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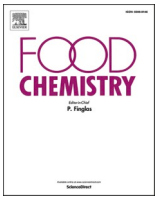

Impact of *Saccharomyces cerevisiae* yeast inoculation mode on wine composition

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ABSTRACT

Inoculation modes are known to affect yeast behavior. Here, we characterized the impact of ADY and pre-culturing on the composition of the resulting wine, fermented by four commercial strains of *Saccharomyces cerevisiae*. Classical oenological parameters were not affected by the yeast inoculation mode. Using an untargeted metabolomic approach, a significant distinction in wine composition was noted regardless of the strain between the two inoculation modes, each associated with a specific metabolomic signature. 218 and 895 biomarkers were annotated, respectively, for ADYs associated with the preservation of wine polyphenols, and for pre-cultures related to the modulation of yeast nitrogen metabolism. Volatilome analysis revealed that the ester family was that most impacted by the inoculation mode whatever the strain. Ester production was enhanced in ADY condition. For the first time, the complete reprogramming of the yeast metabolism was revealed as a function of yeast preparation, which significantly impacts its volatilome and exometabolome.

1. Introduction

Winemaking is a biotechnological process involving various stages driven by microorganisms. Alcoholic fermentation, the transformation of sugars in the must into ethanol and gas, is carried out by fungi, including yeasts, mainly belonging to the *Saccharomyces* genus. More specifically, the species *Saccharomyces cerevisiae* performs most of this transformation. Its capacity to evolve under harsh environmental conditions is greater than that of other species. Its faculties to adapt to environmental changes have been well documented (Varela et al., 2004). This species also displays a high fermentative capacity which makes it a valuable species for winemakers. These capacities currently make it a widely selected and commercialized species. These yeasts are mainly marketed and used as active dry yeast. This process is employed to ensure reproducible quality between different vintages. It also reduces the lag phase before the start of alcoholic fermentation and thus ensures a microbial control with reduced risk of wine spoilage (Beltran et al., 2002; Santamaria et al., 2005).

In most cases, they are rehydrated before inoculation into the must. However, there are other processes for inoculating these yeasts. They can be inoculated directly or multiplied before inoculation. The “pieds de cuve” approach makes it possible to inoculate a wine tank from a previous fermentation initiated by the addition of active dry yeast or indigenous population. Increasingly, propagation processes of active dry yeasts in a specific nutrient medium are used (Manzano et al., 2019).

In 2005, Bely and collaborators noted a modification of volatile acidity depending on the inoculation mode applied in the context of botrytized grape musts. Three modes of inoculation were tested. The preparation of the inoculum was a determining factor in the conduct of fermentation and the production of metabolites (Bely et al., 2005). This was related to the physiological state of the inoculated yeasts (Salvadò et al., 2008). Yeasts do not undergo the same conditions during the propagation or production of active dry yeast (Gómez-Pastor et al., 2010). After the production process and drying, which induce modifications in the cellular structure, the rehydration step of active dry yeast leads to the recovery of cell organization, including intracellular structure, membrane integrity, enzymatic system activities. Gene expression is modulated to deal with production and rehydration conditions (Novo

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et al., 2007;Matallana & Aranda, 2017; Pérez-Torrado et al., 2005). Another practice is to process a "pieds de cuve" phase, aiming at acclimating yeast in the medium in which they will be inoculated. Biomass is produced in must before being transferred to another must with similar physicochemical parameters.

The studies that address this topic mainly used transcriptomic or proteomic approaches (Gómez-Pastor et al., 2010; Pérez-Torrado et al., 2005; Rossignol et al., 2006). These approaches, although essential, do not precisely reflect for what is really occurring in the wine.

In this study we aimed to evaluate the impact of the inoculation method on the final composition of the wine, more specifically the inoculation of yeasts in Active Dry Yeast (ADY) form or in pre-cultured form. Does the physiological state of the yeast during inoculation induce a modification in the finished wine? For the first time, we were interested in addressing this question through the use of metabolomics.

2. Materials and methods

2.1. Saccharomyces cerevisiae strains

Four commercial strains of *Saccharomyces cerevisiae* (Lallemand Inc., Montreal, QC, Canada) were selected for this work. These commercial strains were coded SA, SB, SC and SD, supplied as ADY and stored at 4 °C once opened.

2.2. Preparation / inoculation yeast conditions

Two modes of inoculation of *S. cerevisiae* yeasts were tested for this study.

2.2.1. Post-rehydration inoculation (ADY)

Each strain was rehydrated from ADY stock according to the supplier's instructions (rehydration in 10 times its weight of water at 37 °C, shake and leave to stand for 20 min). This inoculation modality will be indicated throughout the manuscript with the code ADY.

2.2.2. Preculture inoculation (PC)

Each strain was rehydrated from ADY stock according to the supplier's instructions before being diluted at 0.1 % (v/v) concentration in 250 ml sterile Erlenmeyer flasks containing 150 ml of modified YPD medium (0.5 % (w/v) yeast extract, 1 % (w/v) bactopeptone, 2 % (w/v) glucose, and 0.02 % (w/v) chloramphenicol) and closed with dense cotton plugs. After incubation for 18 h at 28 °C under agitation (150 rpm), the second culture, in 150 ml of pasteurized Chardonnay must from a Languedoc harvest 2018, filtered on a 0.22 μm membrane (Steritop-GP, MERCK-Millipore, Burlington, Massachusetts, United States), was performed in Erlenmeyer flasks (250 ml) with an initial DO_{600} of 0.01. The latter was incubated at 28 °C without stirring for 18 h. This inoculation modality will be indicated throughout the manuscript with the code PC.

2.3. Fermentation conditions

Fermentations were carried out in three biological replicates in pasteurized Chardonnay must from a Languedoc harvest 2018, containing 226 $g.L^{-1}$ glucose/fructose, pH 3.92, and 343 $mg.L^{-1}$ total assimilable nitrogen. Fermentations were conducted in 2 L sterile bottles containing 1 L of Chardonnay must and closed with sterile cotton wool. For both inoculation methods, each strain was inoculated at 1×10^6 viable $cells.mL^{-1}$ from Chardonnay must cultures or from the rehydration step according to flow cytometry analysis as previously described (Bordet et al., 2021). Each fermentation was conducted at 20 °C in static mode. Daily sampling was carried out. The end of fermentation was considered as the total depletion of sugars.

2.4. Analytical methods

2.4.1. Oenological analysis

Samples were centrifuged at 8000*g* for 5 min at 4 °C. The supernatant was used for the following analyses. Sugar concentration, malic acid concentration, volatile acidity and ethanol degree were monitored daily by FTIR (Fourier-Transformed InfraRed) spectroscopy (OenoFOSS™, FOSS, Hilleroed, Denmark). A T-test was performed to compare these parameters between ADY and PC inoculation mode conditions for each *S. cerevisiae* strain using R software (R-4.0.4).

2.4.2. Volatilome analysis

Two types of high-performance gas chromatography were used to determine the volatile compounds as reported previously (Bordet et al., 2023). The GC-FID method developed by Ortega et al., 2001 for the major volatile compounds, based on gas chromatography with flame ionization detection (GC-FID) analysis, was used to determine the major compounds concentrations. A DB-20 column from J&W Scientific (Folsom, CA, USA), with a length of 50 m, an internal diameter of 0.32 mm and a film thickness of 0.5 μm, was used to separate the analytes. The column temperature was held at 40 °C for 5 min, then increased to 200 °C at a rate of 3 $°C.min^{-1}$. The injection was fixed at a rate of 30 $mL.min^{-1}$ for 3 μL in split flow. Hydrogen was used as the carrier gas at a flow rate of 3 $mL.min^{-1}$. The concentration of each specific compound was determined by computing the relative response areas to the appropriate internal standard (i.e.; 2-butanol, 4-methyl-2-pentanol, 4-hydroxy-4-methyl-2-pentanone, 2-octanol) as described by Ortega et al., 2001.

