also had the highest production of β-phenylethyl acetate, geraniol, and branched-chain ethyl esters. The latter compounds were highly increased by aging, while acetates and some terpenes decreased. *S. kudriavzevii* strains showed a remarkable ability to release polyfunctional mercaptans, with SK1 strain yielding up to 47-fold and 8-fold more 4-methyl-4-mercaptopentan-2-one (4MMP) than *S. cerevisiae* and *S. uvarum* strains, respectively. The wild *S. cerevisiae* beer isolate showed a particular aroma profile due to the highest production of ethyl 4-methylvalerate (lactic and fruity notes), γ-octalactone (coconut), and furfurylthiol (roasted coffee). The latter compound is possibly produced from the pentose phosphate pathway (PPP). Since erythritol, another PPP intermediate was largely produced by this strain.

1. Introduction

The aroma of white wines is an essential factor defining their quality and varietal character. It results from the sensory contribution of aromatic metabolites proceeding from grapes (varietal aromas) and fermentation, including those produced during alcoholic fermentation and bottle aging. Among the most important varietal aroma compounds family, we found terpenes, C_{13}-norisoprenoids, and polyfunctional mercaptans (also known as thiols) (Parker et al., 2017). In grape musts, these varietal aromas can be found in a free (i.e., volatile) state or a non-volatile state when linked to a so-called non-volatile varietal precursor, except for polyfunctional mercaptans, which are only found in non-volatile form (Roland et al., 2011). This distinction allows discriminating between neutral (or non-aromatic) grapevines whose varietal aromas composition is mainly made of linked aromatic compounds and aromatic grape varieties containing a substantial fraction of volatile varietal aromas (Ferreira and López, 2019).

During winemaking, two mechanisms can participate in the release of the odorous compounds linked to varietal precursors. On the one hand, acid-catalyzed hydrolysis, resulting from the acidic nature of grape must, occurs throughout the winemaking process and participates in the release of bound-aromas (López et al., 2004; Liu et al., 2017). On the other hand, enzymatic hydrolysis of bound aroma compounds can be carried out by enzymes provided by grapes or, to a lesser extent, by

* Corresponding author.
E-mail address: aquerol@iata.csic.es (A. Querol).

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Saccharomyces cerevisiae, the main species used in winemaking (Fernández-González et al., 2003; Sieiro et al., 2014; Ugliano, 2009). Further reactions differentially affect aromas during bottle aging, such as esterification, chemical rearrangements, and hydrolysis. These chemical reactions mainly occur in the absence of oxygen, at low pH, and are time-dependent (Ferreira and López, 2019). Besides, it has recently been determined that the yeast strain used during alcoholic fermentation also affects the way aromas are modulated during bottle aging, like the level of massoia lactone, guaiacol, or TDN produced (Oliveira and Ferreira, 2019; Denat et al., 2021). Similarly, esters produced by yeast during alcoholic fermentation are further differentially affected by aging. For instance, while straight-chain esters are rapidly hydrolyzed, branched ethyl esters increase by esterification with their free fatty acids (Díaz-Maroto et al., 2005).

In this regard, yeast strains other than *Saccharomyces cerevisiae* have gained attention in wine research not only for their ability to produce higher levels of fermentative aromas (e.g., fusel alcohols and esters) (Querol et al., 2018). In addition, they could contribute to liberating a higher quantity of bounded varietal aromas, thus providing wines with more complex flavor profiles (Swiegers et al., 2005; Oliveira and Ferreira, 2019; Borren and Tian, 2021; Feng et al., 2021). For example, cryotolerant species *S. uvarum* can release terpenes due to its enhanced hydrolytic activity (Ugliano et al., 2006) and biosynthesize some of them from sugar metabolism (Fernández-González and Di Stefano, 2004; Gamero et al., 2011). Likewise, *S. uvarum* and *S. kudriavzevii* species in their interspecific hybrid forms with *S. cerevisiae* exhibit a strong capacity to release polyfunctional mercaptans from their odorless grape precursors during wine fermentation (Murat et al., 2001; Dubourdieu et al., 2006). Finally, *S. uvarum* and *S. kudriavzevii* species, along with some wild strains of *S. cerevisiae,* have also been characterized as high-producers of relevant fermentative by-products such as glycerol, erythritol, succinic acid, 2,3-butanediol, ethyl, and acetate esters that could contribute to improving the aromatic and organoleptic quality of the wine (Oliveira et al., 2014; Stribny et al., 2015; Minebois et al., 2020).

In this context, the present work attempts to study how non-wine strains of different *Saccharomyces* species and bottle aging modulate the production of a high range of volatile compounds in *Vitis vinifera* L. Albariño wines. This cultivar was selected for this study because it exhibits a high content of monoterpenes, C_{13}-norisoprenoids, and polyfunctional mercaptans. These aromas constitute an important varietal aroma reserve that can be significantly affected by the aforementioned factors (Vilanova and Vilariño, 2006; Mateo-Vivaracho et al., 2010; Carrascosa et al., 2012). Additionally, by combining the aroma data with that of the rest of the fermentative by-products quantified, we propose several hypotheses of metabolic pathways involved in producing some varietal flavors still little described in the literature.