The minor and trace volatile compounds were determined by solid-phase extraction (SPE) and gas chromatography with mass spectrometric detection (GC–MS) carried out as detailed by López et al., 2002. Fifty milliliters were added to 25 μL of BHA (3-*tert*-butyl-4-hydroxyanisole) solution and passed through the SPE cartridge at 2 $mL.min^{-1}$. Then, the sorbent was dried by letting air pass through it (-0.6 Bar, 10 min). This fraction was analyzed with a Star 3400 CX gas chromatograph coupled to a Saturn 4 electronic impact ion trap mass spectrometer (Varian). For the preliminary analysis, the metabolites were separated on a DB-WAXetr (J&W, Folsom, USA) with a length of 60 m, an internal diameter of 0.25 mm and a film thickness of 0.5 μm and preceded by a 30 m × 0.32 mm uncoated precolumn. The chromatographic oven temperature gradient was as follows: temperature maintained at 40 °C for 5 min, then increased to 230 °C at a rate of 2 $°C.min^{-1}$. The carrier gas was helium at 1 $mL.min^{-1}$. 3 μL of samples after extraction by SPE (Vac ELUT 20 station from Varian) was injected in a 1093 Septum Equipped Programmable Injector (SPI) (Varian). The injector temperature gradient was as follows: initial temperature of 30 °C for 0.6 min and then increased to 230 °C at a rate of 200 $°C.min^{-1}$. The extracted ion chromatogram was then compared with the chemical standards and quantified by peak area.

Volatile compound quantification data were processed by performing a one-way ANOVA (p-value < 0.05) followed by a Tukey test. All the results were processed using R software (R-4.0.4).

2.4.3. Non-volatile metabolome analysis

Post AF wine samples were analyzed by ultra-high-performance liquid chromatography (Dionex Ultimate 3000, ThermoFischer, Waltham, MA, USA) coupled to a MaXis plus MQESI-Q-ToF mass spectrometer (Bruker, Bremen, Germany). Reverse phase liquid chromatography (RP-LC) was used to separate non-polar compounds on a 1.7 m 100 × 2.1 mm Acquity UPLC BEH C18 column (Waters, Guyancourt, France). Eluent A (5 % (v/v) acetonitrile with 0.1 % (v/v) formic acid) and eluent B (acetonitrile with 0.1 % (v/v) formic acid) constituted the mobile phase used for eluting the metabolites according to the following gradient: 5 % (v/v) solvent B from 0 to 1.10 min followed by a linear increase in the proportion of solvent B from 1.10 to 6.40 min to reach 100 % of the latter for 3.6 min, maintaining a constant

flow rate of 0.4 mL.min^{-1} during the analysis. Both negative and positive ionization modes were performed with an electrospray ionization source at a nebulization pressure of 2 bar and a dry nitrogen flow rate of 10 L.min^{-1}. The mass spectrometer parameters were as follows: ion transfer (end plate offset at 500 V), capillary voltage (at 4500 V in positive ionization mode and at 3500 V in negative ionization mode), and acquisition (mass range 100–1500 *m/z*). Fragmentation was performed at an 8 Hz spectra rate using autoMS/MS function (20 – 50 eV).

The samples were centrifuged at 10500 × g for 10 min and preserved at 10 °C during batch analysis. A cluster of Na formate was injected directly into the source for external calibration of the mass spectrometer before each batch analysis. This calibration was carried out in "enhanced quadratic" mode with an error of less than 0.5 ppm. Post-acquisition recalibration was also carried out internally. Both inter-batch (standard peptide and polyphenol mix) and intra-batch (experimental QC, sample mix) quality controls were used to guarantee the batch repeatability and stability of the system during analysis (Supplementary Fig. 1).

All the samples were randomly injected from one batch to avoid batch-to-batch variability. The original mass spectra of each sample were re-calibrated using Compass MetaboScape software (v. 8.0.1, Bruker, Bremen, Germany), to obtain a mass deviation after calibration of 0.5 ppm. Both *m/z* associations and retention times (RT), called features, were extracted (S/N > 30 and intensity thresholds > 1000). Features were kept if they were present in more than 20 % of the samples. Both positive and negative ionization mode data were merged into a single data set, with a tolerance of 5 ppm for *m/z* and 10 s for retention time. 5127 features were obtained. Smart formula was established using isotopic profile (tolerance: 5 ppm and mSigma < 20). R (R-4.2.1) and Perseus (Perseus_1.5.1.6) softwares were used for statistical analysis and data visualization (PCA, HeatMap, ANOVA and *t*-test). The Multidimensional Stoichiometric Compounds Classification (MSCC) script developed by Rivas-Ubach et al., 2018 was used to establish the hypothetical membership of the highlighted elementary formulae. Isolated significant features were annotated using the online database of Metlin, KEGG, YMDB, and online tools, MassTrix and Oligonet. Identification confidence was determined according to Schymanski et al., 2014.

3. Results and discussion

All 24 fermentations in Chardonnay were achieved with complete sugar degradation. The growth kinetics were also monitored until the end of the alcoholic fermentation and are represented in Supplementary Fig. 2. The samples were collected at the end of the alcoholic fermentation.

3.1. Classical oenological parameters

All the conditions studied presented complete degradation of sugars, i.e. < 2 g.L^{-1}. At the end of this alcoholic fermentation, the wines showed an ethanol content between 13.7 and 13.9 % (Supplementary Table 1). The wines derived from the modalities inoculated with Active Dry Yeast (ADY) showed a longer alcoholic fermentation (3 days more on average with ADY modalities) (Supplementary Fig. 2). This may be associated with the presence of a latency phase in the growth of the yeast, which delays the start of alcoholic fermentation.

This lag phase, also observed in real field conditions reflects the time needed for the yeasts to adapt to the environment (Ferreira et al., 2017). The PC yeasts also encountered two different culture media with an increase in sugar concentration allowing for adaptation to must conditions. This adaptation step could be associated with activation of signaling pathways, and metabolic redirection. Nutrient sensing and protein synthesis pathways to transport the various nutrients available in the environment could be promoted (Rossignol et al., 2003).

The pH was on average between 3.79 and 3.84 for ADY and PC respectively. As for ethanol content, no significant difference was observed between the different inoculation conditions for ADY and PC. This was also verified regarding malic acid concentrations. Malic acid concentrations ranged from 3.2 for the SB strain to 3.6 for the SC strain, both of which were inoculated with ADY.

In this study, the inoculation methods tested did not affect the oenological parameters of the wines after alcoholic fermentation.

In this work, the matrix was identical for all modalities, the same Chardonnay must was fermented by the same four strains. The differences highlighted in the compositions of the final wines were induced only by the mode of inoculation of the yeasts, the PC and ADY inoculation modes and the yeast itself, respectively. The composition of the wines, aided by metabolomics, corresponded to the metabolites produced by the yeasts and found in the extracellular medium.

3.2. Non-volatile metabolome analysis

Untargeted LC-MS analyses were conducted on wines resulting from the alcoholic fermentation of four *Saccharomyces cerevisiae* yeasts using two inoculation modes preculture (PC) and Active Dry Yeast (ADY). 5127 features were extracted from the LC-MS analysis of all the samples. First, a PCA representing the distribution of the samples at the end of alcoholic fermentation according to the relative intensity of each of the 5127 extracted features was established (Fig. 1A). The first two axes of the PCA represent 35.2 % of the metabolic variation existing between the different samples. This representation confirmed the very close proximity of biological replicates for a given sample. It was also possible to note that the separation of samples along axis 1 which represents 24.5 % of the variation between samples is mainly driven by the two inoculation modes. This suggests that there were considerable metabolic differences between the two inoculation modes except for the SA strain, which seemed to be associated with post AF wines with a similar chemical composition whatever the inoculation mode. Among all the features extracted during the analysis, 2067 could be associated with a molecular formula (Level 4). Only the features present in at least 2 replicas out of 3 in at least one modality were kept for further data processing. As a result, 2007 molecular formulas (Level 4) were retained. Among these molecular formulas, 44 and 70 were found to be unique to the PC mode and ADY mode respectively. It was possible to distinguish these molecular formulas associated with only one of both inoculation modes from common molecular formulas (Fig. 1B). 1893 were common to the two modes of inoculation whatever the strain. Then, we considered molecular formulas that presented significant differences in their mean intensity between the two inoculations, including all strains. These molecular formulas were extracted by performing ANOVA statistical analyses (p-value < 0.05) and considered as biomarkers. 1484 biomarkers were extracted and presented significant variations of intensity depending on the two inoculation modes regardless of the strain. These biomarkers are represented on the Heat map (Fig. 1C). It was possible to discriminate the biomarkers of the ADY inoculation mode from those of the PC mode. The biomarkers in this case corresponded to the molecular formulas that were significantly more intense in the condition mentioned. Interestingly, we were able to note that 409 molecular formulas were common to both yeast inoculation methods and were not impacted in any way in terms of their intensity by the latter.