2. Materials and methods

2.1. Fermenters setup

2.1.1. Semi-synthetic must composition

The synthetic grape must was prepared as reported Hernández-Orte et al. (2006) with some modifications. Reducing sugars, 100 g/L glucose +100 g/L fructose; 2.5 g/L L-tartaric acid; 0.4 g/L citric acid; 3 g/L L-malic acid. The nitrogen composition was prepared as described Hernández-Orte et al. (2002), simulating the nitrogen content profile of Spanish varieties. Synthetic glutathionylated (Glu) and cysteinylated (Cys) precursors of volatile mercaptans compounds were added in the following composition and proportions: 0.1 mg/L of Cys-MH, 0.05 mg/L Cys-MMP, 1 mg/L Glu-MH, 0.05 mg/L Glu-MMP. This precursor solution was added as they are estimated to be removed together with the amino acid fraction during the extraction of the Albariño aromatic and phenolic fraction (PAF). Then, the pH of the must was adjusted to 3.3 and filtered by sterilization (0.2 μm).

Phenolic and Aroma extract from Albariño grapes (PAF) was provided by Zaragoza University (Spain) in ethanolic solution (45% v/v of ethanol) prepared as described by Alegre et al. (2020). Once the volume of ethanol was removed by vacuum, it was replaced by the same amount of sterile water and was added to the must (100 mL/L) just before inoculation. This 10% PAF addition represents the initial quantity of grapes from which the concentrated alcoholic solution was obtained.

2.1.2. Yeast strains and fermentation conditions

The yeast strains used in this study belonged to the species *S. cerevisiae, S. uvarum,* and *S. kudriavzevii*. Lalvin MSB™, Lalvin T73™, and BMV58™ are wine strains provided by Lallemand Bio SL, Spain. The commercial *Saccharomyces cerevisiae* strains T73 and MSB were included as controls. T73 is a reference wine and commercial strain that we usually employ in our studies; MSB is a wine and commercial strain isolated in New Zealand as a great thiol producer. The rest of the strains were isolated from natural habitats and spontaneous fermentations (Table 1).

Fermentations were carried out in triplicates at 16 °C in 100 mL-glass flasks containing 70 mL of must, a stirrer magnet (100 rpm), and closed with an airlock valve. Each fermenter was inoculated with the precultures at an initial population of 1×10^6 cells/mL. In addition, the same non-inoculated grape must was used as a control during fermentation and aging processes and is referred to as "young must" and "aged must" in the following sections.

Fermentation progress was monitored by measuring daily weight loss and sugar consumption, and the total yeast population was monitored by flow cytometry. The obtained curves were fitted to the non-linear regression Gompertz model (Zwietering et al., 1990). The process was considered finished at residual sugars <1 g/L.

Inside a free-O_2 chamber (Jacomex, Dagneux, France), 18 mL-vials containing each wine sample were placed in plastic bags with oxygen scavengers, and then, bags were vacuum-sealed. Once the samples were free-O_2 conditioned, they were incubated at 50 °C for 5 weeks for the accelerated bottle aging (Vela et al., 2017; Oliveira and Ferreira, 2019).

2.2. Analysis of main metabolites

Concentrations of ethanol (% v/v), glycerol (g/L), erythritol (g/L), succinic, citric, and malic acid (g/L), glucose (g/L), fructose (g/L) were analyzed in the finished wines and during fermentation by HPLC (High-Performance Liquid Chromatography, Thermo Fisher Scientific, Waltham, MA, USA) using the same methodology, standard calibration curves and conditions previously described in Pérez et al. (2021). Additionally, pH levels were determined in the final wines.

2.3. Determination of volatile aromatic compounds in young and aged wines samples

Only in young samples (immediately after fermentation) higher

Table 1
Natural and commercial *Saccharomyces cerevisiae*, *Saccharomyces kudriavzevii,* and *Saccharomyces uvarum* strains used in this study.

Species	Code used	Source of isolation	Geographic origin
S. cerevisiae	T73	Wine, Commercial (Lallemand)	Spain
	MSB	Wine, Commercial (Lallemand)	New Zealand
	SC1	Cachaça fermentation	Brazil
	SC2	Sorghum beer	Burkina Faso
S. kudriavzevii	SK1	Monosporic derivative of CR89, oak (*Q. Faginea)*	Spain
	SK3	Monosporic derivative of CA111, oak (*Q. Faginea)*	Spain
S. uvarum	BMV58	Wine, Commercial (Lallemand)	Spain
	U1	Non fermented liquor (Mistela)	Spain

alcohols, volatile fatty acids, and major esters, which are usually present in concentrations above 0.2 mg/L, were acquired by liquid-liquid microextraction and analyzed by GC-FID (Gas Chromatography with flame ionization detector), following the methodology of Ortega et al. (2001).

Polyfunctional mercaptans were analyzed as described in Mateo--Vivaracho et al. (2010) with some modifications. Briefly, the sample extract was obtained by derivatization, in solid-phase extraction (SPE) in which deuterated analytes as internal standards were added: 3 MH-d5 at 700 ppt, 3MHA-d5 at 200 ppt, 4MMP-d10 at 100 ppt (Eptes Sarl, Switzerland). After washing with brine and drying with anhydrous sodium sulfate, extracts were injected into the GC-GC-MS (NCI) system. Concentrations were obtained using a response factor calculated by analyzing table wines spiked with known amounts of the analytes.

Minor aromatic compounds (0.1–200 μg/L) in aged and young wines were acquired by SPE following the protocol described by López et al. (2002) Their identification and quantification were carried out using a GC coupled to a mass detector (Shimadzu QP 2010, Quioto, Japan).

2.4. Statistical analysis

Each metabolite was expressed as the arithmetic means of biological triplicates with its corresponding standard deviation. One-way ANOVA followed by Tukey's HSD test was applied, considering statistical significance when the *p*-value was below 0.05. A hierarchical clustering (Euclidean distance and average linkage) was applied to determine the distribution and grouping of treatments according to the multiple variables. All statistical analyses and plots were obtained using Infostat software, version 2011 (Grupo Infostat, Córdoba, Argentina) and GraphPad Prism version 8.0 (Graph-Pad Software, Inc., La Jolla, CA).

3. Results

3.1. Fermentation activity and main metabolites produced

All fermentations, carried out at 16 °C, finished successfully reaching trace sugars in less than 14 days. As shown in Fig. 1A and B, yeast strains of cryotolerant species (squares and diamonds) broadly consumed hexose sugars faster than *S. cerevisiae* strains (circles). For instance, besides glucose, *S. kudriavzevii* strain SK1 was also the quickest in consuming fructose. Considering cell growth, the highest biomass producers were the two *S. uvarum* strains (Fig. 1C). On the contrary, from the early stages of fermentation, the *S. cerevisiae* strain SC2 mainly showed sluggish fructose consumption, resulting in a low fermentation and a slow biomass production (Fig. 1B and C).

Regarding fermentation by-products, an influence of species and isolation origin was observed (Fig. 2). Wine strains (T73, MSB, and BMV58) were associated with slightly higher malic acid content in the final wines compared to the initial content in the grape must, suggesting production of this compound across fermentation. On the other hand, wines fermented with *S. uvarum* strains presented the highest succinic acid content, with concentrations of 4.7 and 7.3 g/L reported for SU1 and BMV58, respectively. For the rest of the yeast strains, the succinic acid content ranged between 0.40 and 1.3 g/L. This difference observed in the succinic acid yielded by *S. uvarum* strains could be due to a great production of this acid through the GABA shunt, which aligns with the differential production by *S. uvarum* strains of certain aromas related to this pathway, as detailed below.

Interestingly, the wine produced with SC2 strain was characterized by the lowest malic and citric acid content, which correlated with the highest pH level (Table S2). Wines of *S. cerevisiae* strains, T73 and MSB, were also characterized by their highest ethanol and lowest glycerol and erythritol amounts. On the contrary, natural *S. kudriavzevii* strains SK1 and SK3 produced the highest levels of glycerol (Fig. 2).

3.2. General effect of yeast strain and aging on Albariño wine and must aromas

Sixty volatile compounds classified into ten categories (volatile acids, higher alcohols, acetate esters, ethyl esters, miscellaneous and carbonyls compounds, phenols, lactones, C_{13}-norisoprenoids, monoterpenes, and polyfunctional mercaptans) were determined in young wines and musts (Fig. 3A). After accelerated anoxic aging, thirty-eight minor volatile compounds (0.1–200 μg/L) were also determined in these samples (Fig. 3B). The values displayed in Fig. 3A and B represent the relative concentration of each aroma compound compared to the mean calculated from the concentrations of all strains and the non-inoculated must.

The following compounds were detected in young control grape must: volatile phenols, lactones, terpenes except for citronellol, polyfunctional mercaptans, and β-damascenone (Fig. 3A and B). We assumed that they represented the initial Albariño volatile fraction or could also have been released by acid hydrolysis during the time of fermentation. The aromas only found in the young wines represented fermentative aromas, which showed a great variability according to the strain used. Similarly, we could discern the aroma fraction that proceeded entirely from the aging process, aromas that were only found in the aged must and the aged wines. So, we identified massoia lactone, vitispirane A and B, TDN, riesling acetal, vanillin, and 1,8-cineole as aromas formed or liberated during the aging process. The data also suggested a combined effect between yeast and aging on vanillin derivatives, ethyl cinnamate, and ethyl dihydrocinnamate. During fermentation, their precursors were modified by yeast, and subsequent aging led to the formation of volatiles.

The percentage variability between the average concentration of each compound in young and aged wines determined that acetates, linalool, β-citronellol, *p*-propylguaiacol, 4-vinylguaiacol, and 4-vinylphenol decreased due to the aging process. These compounds underwent a decrease of between 40 and 90%, while the rest of the compounds increased by between 20 and 7000%. While lactones

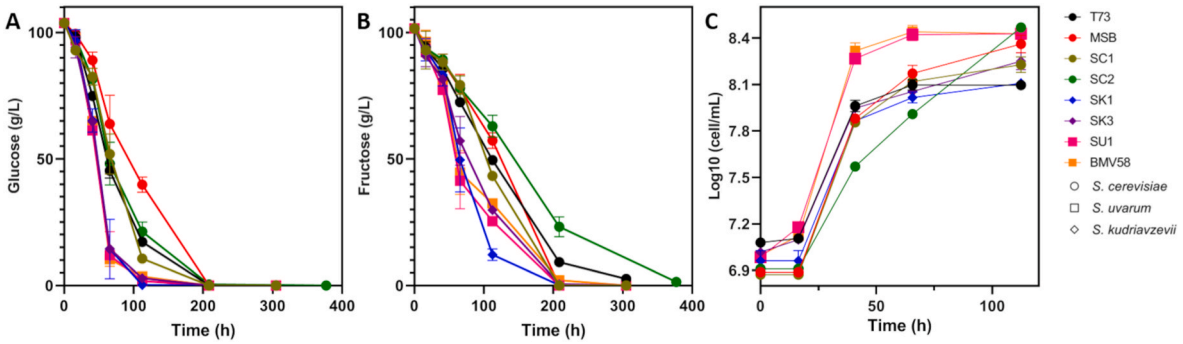

Fig. 1. Sugar consumption and cell growth curves by yeasts during fermentation in semi-synthetic Albariño musts.