Among all the biomarkers, 218 were associated with ADY while 895 were related to the PC inoculation mode. It should be noted, that as previously shown through the PCA representation of all the extracted features, the wine from the SA strain exhibited no difference in chemical composition between the two inoculation modes. This observation may be related to the genetic background of the SA yeast strain. This yeast resulted from an adaptive evolution inducing a redirection of the metabolic flux towards the pentose phosphate pathway (Cadière et al., 2011). This metabolic flow reorientation seemed to mask the effect of the inoculation mode on the yeast metabolism. To go further it was important to investigate the nature of these biomarkers. Biomarkers for

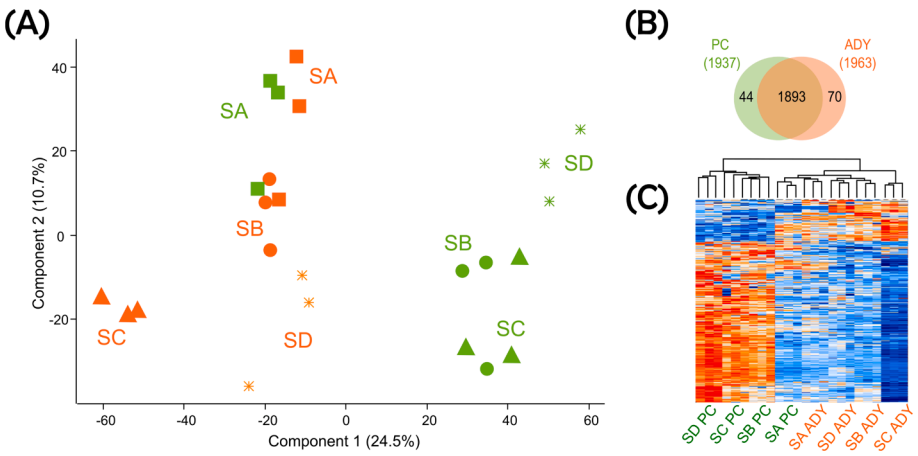

Fig. 1. Discrimination of two yeast inoculation modes (ADY and PC) using HRMS. (A) Principal-component analysis of two different inoculation modes (ADY: orange, PC: green) of four *Saccharomyces cerevisiae* strains (SA: square, SB: circle, SC: triangle, and SD: star) based on UPLC-qToF-MS data. (B)Venn diagram of features associated with elemental formulas. ANOVA ($p < 0.05$) was used to extract significant biomarkers for each inoculation mode. (C) Heatmap and HCA representing extracted biomarkers in the two inoculation modes. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

each mode of inoculation are represented according to their molecular formula on the van Krevelen diagram (Fig. 2). Biomarkers for the ADY inoculation mode (218) are considerably fewer than for the PC inoculation mode (895). Moreover, it is interesting to observe a completely different compositional diversity according to the inoculation mode regarding Fig. 2.

Putative CHON compounds represent only 30.3 % of the biomarkers (Fig. 2B1) in the ADY inoculation mode compared to 58.9 % for the PC

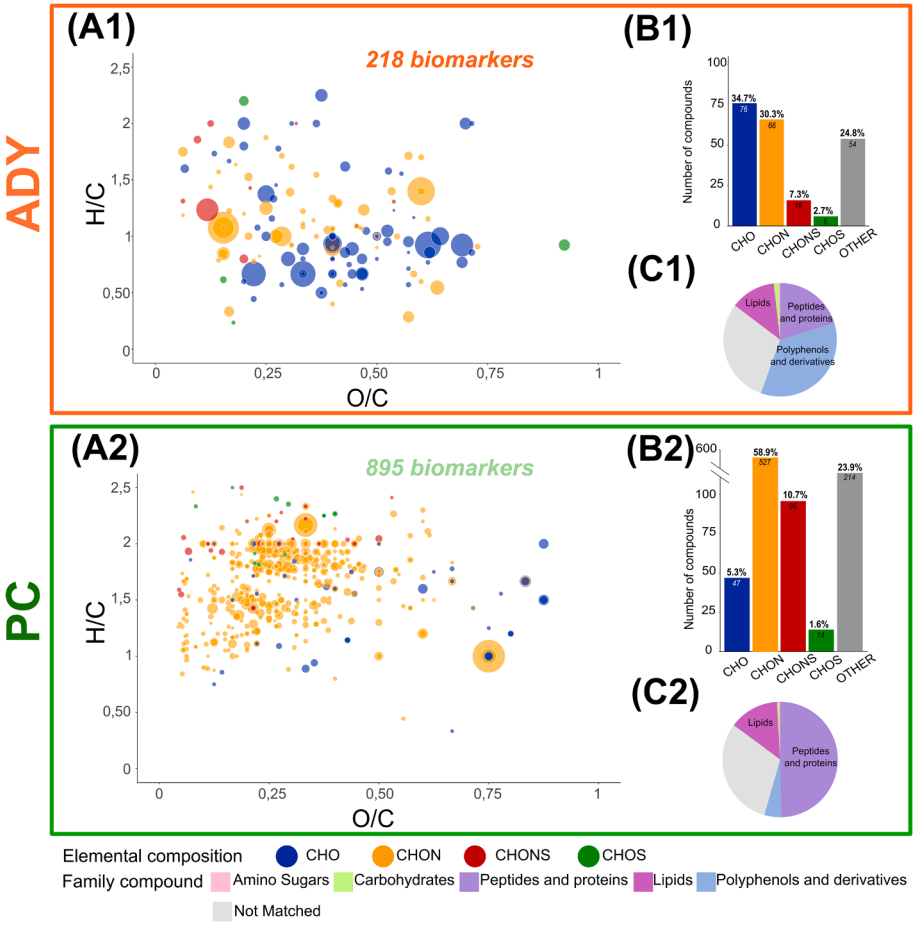

Fig. 2. Characterization of the impact of two yeast inoculation modes (ADY and PC) on exometabolome. For each inoculation mode (ADY (orange), PC (green)), H/C vs O/C van Krevelen diagrams (A1, A2), histogram proportions that show their elemental compositions (B1, B2), and pie charts (C1, C2) representing the distribution of these biomarkers by hypothetical families of common wine compounds are presented. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

inoculation mode (Fig. 2B2). The ADY inoculation mode was associated with a majority of molecular formulas in CHO (34.7 %) which represented only 5.3 % of the biomarkers in PC conditions. These molecular formulas mostly belong to the family of polyphenols and derivatives while the nitrogenous compounds in CHON are associated with the family of peptides, proteins and amino acids (Fig. 2C2).

To obtain greater detail, the chemical composition of the wines from the two inoculation modes were compared for each of the strains respectively.

T-tests were performed (p-value < 0.05) on extracted features associated with the molecular formulas exhibited by each strain as part of the two different inoculation modes. Features that displayed significant differences of intensity within each strain were considered as biomarkers. Biomarkers for both each of the strains and inoculation modes are shown in Fig. 3. For better understanding, our research team choose to focused on the SB and SC strains which are strains widely used by professionals in the sector.

Regarding the ADY modality of inoculation of the two strains, 362 and 470 biomarkers were found to be characteristic for strains SB and SC, respectively. As previously observed when considering all yeast strains, the majority of biomarkers were composed of CHO and CHON. When considering the metabolomic signature for both strains, considerable diversity in the nature of the compounds was observed. When examining the coordinates of the compounds represented by the bubbles and their color on graphs A1 and A2 (Fig. 3), a different pattern can be noticed. Indeed, despite similar proportions of compounds with the same elemental category, there was a considerable diversity of compounds. Those compounds seemed to be associated with polyphenols compounds and their derivatives or lipids. Moreover, for the modalities inoculated with pre-culture, the number of biomarkers was much higher (more than twice as many) than for the modalities inoculated with ADY, regardless of the yeast strain. For both strains, more than 40 % of the elemental formulas were composed of CHON which can be associated with the peptide and protein family. It should be noted that unlike the modalities with ADY the metabolomic signature of the two strains seemed to be very close. This was verified by comparing the biomarkers of both strains inoculated by preculture. 37 % of the biomarkers of SB and SC strains associated with the PC inoculation mode were common to both strains. For the ADY inoculation mode, this proportion of common biomarkers between strains was different, representing only 20 %. This was confirmed by comparing the SB vs SD and the SC vs SD strains. This would indicate that the inoculation mode may have an impact on the diversity of wine composition, regardless of the strain. On average, the ADY mode of inoculation was associated with higher variability among strains (+18.3 %). This would suggest that this mode of inoculation preserves the specific metabolomic signature linked to the strain. This could also be associated with the same physiological state of the different strains during the PC inoculation due to the standardization during the two pre-culture phases.

Each of the biomarkers was subjected to comparison to the KEGG database. Interestingly, regardless of strain (SB, SC and SD), the metabolite annotated as glutathione (annotation level 2) was found to be significantly more intense in the ADY inoculation mode. The presence of glutathione undoubtedly reflects the conditions undergone by the yeast during the production process of active dry yeasts. Gòmez-Pastor and collaborators demonstrated an increase in glutathione during the desiccation step (Gómez-Pastor et al., 2010). This could suggest that yeasts subjected to the ADY production process during which they undergo oxidative conditions have developed a metabolism associated with resistance to future oxidative conditions. This condition (ADY) was also associated with a significant increase in octanoic acid intensity (annotation level 2). Medium-chain fatty acids have been described as possible stress markers (Czabany et al., 2007; Mannazzu et al., 2008). This could also reflect an involvement of beta oxidation. A rise in the

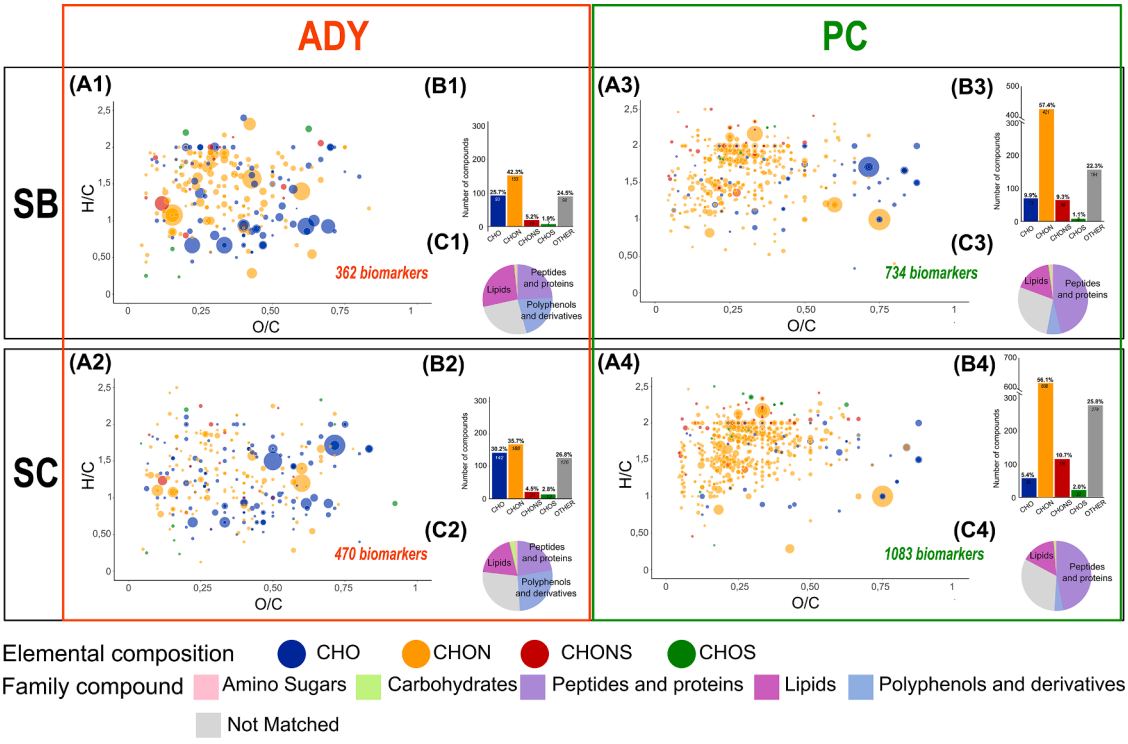

Fig. 3. Comparison of the metabolomic footprint according to two inoculation modes (ADY and PC) of two strains of *Saccharomyces cerevisiae*. (A) H/C vs O/C van Krevelen diagrams combined with intensity vs *m/z* diagrams coupled to (B) histogram proportions of the elemental formula compositions exhibit specific strain biomarkers significantly more intense in modality PC (green) and more intense in modality ADY (orange) for the strains SC and SB. Bubble sizes indicate relative intensities of corresponding features. Color code: CHO, blue; CHON, orange; CHONS, red; CHOS, green. (C) The pie chart represents the distribution of these markers by hypothetical families of common wine compounds. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

expression of genes involved in the beta-oxidation of long-chain fatty acids was observed by Singh and colleagues in a transcriptomic analysis (Singh et al., 2005).

Fragmentation using LC-MS/MS analysis was performed to confirm the structure of annotated compounds and refine compound identification (Supplementary Table 2). Regarding the ADY inoculation modality 226 and 282 highlighted biomarkers were fragmented, for both SB and SC strains, respectively. Among these fragmented biomarkers, 18 and 26 were annotated (Level 2), for SB and SC strains respectively, according to a comparison with the internal and external fragmentation databases (MassBank and Metlin).

Of the annotated biomarkers associated with the ADY inoculation mode, four phenolic compounds were found regardless of the strain studied: epicatechin, quercetin, catechin and caffeic acid. Their structure was confirmed by MS/MS analysis and comparison to standard compounds from the internal library (Level 2) (MassBank).

Quercetin and catechin were found to be more abundant in the conditions inoculated with ADY. Thus, the mode of inoculation without pre-culturing could induce the conservation of these compounds in wine. Different hypotheses could be proposed regarding the difference in abundance of these polyphenols between the two inoculation methods. Indeed, it seems that they were not complexed with other compounds or with the cell walls and plasma membranes of the yeast. Many authors have identified interactions between polyphenols and compounds such as polysaccharides like mannoproteins or peptides and proteins (Le Bourvellec & Renard, 2012; Mekoue Nguela et al., 2015). It has also been shown that nutrient conditions are associated with a modulation of polyphenol adsorption by yeast (Mekoue Nguela et al., 2015; Sidari & Caridi, 2016). A difference in nutrient status was indeed induced between the two modes of inoculation and could contribute to this observation.

These polyphenols are known to be natural antioxidants through different reactions such as the chelation of metal ions, radical scavenging activity, direct or indirect interactions with reactive oxygen species (ROS) or the inhibition of enzymes involved in ROS production (Bayliak et al., 2016; Romanet et al., 2021). They have also been reported to increase yeast cell stress resistance and longevity (Belinha et al., 2007). For example, quercetin has been described to induce the modulation of signaling pathways involved in cell integrity via the regulation of actin synthesis (Vilaça et al., 2012), as well as to positively regulate the transcription of genes involved in central carbon metabolism in order to increase the biosynthesis of trehalose, a source of energy and a protector against stress (Gancedo & Flores, 2004).

Moreover, the yeasts inoculated in the ADY form were all associated with an increased presence of caffeic acid in the wine compared to the pre-culture modality. In addition, SB ADY also presented p-coumaric acid as a biomarker (level 4). In yeasts, caffeic and p-coumaric acids could be metabolized into vinyl phenols dues to the activity of the decarboxylase possessed by certain strains associated with a phenotype called POF +. The inoculation mode could impact on the previously described decarboxylase activity of the strain (Grando et al., 1993, Shinoara et al., 2000).

Regarding PC conditions, the annotated compounds (Level 1) were peptides and amino acids (L-isoleucine, leucine, phenylalanine, peptides including leucine). As the sample was collected at the end of alcoholic fermentation, it seems unlikely that autolysis occurred. Also, it was possible that the pool of amino acids present in the medium could also be due to the passive exsorption of amino acids. This phenomenon occurs upstream of autolysis at the beginning of cell degeneration and induces a rapid release of the pool of amino acids stored in the cell vacuole (Fornairon-Bonnefond et al., 2002). Another hypothesis concerns the nitrogen status of the inoculated yeasts. Indeed, during the ADY production phase, the yeasts are starved of nitrogen resources (Gómez-Pastor et al., 2010; Matallana & Aranda, 2017), unlike yeasts in pre-culture which grow in the presence of high concentrations of these nutrients. The latter could be at the origin of an increase in the uptake of surrounding nitrogenous nutrients during must inoculation, thus depleting the environment. This difference could induce a variation in metabolism expression described as a modulation of nutrient uptake under harsh conditions previously reported by Rossignol et al., 2003. Nutrient sensing pathways were found to be differentially regulated during nutrient stresses involving the *TOR* genes for nitrogen resources and *PKA* and *SNF1* for sugars.

High resolution mass spectrometry revealed a significant modification of the yeast metabolome for three of the four *S. cerevisiae* strains depending on the inoculation method. A specific metabolome for each of the inoculation modes could be established under our experimental conditions. Thus, it should be noted that the yeasts presented different metabolisms according to the preparation conditions before inoculation. The yeasts produced in the ADY form encountered various production conditions for which they were still marked. The pre-culture stages undergone by PC yeasts seem to have caused them to lose these stress resistance capacities, inducing changes in the metabolome. Moreover, when inoculated into the must, they switched to a fermentative metabolism, unlike the yeasts in pre-culture, which were already fermentative. The different types of yeast preparation induced significant metabolism redirections, leading to the production of specific metabolites and a significant modulation of the final composition of the wine.