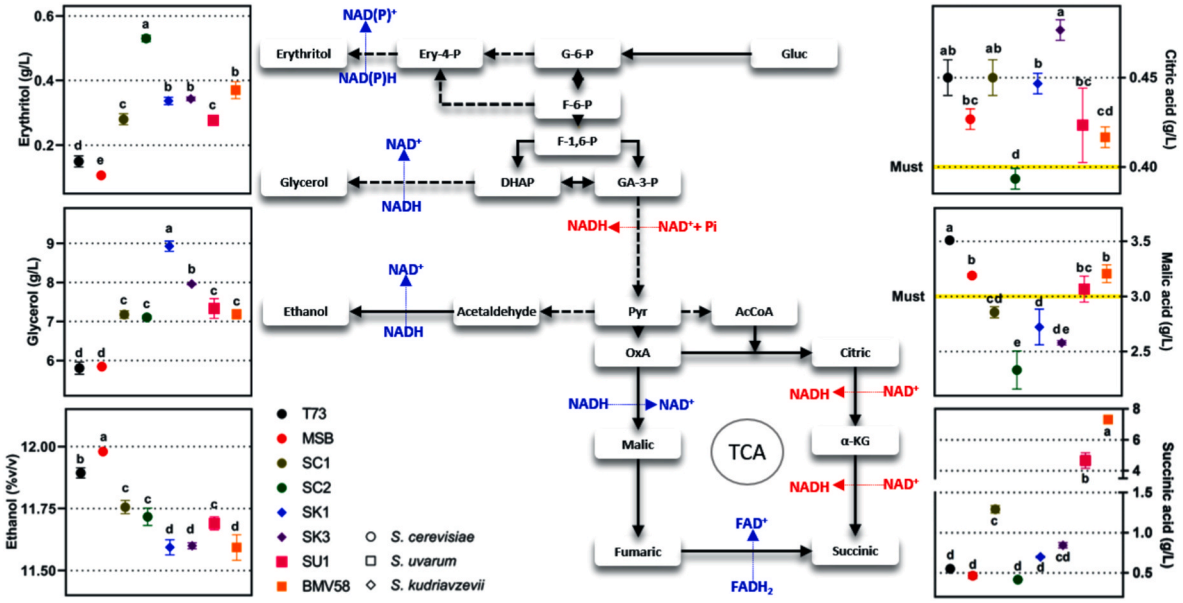

Fig. 2. Primary metabolites (mean ± SD) determined in young wines fermented by the different strains and a simplified representation of their yeast's synthesis routes. Gluc: glucose; G-6-P: glucose-6-phosphate; F-6-P: fructose-6-phosphate; Ery-4-P: erythrose-4-phosphate; DHAP: dihydoxyacetone phosphate; GA-3-P: glyceraldehyde-3-phosphate; Pyr: pyruvate; AcCoA: acetyl coenzyme A; OxA: oxaloacetate; α-KG: α-ketoglutarate; TCA: tricarboxylic acid cycle.

increased slightly, the highest increases were found for linalool oxide (4525%), ethyl isovalerate (6694%), ethyl 2-methylbutryate (4457%), ethyl isobutyrate (3886%), and ethyl leucate (1420%). On the contrary, among the compounds that were no longer detected after aging, we found nerol, geraniol, rose oxide and, 4-ethylphenol. These compounds may have undergone isomerization reactions leading to the formation of other compounds or degradation. On the other hand, β-citronellol was the only monoterpene not found in the unfermented must.

3.2.1. *Aromatic diversity in young Albariño wines according to yeast strains*

The hierarchical cluster analysis applied on the aroma's concentration data sets of young wines (Fig. 3A) grouped yeasts into 3 groups. The SC2 strain stood alone on the left side of the plot and opposite the two *S. uvarum* strains were clustered together. In the middle, we found strains T73, MSB, SK1, SK3, and SC1. Yeasts were sub-grouped according to species within this last group, *S. cerevisiae* wine strains (T73 and MSB), and the environmental *S. kudriavzevii* strains (SK1 and SK3). The lowest levels of 2,3-butanediol characterized T73 and MSB strains. SC1 was in between these subgroups, sharing some traits, such as the high R-limonene content found equally in T73, SC1, SK1, and SK3 and was 13-fold higher than the one detected in the young must (Fig. 3A).

SK1 and SK3 strains were distinguished from T73, MSB, and SC1 by yielding the highest amounts of compounds related to 2,3-butanediol (i.e., acetaldehyde, acetoin, and 2,3-butanediol). Besides, their most distinctive trait was their great release of polyfunctional mercaptans (PFMs), particularly 4-methyl-4-mercaptopentan-2-one (4MMP), derived from the added precursors. For instance, strain SK1 reached a concentration of 4MMP 47-fold and 8-fold higher than the concentration yielded by *S. cerevisiae* and *S. uvarum* strains, respectively.

Regarding the *S. uvarum* cluster, their young wines were characterized by the highest yields of linear and branched-chain fatty acids, higher alcohols, ethyl, and acetates esters (Fig. 3A). The most notable aromas were β-phenylethanol and its acetate, with concentrations for both compounds almost 3-fold higher than the mean value. Additionally, octanoic and decanoic medium-chain fatty acids were highly present in these wines, as well as γ-butyrolactone and diethyl succinate, whose concentrations determined in these young wines doubled the average value (Fig. 3A). Curiously, SC2 was the only strain that produced detectable amounts of ethyl 4-methylvalerate (strawberry notes), while it yielded the lowest concentrations for the rest of the ethyl and acetate esters. Besides, this strain was also the one that produced the highest level (5-fold higher) of γ-octalactone in young wines.