Here we have shown for the first time that the use of the same yeast strain in two different physiological states to inoculate musts leads to different wine compositions. Thus, the metabolome keeps traces of yeast history.

In view of this significant metabolic difference according to the mode of inoculation, this suggest that from a volatilome perspective, there could be a modulation of the concentrations of volatile compounds that may be related to the aromatic profiles of wines.

3.3. Volatilome analysis

The volatilome of wines from both inoculation modes were analyzed. The concentration of 67 volatile compounds determined by GC-FID or GC–MS is reported in Supplementary Table 2. Volatile compounds detected in the wine resulting from PC and ADY for each strain were separated using Principal Component Analysis (PCA) (Fig. 4A). These projections were able to separate the PC fermentations from the ADY fermentations for each modality according to the component 2. The strongest effects of the inoculation mode were observed for all of the strains, shown in Fig. 4A. In addition to this substantial impact for all the strains, it should also be noted that the SA strain stands out from the other strains studied. This strain appeared separate from the three other yeast strains according to PCA component 1, explaining 30.29 % of the variability data. This significant discrimination supported a previous observation, noted at the exometabolome level. This variation could be associated with the redirection of the metabolic flux of carbohydrates towards the pentose phosphate pathway (Cadière et al., 2011). Indeed, the volatile compounds involved in the discrimination of this strain are esters, in green, on the PCA of variables (Fig. 4B). This confirmed the overexpression of volatile ester-like compounds such as isoamyl acetate, phenylethyl acetate and isobutyl acetate displayed by this strain numerous times (Cadière et al., 2011). As can be seen, PCA component 2, explaining 23.38 % of the variability data, allowed discriminating PC inoculation from the corresponding ADY inoculation mode, which demonstrates that the wines produced by the two different inoculation mode are significantly different.

For each family of compounds, esters, acids, higher alcohols, norisoprenoids and phenols, the total concentration was compared for each of the wines derived from the inoculated strains according to the two modalities studied (Fig. 5). According to a T-test (p-value < 0.05) regarding the levels of acids and higher alcohols, no significant difference was observed between the two inoculation conditions, except for the level of higher alcohols for strain D. The production of these two families of compounds seems to be only slightly or not impacted by the

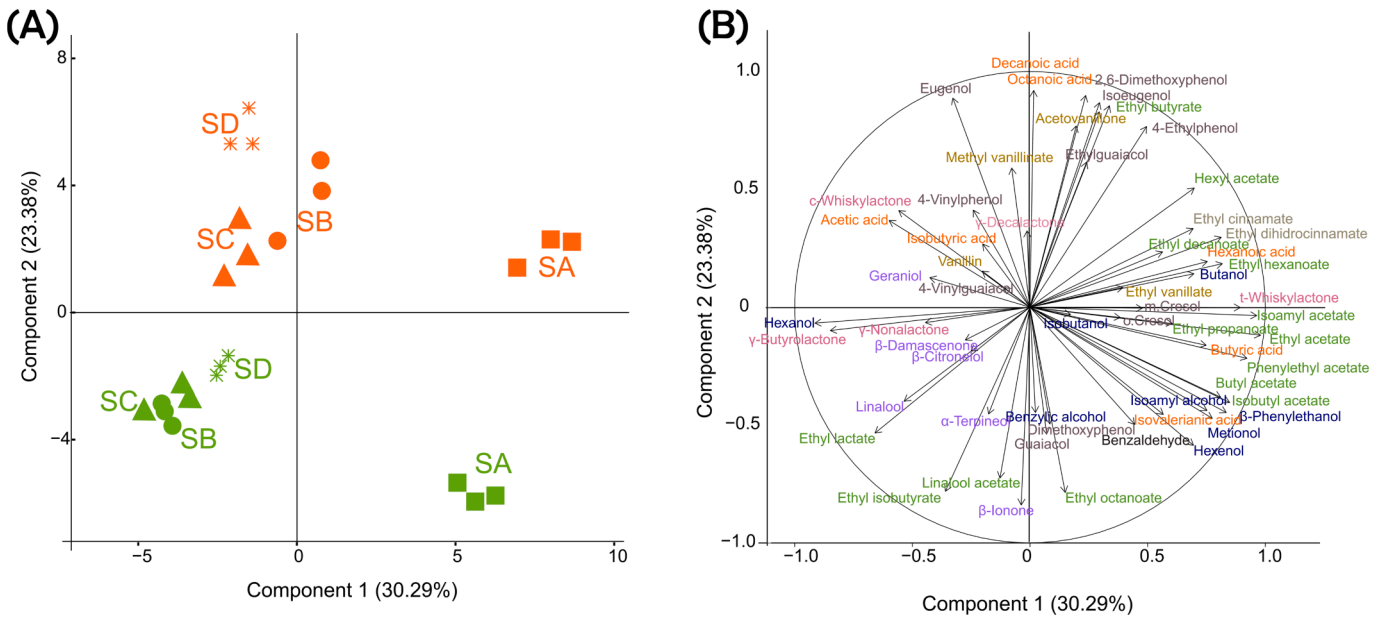

Fig. 4. Characterization of the impact of two yeast inoculation modes (ADY and PC) on volatilome. PCA analysis of samples (A1) and variables (A2) (Component 1 vs Component 2) applied to all volatile compounds considered in the eight fermentations carried out by the different strains of *Saccharomyces cerevisiae* with two different inoculation modes. A1: ADY is represented in orange and PC in green. A2: Colored variables represent different families of compounds (green: esters, orange: acids, blue: higher alcohols, pink: lactone, purple: norisoprenoids, grey: phenols). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

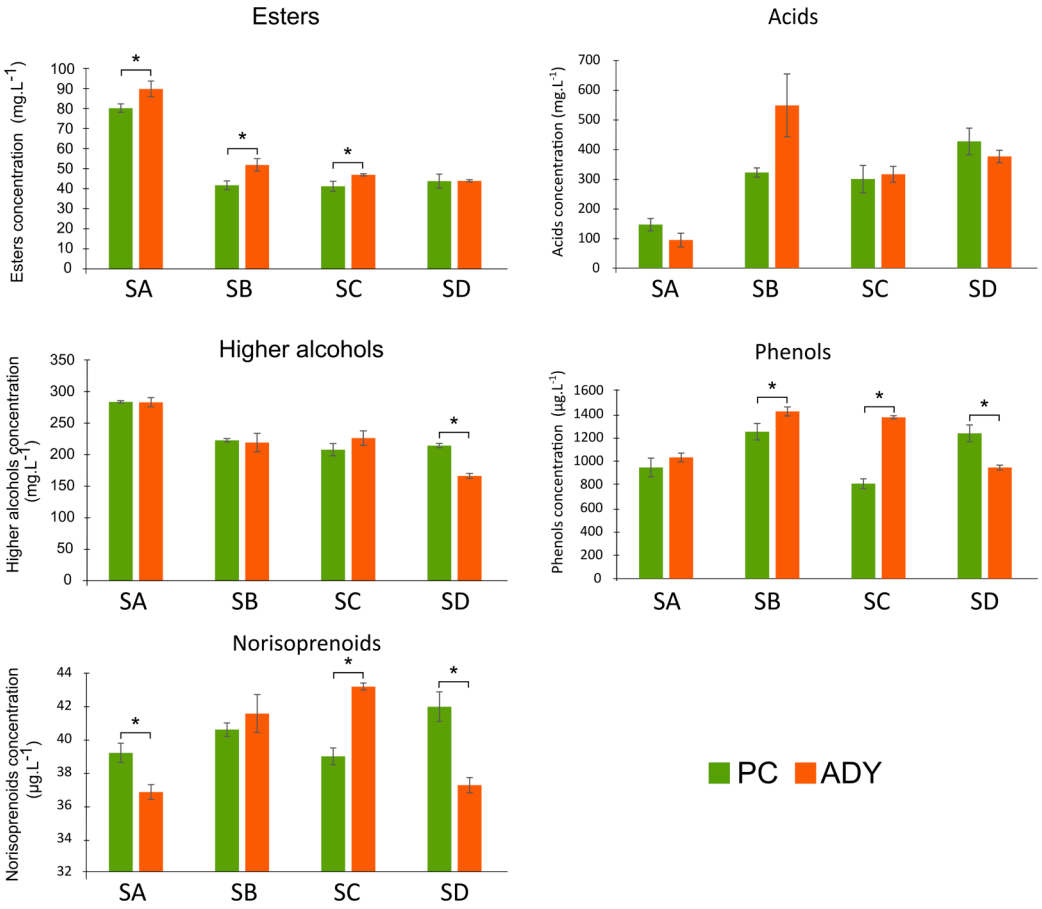

Fig. 5. Impact of two yeast inoculation modes (ADY and PC) on the major families of volatile compounds in wine. Comparison of volatile organic compound content between the two modes of inoculation of the four strains of *Saccharomyces cerevisiae*. *Values correspond to the average of three biological replicates ± standard deviation. Statistical analysis was performed between both inoculation modes for each yeast strain (test, p-value ≤ 0.05) (* significant difference).

inoculation mode of the yeast during alcoholic fermentation. The concentrations of norisoprenoids and phenols were significantly affected by the inoculation mode for 3 out of 4 strains. For example, considering the SD strain, the inoculation mode seemed to induce a modification of the concentration of norisoprenoids and total phenols. Total norisoprenoid concentrations of 39.1 and 43.2 $\mu g.L^{-1}$ were associated with the PC and ADY inoculation modes respectively. For phenols, a difference of 290.7 $\mu g.L^{-1}$ was observed between the two inoculation modes. The PC inoculation mode was reported to reach a concentration of 1237.2 $\mu g.L^{-1}$ while the ADY inoculation mode was reported to lead to a concentration of 946.4 $\mu g.L^{-1}$.

However, these modulations of concentration did not follow the same trend. Thus, these variations in concentration seemed to be strain-dependent.

The ester family seemed to be the family of compounds most impacted by the mode of inoculation according to the same trend, with an increase in ester production during the inoculation of the SA, SB and SC strains following the rehydration of ADY. Moreover, these compounds, mainly produced by yeasts, were previously described to be the main contributors to the aromatic profile of the wines (Sumby et al., 2010). In our study, in most cases and individually these compounds were present at concentrations higher than the perception threshold. Therefore, we focused on these compounds.

Among the 13 esters quantified, 5 were considered as examples and are detailed in this article (Fig. 6). The concentration of these five compounds was particularly affected by the inoculation mode used.

Ethyl lactate, ethyl esters of organic acids, exhibited similar trends, regardless of strain. The PC modality displayed significantly higher concentrations than the ADY modality. For example, a factor of 1.7 was found between the concentration of this compound for strain SC between the two inoculation modes, PC (0.50 $mg.L^{-1}$) and ADY (0.29 $mg.L^{-1}$). Similar results were observed for ethyl octanoate. Moreover, it should be noted that octanoic acid, the precursor of this ester (Saerens et al., 2008), was found with a significantly higher intensity under ADY conditions with a putative annotation (level 2) by metabolomics analysis. Thus, an increased intensity of this precursor compound could participate in a lower concentration of the final product, ethyl octanoate, in these same conditions. Indeed, the synthesis of ethyl esters is largely dependent on the concentration of the precursors of these compounds, the fatty acids. Moreover, redox conditions are known to affect the activity of acetyl-coA carboxylase and thus the concentration of the fatty acid pool (Saerens et al., 2010). It is therefore possible that the two inoculation methods are subject to different redox conditions that could induce the difference in wine ethyl ester concentration as verified by (Fariña et al., 2012).

In contrast, ethyl butyrate was found to be significantly more concentrated in the wines produced under ADY conditions. All the wines from the four strains were concerned. Concentrations ranged from 0.09 for wines associated with strains under PC conditions to 0.16 $mg.L^{-1}$ for those under ADY conditions. On the other hand, wines from strains inoculated with PC were significantly richer in ethyl lactate than wines from ADY strains, regardless of the strain.

The enhanced occurrence of phenolic acids such as caffeic acid in the wines from the ADY yeast inoculation modalities could contribute to the persistence of fruity aromas during wine ageing. These phenolic acids contribute to the protection of the ethyl esters of fatty acids from hydrolysis (Lambropoulos & Roussis, 2007).

Isoamyl acetate showed the same pattern as ethyl butyrate in the wines produced by the SB, SC and SD strains in the ADY modality compared to the PC modality. However, it should be noted that the same trend was observed for the wine associated with the SA strain. This lack of significant difference could be related to the overexpression of the pentose phosphate pathway in the SA strain. This strain is indeed associated with an increased production of acetate esters including isoamyl acetate (Cadière et al., 2011; Rollero et al., 2015). The same description was given for 2-phenylethyl acetate by the same authors. This was verified in our work on the Chardonnay must studied with a 3 to 4-fold increase in concentration compared to other strains. For the latter compound, it would appear that the impact of the mode of inoculation on the compound concentration is strain dependent. There was a higher production of 2-phenylethyl acetate for the PC inoculation condition for the SB and SD strains. In contrast, production was significantly

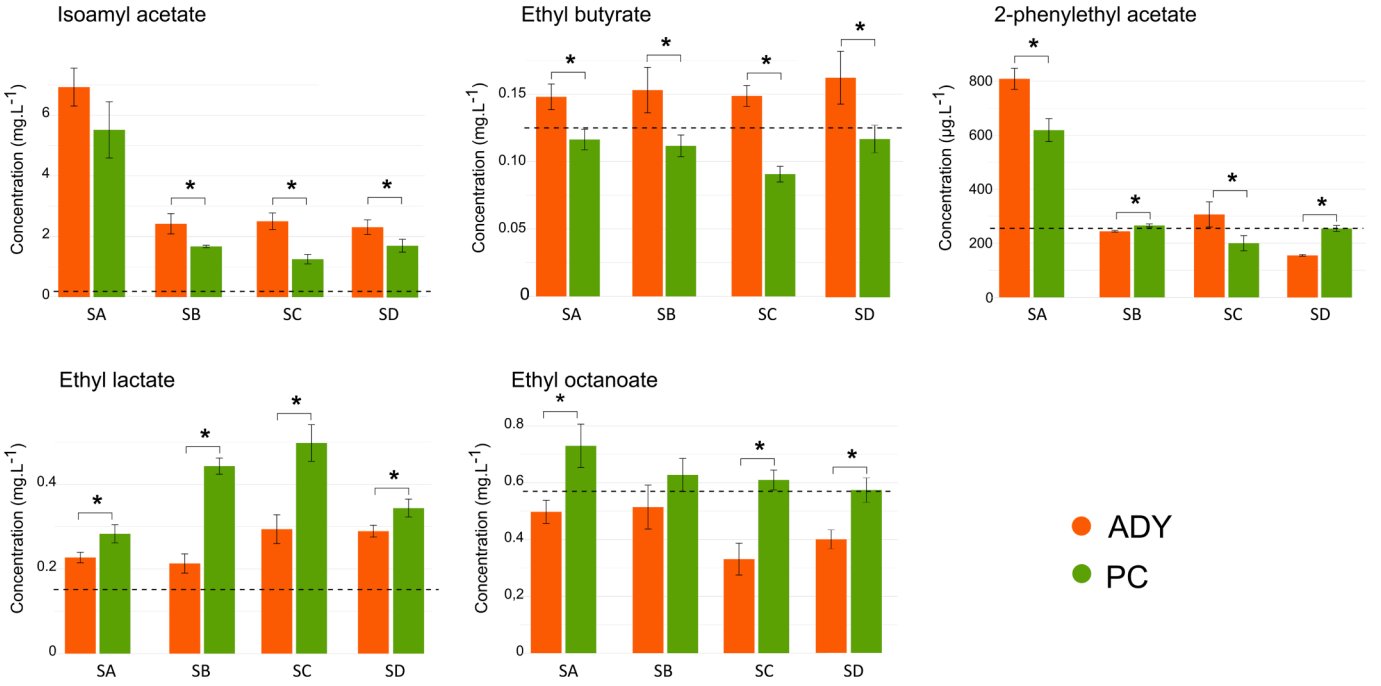

Fig. 6. Comparison of esters content between two inoculation modes (ADY and PC) for four strains of *Saccharomyces cerevisiae*. Comparison of five ester concentrations between both modalities of inoculation for the four strains of *S. cerevisiae* (SA, SB, SC and SD) in Chardonnay. The dotted line corresponds to the perception threshold of the quantified compounds. *Values correspond to the average of three biological replicates ± standard deviation. Statistical analysis was performed between both inoculation modes for each yeast strain (test, p-value ≤ 0.05) (* significant difference).

lower for the SA and SC strains.