Finally, geraniol amounts in young wines of *S. uvarum* strains were above the odor threshold (OT) and 3.5-fold higher than the average value. However, for nerol, the levels detected in SU1 and BMV58 wines were statistically equal to unfermented young musts and two orders of magnitude lower than in the other strains (Fig. 3A).

3.2.2. *Aromatic diversity of aged Albariño wines according to yeast strains*

Like young wines, the hierarchical cluster analysis applied on aromas of aged wines grouped yeasts into 3 groups (Fig. 3B). *S. uvarum* strains BMV58 and SU1 still clustered together, mainly because of their highest concentrations of most esters, except ethyl cinnamate, which was not detected (Fig. 3B). Interestingly, ethyl isobutyrate significantly increased by aging in these strains, exceeding its OTs (19.23–647.3 μg/L in SU1 and 24.7–848.5 μg/L in BMV58; Tables S4 and S5).

Again, SC2 strain stood alone at the opposite end of the dendrogram. It was characterized by having the lowest amounts of most aroma compounds but was still the strain with the highest γ-octalactone levels after aging (Fig. 3B). SC2 aged wines were also characterized by 2.5-fold higher massoia lactone and 2-fold higher 4-vinylguaiacol concentrations.

After aging, wines fermented with *S. kudriavzevii* strains SK1 and SK3 developed an aromatic profile more similar to those fermented with T73 and SC1 strains and were characterized by the highest levels of R-limonene and the lowest amount of γ-octalactone. On the contrary, the wine fermented with MSB strain evolved after aging towards an aromatic profile similar to *S. uvarum* strains, coinciding in the detection of vanillin compounds which were only found with these strains.

4. Discussion

Albariño grapevine is a cultivar with an important reservoir of varietal aromas and few studies have been carried out to determine the influence of different yeast strains and bottle aging on the release of their volatile form in the resulting wine. The existing bibliography generally focused on specific aroma groups, including C13-norisoprenoids, monoterpenes, and fermentative aromas, often using *S. cerevisiae*