It was observed that the concentration of 2-phenylethyl acetate was significantly lower in the wines produced by the SA and SC strains in the PC inoculation modality compared to the ADY modality. This was not noted for all the strains. Interestingly, this could have been paralleled with the precursor amino acid content in the medium for the SC strain. Although amino acids were described as precursors of certain volatile organic compounds as esters (Saerens et al., 2010), it is known that the production mechanisms of these volatile compounds depend on many factors (Lilly et al., 2000). Moreover, it was shown by Crépin and co-workers that the assimilated amino acids were mainly used for the de novo synthesis of proteogenic amino acids and not for the production of volatile compounds (Crépin et al., 2017).

The carbon skeleton of these volatile compounds appears to come mainly from resources such as glucose. Therefore, a difference in the assimilation of these resources could contribute to a difference in the production of volatile compounds like 2-phenylethyl acetate and isoamyl acetate.

These observations could indicate a difference in the assimilation of the precursors, carbon compounds, amino acids and fatty acids, or a modulation of their catabolism in the cell.

The mode of inoculation affected the volatilome significantly. Different families of volatile compounds were concerned: phenols, norisoprenoids and esters. The total concentration of the ester family was mostly impacted by the inoculation mode, whatever the strain. The majority of these compounds exhibited a concentration above the perception threshold. This concentration suggests potential participation in the aromatic profile of wines. Therefore, the inoculation method could have a significant impact on the aromatic profile of post alcoholic fermentation wines.

4. Conclusion

More and more yeast inoculation methods are used to initiate the alcoholic fermentation of wines. Up to now, few studies have focused on the impact of these inoculation methods on the composition of wine. Through this study, we wanted to describe this impact using a metabolomic approach that would account for changes in wine composition post alcoholic fermentation. Although the wines produced by each of the strains studied according to the two inoculation modes did not present differences in the classical oenological parameters, we were able to observe a significant modification of the metabolomic signature. High resolution mass spectrometry revealed a modification of the exometabolome of 3 out of 4 strains according to the preparation mode of the yeasts for inoculation. The intensity of 1484 biomarkers was modulated. This revealed that yeasts have different metabolisms depending on the production or propagation stages undergone before inoculation. The biomarkers impacted and associated with these differences in metabolism are mainly nitrogenous compounds and polyphenols. Like the non-volatile metabolome, the volatilome was impacted by the inoculation mode regardless of the strain studied. Esters were the compounds most impacted according to the same trend. Most of them had concentrations above their perception threshold. This suggests a direct impact on the aromatic profile of the wines. The modulations of ester concentrations according to inoculation modes could be compared to the modulations of composition observed during the metabolomic analysis. Thus, a modification of the assimilation of precursors or a difference in their catabolism could be at the origin of the modulation of the volatilome. Through this study, we were able to observe that the yeast inoculation method has a significant impact on the composition of wines after alcoholic fermentation. Wines obtained with PC yeasts exhibit similar metabolomic signatures whatever their genetic background whereas wines fermented by the ADY showed very distinct metabolomic footprints, respecting their different genetic backgrounds. The mode of yeast inoculation should therefore be considered by winemakers when choosing a must inoculation method.

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CRediT authorship contribution statement

Fanny Bordet: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Validation, Visualization, Writing – original draft, Writing – review & editing. **Rémy Romanet:** Data curation, Formal analysis, Investigation, Writing – review & editing. **Florian Bahut:** Data curation, Formal analysis, Writing – review & editing. **Vicente Ferreira:** Writing – review & editing. **Cristina Peña:** Formal analysis, Writing – review & editing. **Anne Julien-Ortiz:** Funding acquisition, Project administration, Supervision, Writing – review & editing. **Chloé Roullier-Gall:** Conceptualization, Funding acquisition, Methodology, Project administration, Supervision, Validation, Writing – review & editing. **Hervé Alexandre:** Conceptualization, Funding acquisition, Methodology, Project administration, Supervision, Writing – review & editing.

Declaration of competing interest

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

Data availability

The data that has been used is confidential.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodchem.2024.138391.

References

Bayliak, M. M., Burdylyuk, N. I., & Lushchak, V. I. (2016). Quercetin increases stress resistance in the yeast Saccharomyces cerevisiae not only as an antioxidant. *Annals of Microbiology, 66*(2), 569–576. https://doi.org/10.1007/s13213-015-1136-8

Belinha, I., Amorim, M. A., Rodrigues, P., de Freitas, V., Moradas-Ferreira, P., Mateus, N., & Costa, V. (2007). Quercetin Increases Oxidative Stress Resistance and Longevity in *Saccharomyces cerevisiae*. *Journal of Agricultural and Food Chemistry, 55*(6), 2446–2451. https://doi.org/10.1021/jf063302e

Beltran, G., Torija, M. J., Novo, M., Ferrer, N., Poblet, M., Guillamón, J. M., Rozès, N., & Mas, A. (2002). Analysis of yeast populations during alcoholic fermentation : A six year follow-up study. *Systematic and Applied Microbiology, 25*(2), 287–293. https://doi.org/10.1078/0723-2020-00097

Bely, M., Masneuf-Pomarède, I., & Dubourdieu, D. (2005). Influence of physiological state of inoculum on volatile acidity production by Saccharomyces cerevisiae during high sugar fermentation. *OENO One*, *39*(4), 191. 10.20870/oeno-one.2005.39.4.886.

Bordet, F., Romanet, R., Bahut, F., Ballester, J., Eicher, C., Peña, C., Ferreira, V., Gougeon, R., Julien-Ortiz, A., Roullier-Gall, C., & Alexandre, H. (2023). Expanding the diversity of Chardonnay aroma through the metabolic interactions of Saccharomyces cerevisiae cocultures. *Frontiers in Microbiology, 13*, 1032842. https://doi.org/10.3389/fmicb.2022.1032842

Bordet, F., Roullier-Gall, C., Ballester, J., Vichi, S., Quintanilla-Casas, B., Gougeon, R. D., Julien-Ortiz, A., Kopplin, P. S., & Alexandre, H. (2021). Different Wines from Different Yeasts? "Saccharomyces cerevisiae Intraspecies Differentiation by Metabolomic Signature and Sensory Patterns in Wine". *Microorganisms, 9*(11), 2327. https://doi.org/10.3390/microorganisms9112327

Cadière, A., Ortiz-Julien, A., Camarasa, C., & Dequin, S. (2011). Evolutionary engineered *Saccharomyces cerevisiae* wine yeast strains with increased in vivo flux through the pentose phosphate pathway. *Metabolic Engineering, 13*(3), 263–271. https://doi.org/10.1016/j.ymben.2011.01.008

Crépin, L., Truong, N. M., Bloem, A., Sanchez, I., Dequin, S., & Camarasa, C. (2017). Management of Multiple Nitrogen Sources during Wine Fermentation by Saccharomyces cerevisiae. *Applied and Environmental Microbiology, 83*(5). https://doi.org/10.1128/AEM.02617-16

Czabany, T., Athenstaedt, K., & Daum, G. (2007). Synthesis, storage and degradation of neutral lipids in yeast. Biochimica et Biophysica Acta (BBA) - Molecular and Cell Biology of. *Lipids, 1771*(3), 299–309. https://doi.org/10.1016/j.bbalip.2006.07.001

Fariña, L., Medina, K., Urruty, M., Boido, E., Dellacassa, E., & Carrau, F. (2012). Redox effect on volatile compound formation in wine during fermentation by Saccharomyces cerevisiae. *Food Chemistry, 134*(2), 933–939. https://doi.org/10.1016/j.foodchem.2012.02.209

Ferreira, D., Galeote, V., Sanchez, I., Legras, J.-L., Ortiz-Julien, A., & Dequin, S. (2017). Yeast multistress resistance and lag-phase characterisation during wine fermentation. *FEMS Yeast Research, 17*(6). https://doi.org/10.1093/femsyr/fox051

Fornairon-Bonnefond, C., Camarasa, C., & Moutounet, M. (2002). NEW TRENDS ON YEAST AUTOLYSIS AND WINE AGEING ON LEES: A BIBLIOGRAPHIC REVIEW. *J. Int. Sci. Vigne Vin.*

Gancedo, C., & Flores, C. (2004). The importance of a functional trehalose biosynthetic pathway for the life of yeasts and fungi. *FEMS Yeast Research, 4*(4–5), 351–359. https://doi.org/10.1016/S1567-1356(03)00222-8

Gómez-Pastor, R., Pérez-Torrado, R., Cabiscol, E., & Matallana, E. (2010). Transcriptomic and proteomic insights of the wine yeast biomass propagation process : Transcriptomics and proteomics during biomass propagation. *FEMS Yeast Research, 10*(7), 870–884. https://doi.org/10.1111/j.1567-1364.2010.00667.x