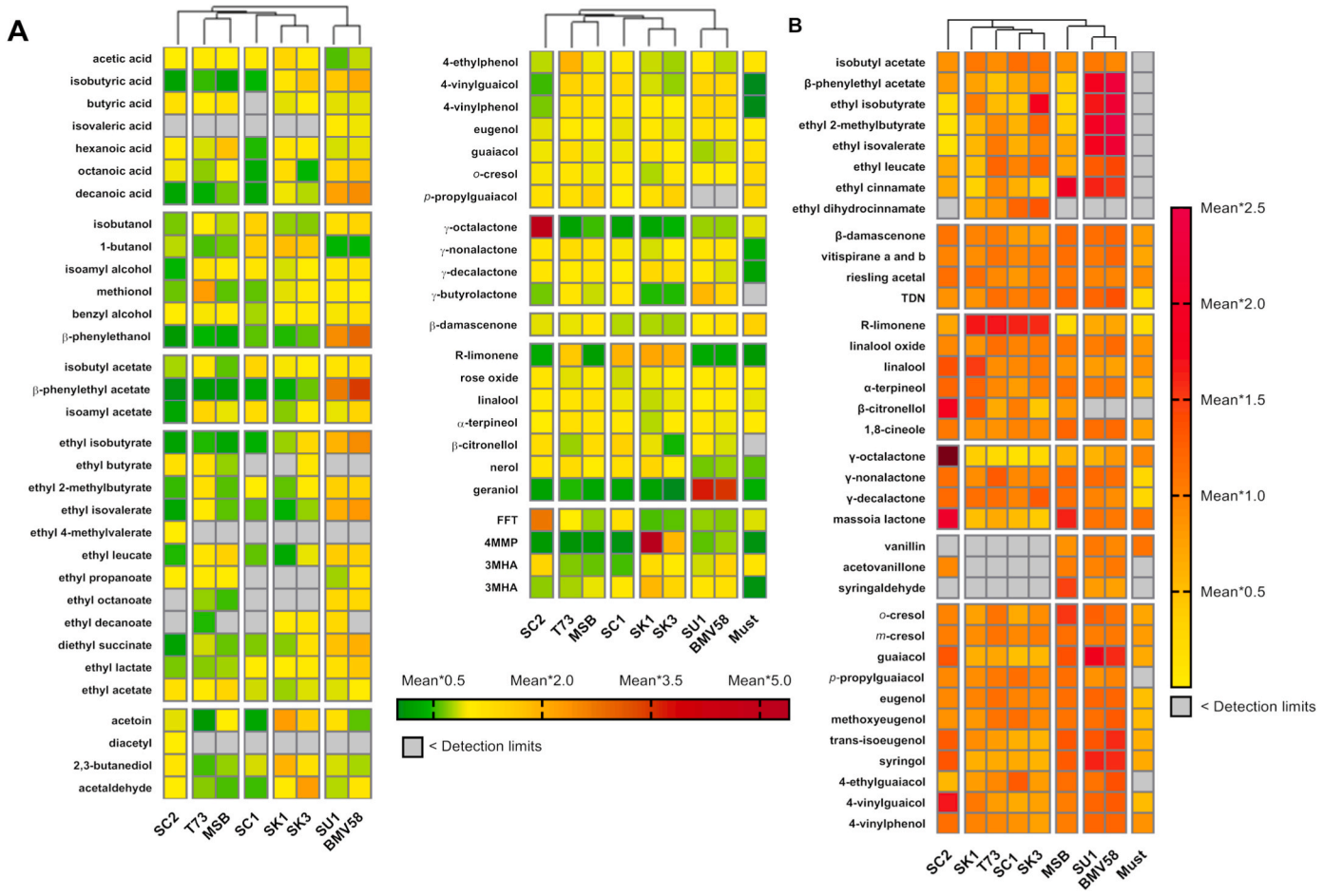

Fig. 3. Heat map represents by a color scale the relative aroma values around the average concentration obtained in young (A) and aged (B) wines resulting from fermentation with different yeasts strains. Dataset of each condition was submitted to hierarchical clustering analysis resulting in a dendrogram representing the separation of the yeasts according to their similarities in the aroma profiles. Gaps between volatile indicate the different aromatic groups, starting from the top in A: acids, higher alcohols; acetate esters; ethyl esters; compounds related to 2,3-butanediol synthesis, volatile phenols, lactones, C_{13}-norisoprenoids, terpenes, polyfunctional mercaptans; in B, acetate esters and ethyl esters; C_{13}-norisoprenoids, terpenes, lactones, vanillin derivatives, volatile phenols. The average concentration of aromas in the aged wines was subtracted and divided by the average value of the young wines and expressed as a percentage: %VAR, thus obtaining the variability caused by the aging. Aromas not found in either of the two conditions: %VAR = n. a, not applicable. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

strains (Lema et al., 1996; Vilanova and Sieiro, 2006; Carrascosa et al., 2012; Oliveira et al., 2008). For this reason, in this study, we aimed to determine how wild *S. cerevisiae*, cryotolerant *S. uvarum,* and *S. kudriavzevii* yeasts and aroma maturation during bottle aging modulate a variety of Albariño aromas. Ultimately some hypotheses on the metabolic origin of these aroma compounds within the yeast metabolic network are presented and summarized in Fig. 4 that will be used in the discussion section.

Regarding the effect caused by aging, we observed several compounds highly modified in Albariño aged wines. As several studies have determined, the anoxic aging favored the esterification of branched ethyl esters, which was visualized in this study in the high increase of ethyl isovalerate and ethyl 2-methylbutyrate. We also corroborated the effect of aging on the degradation of isobutyl and β-phenylethyl acetates by hydrolysis (Díaz-Maroto et al., 2005). Regarding monoterpenes, geraniol, the principal precursor of monoterpenols, was degraded via acid-catalyzed hydrolysis during aging resulting in α-terpineol and linalool. Likewise, linalool initially present in grape must also undergo a chemical evolution resulting in 1,8-cineole and α-terpineol (Waterhouse et al., 2016).

Besides the general effect caused by aging, yeast also played a major role in modulating these aromas. A good example of this is the case of R-limonene observed in wines fermented with SK1, SK3, T73, and SC1 strains. The high concentration could be attributed to the synthesis of R-limonene from GPP through the mevalonate pathway (Fig. 4C). However, the biosynthetic pathway of R-limonene is not fully known on yeast (Duetz et al., 2003). After aging, its concentration still increases, which could be linked to the further release of this compound from yeast cells during the aging process.

4.1. Aroma modulation by S. uvarum strains

The most striking result obtained with the *S. uvarum* strains was their great capacity to release γ-butyrolactone and diethyl succinate. Although the sensory effect was not relevant according to their perception thresholds, their biosynthetic origin can be related to the catabolic pathway of glutamate, most commonly known as the GABA shunt (Fig. 4B). In this pathway, glutamate is decarboxylated to γ-aminobutyric acid (GABA), transaminated to succinate semi-aldehyde (SSA), and oxidized to succinate. When SSA is not oxidized to succinate, it can be reduced to γ-hydroxybutyric acid (GHB) (Bach et al., 2009), which is the substrate of lactonization to γ-butyrolactone (Ribéreau-Gayon et al., 2006). On the other hand, the end product of the GABA shunt is succinate, which after double esterification with ethanol, can lead to diethyl succinate (Sieiro-Sampedro et al., 2019). Therefore, the overproduction of γ-butyrolactone and diethyl succinate by