Lambropoulos, I., & Roussis, I. G. (2007). Inhibition of the decrease of volatile esters and terpenes during storage of a white wine and a model wine medium by caffeic acid and gallic acid. *Food Research International, 40*(1), 176–181. https://doi.org/10.1016/j.foodres.2006.09.003

Le Bourvellec, C., & Renard, C. M. G. C. (2012). Interactions between Polyphenols and Macromolecules : Quantification Methods and Mechanisms. *Critical Reviews in Food Science and Nutrition, 52*(3), 213–248. https://doi.org/10.1080/10408398.2010.499808

Lilly, M., Lambrechts, M. G., & Pretorius, I. S. (2000). Effect of Increased Yeast Alcohol Acetyltransferase Activity on Flavor Profiles of Wine and Distillates. *Applied and Environmental Microbiology, 66*(2), 744–753. https://doi.org/10.1128/AEM.66.2.744-753.2000

López, R., Aznar, M., Cacho, J., & Ferreira, V. (2002). Determination of minor and trace volatile compounds in wine by solid-phase extraction and gas chromatography with mass spectrometric detection. *Journal of Chromatography A, 966*(1–2), 167–177. https://doi.org/10.1016/S0021-9673(02)00696-9

Mannazzu, I., Angelozzi, D., Belviso, S., Budroni, M., Farris, G. A., Goffrini, P., Lodi, T., Marzona, M., & Bardi, L. (2008). Behaviour of Saccharomyces cerevisiae wine strains during adaptation to unfavourable conditions of fermentation on synthetic medium : Cell lipid composition, membrane integrity, viability and fermentative activity. *International Journal of Food Microbiology, 121*(1), 84–91. https://doi.org/10.1016/j.ijfoodmicro.2007.11.003

Manzano, S., Vargas, S., Casaubon, G., & González, Á. (2019). Evaluation of an active yeast propagation system on fermentation traits and quality of C.V. *Carmenère wine. BIO Web of Conferences, 12*, 02009. https://doi.org/10.1051/bioconf/20191202009

Matallana, E., & Aranda, A. (2017). Biotechnological impact of stress response on wine yeast. *Letters in Applied Microbiology, 64*(2), 103–110. https://doi.org/10.1111/lam.12677

Mekoue Nguela, J., Sieczkowski, N., Roi, S., & Vernhet, A. (2015). Sorption of Grape Proanthocyanidins and Wine Polyphenols by Yeasts, Inactivated Yeasts, and Yeast Cell Walls. *Journal of Agricultural and Food Chemistry, 63*(2), 660–670. https://doi.org/10.1021/jf504494m

Novo, M., Beltran, G., Rozes, N., Guillamon, J.-M., Sokol, S., Leberre, V., FranÃ§ois, J., & Mas, A. (2007). Early transcriptional response of wine yeast after rehydration : Osmotic shock and metabolic activation. *FEMS Yeast Research, 7*(2), 304–316. https://doi.org/10.1111/j.1567-1364.2006.00175.x

Ortega, C., López, R., Cacho, J., & Ferreira, V. (2001). Fast analysis of important wine volatile compounds. *Journal of Chromatography A, 923*(1–2), 205–214. https://doi.org/10.1016/S0021-9673(01)00972-4

Pérez-Torrado, R., Bruno-Bárcena, J. M., & Matallana, E. (2005). Monitoring Stress-Related Genes during the Process of Biomass Propagation of *Saccharomyces cerevisiae* Strains Used for Wine Making. *Applied and Environmental Microbiology, 71*(11), 6831–6837. https://doi.org/10.1128/AEM.71.11.6831-6837.2005

Rivas-Ubach, A., Liu, Y., Bianchi, T. S., Tolić, N., Jansson, C., & Paša-Tolić, L. (2018). Moving beyond the van Krevelen Diagram : A New Stoichiometric Approach for Compound Classification in Organisms. *Analytical Chemistry, 90*(10), 6152–6160. https://doi.org/10.1021/acs.analchem.8b00529

Rollero, S., Bloem, A., Camarasa, C., Sanchez, I., Ortiz-Julien, A., Sablayrolles, J.-M., Dequin, S., & Mouret, J.-R. (2015). Combined effects of nutrients and temperature on the production of fermentative aromas by *Saccharomyces cerevisiae* during wine fermentation. *Applied Microbiology and Biotechnology, 99*(5), 2291–2304. https://doi.org/10.1007/s00253-014-6210-9

Romanet, R., Sarhane, Z., Bahut, F., Uhl, J., Schmitt-Kopplin, P., Nikolantonaki, M., & Gougeon, R. D. (2021). Exploring the chemical space of white wine antioxidant capacity : A combined DPPH. *EPR and FT-ICR-MS study. Food Chemistry, 355*, Article 129566. https://doi.org/10.1016/j.foodchem.2021.129566

Rossignol, T., Dulau, L., Julien, A., & Blondin, B. (2003). Genome-wide monitoring of wine yeast gene expression during alcoholic fermentation. *Yeast, 20*(16), 1369–1385. https://doi.org/10.1002/yea.1046

Rossignol, T., Postaire, O., Storaï, J., & Blondin, B. (2006). Analysis of the genomic response of a wine yeast to rehydration and inoculation. *Applied Microbiology and Biotechnology, 71*(5), 699–712. https://doi.org/10.1007/s00253-006-0398-2

Saerens, S. M. G., Delvaux, F. R., Verstrepen, K. J., & Thevelein, J. M. (2010). Production and biological function of volatile esters in *Saccharomyces cerevisiae*. *Microbial Biotechnology, 3*(2), 165–177. https://doi.org/10.1111/j.1751-7915.2009.00106.x

Saerens, S. M. G., Delvaux, F., Verstrepen, K. J., Van Dijck, P., Thevelein, J. M., & Delvaux, F. R. (2008). Parameters Affecting Ethyl Ester Production by *Saccharomyces cerevisiae* during Fermentation. *Applied and Environmental Microbiology, 74*(2), 454–461. https://doi.org/10.1128/AEM.01616-07

Salvadò, Z., Chiva, R., RodrÃguez-Vargas, S., RÃ¡ndez-Gil, F., Mas, A., & GuillamÃ³n, J. M. (2008). Proteomic evolution of a wine yeast during the first hours of fermentation. *FEMS Yeast Research*, *8*(7), 1137-1146. 10.1111/j.1567-1364.2008.00389.x.

Santamaria, P., Garijo, P., Lopez, R., Tenorio, C., & Rosagutierrez, A. (2005). Analysis of yeast population during spontaneous alcoholic fermentation : Effect of the age of the cellar and the practice of inoculation. *International Journal of Food Microbiology, 103*(1), 49–56. https://doi.org/10.1016/j.ijfoodmicro.2004.11.024

Schymanski, E. L., Jeon, J., Gulde, R., Fenner, K., Ruff, M., Singer, H. P., & Hollender, J. (2014). Identifying Small Molecules via High Resolution Mass Spectrometry : Communicating Confidence. *Environmental Science & Technology, 48*(4), 2097–2098. https://doi.org/10.1021/es5002105

Sidari, R., & Caridi, A. (2016). Nutrient depletion modifies cell wall adsorption activity of wine yeast. *World Journal of Microbiology and Biotechnology, 32*(6), 89. https://doi.org/10.1007/s11274-016-2047-y

Singh, J., Kumar, D., Ramakrishnan, N., Singhal, V., Jervis, J., Garst, J. F., Slaughter, S. M., DeSantis, A. M., Potts, M., & Helm, R. F. (2005). Transcriptional Response of Saccharomyces cerevisiae to Desiccation and Rehydration. *Applied and Environmental Microbiology, 71*(12), 8752–8763. https://doi.org/10.1128/AEM.71.12.8752-8763.2005

Sumby, K. M., Grbin, P. R., & Jiranek, V. (2010). Microbial modulation of aromatic esters in wine : Current knowledge and future prospects. *Food Chemistry, 121*(1), 1–16. https://doi.org/10.1016/j.foodchem.2009.12.004

Varela, C., Pizarro, F., & Agosin, E. (2004). Biomass Content Governs Fermentation Rate in Nitrogen-Deficient Wine Musts. *Applied and Environmental Microbiology, 70*(6), 3392–3400. https://doi.org/10.1128/AEM.70.6.3392-3400.2004

Vilaça, R., Mendes, V., Mendes, M. V., Carreto, L., Amorim, M. A., de Freitas, V., Moradas-Ferreira, P., Mateus, N., & Costa, V. (2012). Quercetin Protects Saccharomyces cerevisiae against Oxidative Stress by Inducing Trehalose Biosynthesis and the Cell Wall Integrity Pathway. *PLoS ONE, 7*(9), e45494.