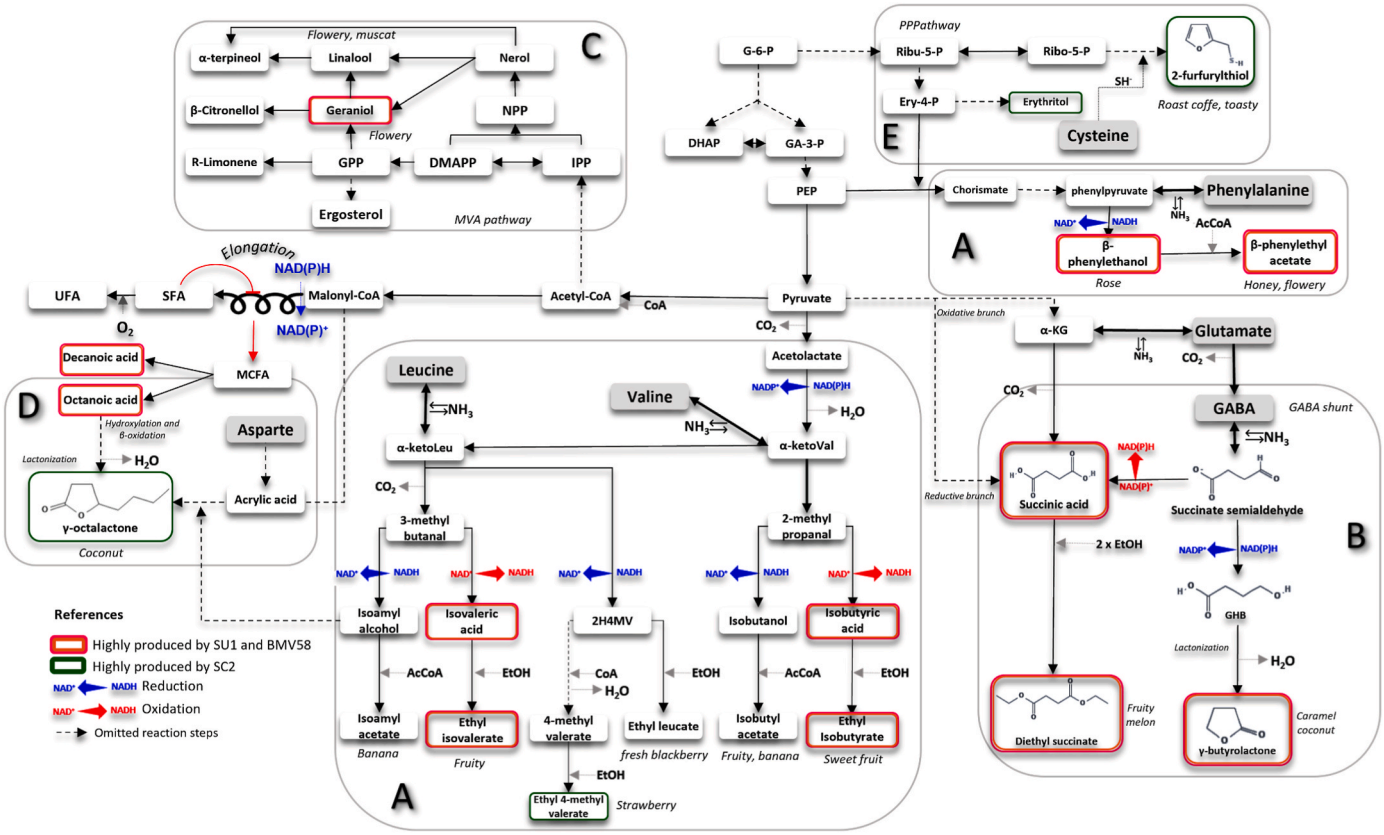

Fig. 4. Summary of yeast metabolic pathways involved in the synthesis of relevant aroma compounds determined in this study. UFA: unsaturated fatty acids; SFA: saturated fatty acids; MCFA: medium-chain fatty acids; 2H4MP: 2-hydroxy 4-methylpentanoic acid; GPP: geranyl diphosphate; DMAPP: dimethylallyl diphosphate; IPP: isopentenyl diphosphate. Odor descriptors were taken from Culleré et al. (2019).

S. uvarum strains is likely the result of a greater flux through the GABA shunt in this species. This is concordant with the higher succinate yields found for SU1 and BMV58 strains in our study. The recent work of Henriques et al. (2021) highlights the role of this pathway in succinic acid synthesis and balancing redox homeostasis in *S. uvarum* strains.

In young and aged wines fermented with *S. uvarum* strains, we also observed a significant amount of higher alcohols, ethyl esters, and acetates related to the catabolism of branched-chain and aromatic amino acids. As shown in Fig. 4A, all these compounds can be formed from their exogenous and endogenous (i.e., *de novo* synthesized from sugars metabolism) amino acids precursors, valine, leucine, or phenylalanine, via the Ehrlich pathway. Briefly, the Ehrlich pathway consists of the catabolism of branched-chain and aromatic amino acids, or their related keto-acids, in a three-step reaction. The last reaction can be a reduction (leading to a fusel alcohol) or oxidation (leading to a fusel acid). As presented in Fig. 4A, the higher amounts of isobutyric and isovaleric acids and their related ethyl esters (i.e., ethyl isobutyrate and ethyl isovalerate) in *S. uvarum* strains suggested that they mainly directed the catabolism of leucine and valine through the oxidative branch of the Ehrlich pathway. In addition, we also observed that *S. uvarum* strains were the fastest in growing while SC2 strain was the slowest, which could be the reason for an initial higher demand for reductive equivalents (NADPH) by *S. uvarum* (Bakker et al., 2001).

Regarding the highest levels of geraniol detected in the young wines of these strains, this phenotype is probably related to the blockage of ergosterol synthesis at the level of squalene in the absence of oxygen during fermentation (Vaudano et al., 2004). The accumulation of geranyl diphosphate (GPP), an intermediate of ergosterol synthesis and situated above squalene, might have contributed to geraniol synthesis in *S. uvarum* strains (Fig. 4C). From this step, the bioconversion of this terpene into other terpenes could have been generated in several of the strains, giving rise, among others, to citronellol (Gamero et al., 2011).

Moreover, under the conditions of our study, we also found *S. uvarum* strains with a high capacity to release various PFMs. This aptitude of *S. uvarum* has also been reported on Sauvignon Blanc fermentation (Masneuf et al., 2002). However, in our work, differences in 4MMP and 3 MH production between commercial strains of *S. cerevisiae* and *S. uvarum* strains were much greater.

According to the literature, ethyl isobutyrate results from the slow esterification of isobutyric acid during aging (Díaz-Maroto et al., 2005). Therefore, the highest amounts found in young wines and the major increase observed after aging are directly related to the high levels of the branched acids found, mainly in BMV58. The most significant increase of this compound by the action of aging was reported in our *S. uvarum* strains.

4.2. SC2 strain a particular strain

Curiously, SC2 had the lowest amount of most ethyl esters, but it was the only strain that produced a detectable amount of ethyl 4-methylvalerate. This ester was first identified in wines by Campo et al. (2006) as an isomer of ethyl hexanoate but with a much lower odor threshold, contributing to the strawberry aroma. Its synthesis has not been entirely determined but is suggested to result from the esterification of 4-methylvaleric acid with ethanol (Gracia-Moreno et al., 2015). Here we hypothesize that the latter acid could derive from 2H4MV (2-hydroxy 4-methylpentanoic acid), the hydroxy acid precursor of ethyl leucate (Shimizu et al., 2016) (Fig. 4A).

Lactones in wine have a relevant flavor role, but their synthesis by yeasts remains to be fully discovered. From the limited literature and observations in this strain, we traced two metabolic pathways that could result in γ-octalactone (Fig. 4D). On the one hand, γ-octalactone could

derive from yeast lipid metabolism. In this scenario, it would be produced from octanoic acid after being hydroxylated, β-oxidized, and finally lactonized (Romero-Guido et al., 2011). The second hypothetical pathway involved acrylic acid, a compound little studied in yeasts and wine but could proceed from aspartate or malonyl-CoA. Acrylic acid could be bound with isoamyl alcohol and, after losing a water molecule by lactonization, γ-octalactone would be formed (Berger and Zorn, 2004).

High content in furfurylthiol also characterized SC2 wines. This compound is another key volatile thiol with a strong roast coffee aroma. The presence of FFT in wine is generally associated with contact with toasted staves either during fermentation or aging (Blanchard et al., 2001; Tominaga et al., 2000). However, its detection in oak-free wines and the variability between cultivars or vintages has raised concerns about its origin (Tominaga et al., 2000). According to Hofmann and Schieberle (1998), FFT is formed from the reaction between ribose and cysteine under dry heat during food processing. In this aspect, we notably found that SC2 produced high levels of erythritol, a pentose phosphate pathway derivative, suggesting that this pathway might be more active in this strain. Consequently, the higher availability of other pentose phosphate intermediates, such as ribose-5-phosphate, might have contributed to the higher synthesis of FFT in SC2.

4.3. The great capacity of *S. kudriavzevii* strains in releasing polyfunctional mercaptans

The most important related trait to *S. kudriavzevii* strains was a large amount of polyfunctional mercaptans (4MMP and 3 MH) found in their young wines. These compounds are considered very potent aroma molecules that impart important tropical notes, even at very low concentrations in wines (Roland et al., 2011). 4MMP has the lowest perception threshold, and its aroma is related to the box tree or cat's pee notes, typical of Sauvignon Blanc wines (Tominaga et al., 1998). The release of 4MMP from its nonvolatile S-cysteinylated precursor, *S*-3-(4-mercapto-4-methylpentan-2-one)-cysteine (Cys4MMP), has been reported to be carried out by different carbon-sulfurylase enzymes whose coding genes have only been identified in laboratory *S. cerevisiae* strain so far (Howell et al., 2005). Among them, protein Irc7p is the main responsible for the release of 4MMP, and its activity is controlled by genes of the nitrogen catabolic repression (NCR) system (Thibon et al., 2008). Therefore, greater activity of carbon-sulfurylase enzymes in SK1 and SK3 or a distinct regulation of their coding genes by the NCR complex could explain the higher levels of 4MMP. In line with this, previous works have already pointed out the higher volatile-thiol release capacity of an interspecific *S. kudriavzevii* × *S. cerevisiae* hybrid widely used in white vinifications (Murat et al., 2001; Erny et al., 2012). Additionally, the first step in the release of 4MMP and 3 MH consists of the incorporation by yeast of Cys4MMP and Cys3MH (S-3-(hexan-1-ol)-cysteine) precursors. This can be accomplished via the general amino-acid permease Gap1p (Subileau et al., 2008) or cysteine-specific permeases (Thibon et al., 2008). In another essay (data not shown), we notably found that *S. kudriavzevii* strains grown better on cysteine as the sole nitrogen source than other strains. This could be a reason for the higher incorporation of S-Cys-conjugates into their cells, cleaving and releasing into the free form of 4MMP and 3 MH.

5. Conclusion

To recapitulate, the most relevant findings in this work regarding the impact of yeast strain and aging on the modulation of Albariño aroma profile were found in young and aged wines obtained with *S. uvarum*, SC2, and *S. kudriavzevii* strains.

First, we give several evidence elements that, besides its contribution to the higher succinic acid production by the *S. uvarum* strains, the GABA shunt probably provides the precursors (SSA and succinate) required for γ-butyrolactone and diethyl succinate synthesis. Secondly, besides the fact that *S. kudriavzevii* strains had the best fermentative performances in Albariño fermentations, they also stood out for their great capacity to release PFMs. We related this with more efficient incorporation of their precursor inside their cells and a consequent better ability to be cleaved and released by these yeasts. At this point, we found that these two groups of yeasts demonstrated the ability to enhance the floral character of this grape variety, providing floral profiles by *S. uvarum* and tropical fruits profiles by *S. kudriavzevii*.

On the other hand, the wild *S. cerevisiae* SC2 strain, isolated from sorghum beer, was noted for its high production of the PFMs furfurylthiol, whose synthesis route we related to the pentose phosphate pathway since this strain also produced the highest concentrations of erythritol. In addition, this strain was noted for the high production of γ-octalactone, and ethyl 4-methylvalerate, although at concentrations of low sensory significance.

Finally, aging favored the increase of several important aromatic compounds, among which the aged wines of *S. uvarum* stood out for their higher content of branched esters with fruity notes.

Authors' contributions

JMH, JMG, VF and AQ conceived and designed the experiments. DP and MD performed the experiments. DP, RM and MD analyzed the data. DP, RM and AQ wrote the paper. AQ funding acquisition.

Declaration of competing interest

As the corresponding author, I warrant that: 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.fm.2022.103981.

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