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Investigating biological mechanisms of colour changes in sustainable food systems: The role of *Starmerella bacillaris* in white wine colouration using a combination of genomic and biostatistics strategies

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A R T I C L E I N F O

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A B S T R A C T

This study explores the biological mechanisms behind colour changes in white wine fermentation using different strains of *Starmerella bacillaris*. We combined food engineering, genomics, machine learning, and physico-chemical analyses to examine interactions between *S. bacillaris* and *Saccharomyces cerevisiae*. Significant differences in total polyphenol content were observed, with *S. bacillaris* fermentation yielding 6 % higher polyphenol content compared to *S. cerevisiae* EC1118. Genomic analysis identified 12 genes in *S. bacillaris* with high variant counts that could impact phenotypic properties related to wine color. Notably, SNP analysis revealed numerous missense and synonymous variants, as well as stop-gained and start-lost variants between PAS13 and FRI751, suggesting changes in metabolic pathways affecting pigment production. Besides that, high upstream gene variants in SSK1 and HIP1R indicated potential regulatory changes influencing gene expression. Fermentation trials revealed FRI751 consistently showed high antioxidant activity and polyphenol content (Total Polyphenol: 299.33 ± 3.51 mg GAE/L, DPPH: 1.09 ± 0.01 mmol TE/L, FRAP: 0.95 ± 0.02 mmol TE/L). PAS13 exhibited a balanced profile, while EC1118 had lower values, indicating moderate antioxidant activity. The Weibull model effectively captured nitrogen consumption dynamics, with EC1118 serving as a reliable benchmark. The scale parameter delta for EC1118 was 23.04 ± 2.63, indicating moderate variability in event times. These findings highlight *S. bacillaris* as a valuable component in sustainable winemaking, offering an alternative to chemical additives for maintaining wine quality and enhancing colours profiles. This study provides insights into the biotechnological and fermented food systems applications of yeast strains in improving food sustainability and supply chain, opening new avenues in food engineering and microbiology.

Abbreviations: WWR, Whatman Water Repellent; OD, Optical Density; CFU, Colony-Forming Units; HPLC, High-Performance Liquid Chromatography; TPC, Total Polyphenol Content; FRAP, Ferric-Reducing Antioxidant Power; DPPH, 2,2-Diphenyl-1-Picrylhydrazyl; PCA, Principal Component Analysis; SNP, Single Nucleotide Polymorphisms; ANOVA, Analysis of Variance; FDR, False Discovery Rate; CO2, Carbon Dioxide; YAN, Yeast Assimilable Nitrogen; SAM, Sequence Alignment/Map; BCF, Binary Call Format; VCF, Variant Call Format; LDA, Linear Discriminant Analysis; CART, Classification and Regression Trees; kNN, k-Nearest Neighbors; SVM, Support Vector Machines; RF, Random Forest; TE, Trolox Equivalent; TPTZ, 2,4,6-Tripyridyl-s-triazine; EtOH, Ethanol; SPOCS, Species Paralogy and Orthology Clique Solver; PAS, Starmerella bacillaris strains (PAS13, PAS66, etc.); EC1118, Commercial S. cerevisiae strain.

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1. Introduction

Food science and technology are currently facing the challenge of integrating knowledge with advancements in sustainability throughout the food supply chain. Sustainable practices in food production, processing, and distribution are gaining momentum globally, driven by environmental concerns and society's demand for ethically sourced products. Simultaneously, there is a growing emphasis on offering healthy products to consumers (Bosona & Gebresenbet, 2013; Clarke & Best, 2017).

Within this context, colour emerges as a relevant parameter, as it is correlated with the quality and safety of foods. Additionally, it serves as a sensory cue that significantly influences consumer perception, preference, and ultimately, consumption attitude (Wei et al., 2012).

The interconnection between colour considerations and sustainable protocols in food production lies in optimizing food properties' integrity while simultaneously reducing the levels of preservative content. Researchers and industry stakeholders can contribute to this goal by exploring innovative methods and technologies that enhance natural colour retention in foods, such as using natural pigments and extracts, gentle processing techniques, and controlled storage environments(Di Salvo et al., 2023; Lante et al., 2016; Novais et al., 2022; Stratil et al., 2008).

Aiming at minimizing the reliance on synthetic additives and preservatives, the creation of healthier, and more nutritious products is promoted, appealing to diverse consumer demand and dietary preferences. This approach aligns with sustainability objectives by reducing environmental impact and promoting the use of renewable resources in food and beverage production processes (Yong et al., 2024). Furthermore, sustainable colouration strategies can serve as alternatives to meet consumer expectations compared to traditional products.

Microbial metabolism encompasses numerous biochemical processes that drive transformations of organic compounds within microbial cells, influencing both the synthesis and degradation of biologically active compounds (Debbab et al., 2010; Lemos Junior, Binati, et al., 2021; Lemos Junior et al., 2022).

Genomic studies employing updated bioinformatics methods can provide insights into the role of microorganisms, their metabolic activity, and their interactions with bioactive compounds in food systems (Basile et al., 2021; Treu et al., 2014; Santos et al., 2022). Indeed, yeast has been previously found to alter the colour profile of various food and beverage products through indirect metabolic activity and direct substrate degradation (Fleet, 2007). For instance, strains of *Rhodotorula* and *Yarrowia lipolytica*, are used for various applications such as in food, pharmaceuticals, and biotechnology industries. In the food sector, yeast is used for the production of natural pigments like carotenoids, which are utilized as food colourants and additives (Di Salvo et al., 2023).

Specifically, in fermentative processes like winemaking, brewing, and baking, yeast species can influence colour development and stability through various mechanisms. They affect the production of pigmented compounds such as anthocyanins, flavonoids, and melanoidins, which contribute to the colour intensity and hue of the final product (Carrau et al., 2020). *Saccharomyces cerevisiae* can metabolize polyphenols, leading to the production of secondary metabolites that may have added beneficial biological activities compared to the original compounds. For example, yeast-mediated fermentation processes can modify the structure of polyphenols, potentially enhancing their bioavailability and bioactivity. Samoticha et al., (2019) reported distinct behaviours between two *S. cerevisiae* strains and a *Saccharomyces bayanus* strain, specifically regarding their impact on the phenolic profile of Aurora white wine. These findings indicated that the wines fermented with *S. cerevisiae* strains contain significantly higher levels of polyphenols compared with *S. bayanus* strain.

Additionally, during fermentation yeasts modify the pH and redox potential of the food matrix, and thus, affect the stability and reactivity of chemical colourants (Novais et al., 2022). It is important to highlight that yeast populations may exhibit intra-species differences related to the enzymatic activities that catalyse the formation or degradation of pigments, which can impact the overall colour profile of the food or beverage (Belda et al., 2016).

Moreover, the interaction of *S. cerevisiae* and *S. bacillaris* on white wine colouration is multifactorial and encompasses various biochemical and physiological processes (Lemos Junior, et al., 2021). These species also contribute to the production of bioactive compounds through their metabolic activities (Binati et al., 2021). During fermentation, these yeast species can metabolize precursors present in grape/apple juice, leading to the formation of alcohols, esters, aldehydes, ketones, acids, terpenes, and phenols. Some of these compounds may interact with pigments and alter the overall colour profile of alcoholic beverages (Lemos Junior et al., 2020).

Advancements in food engineering, combined with genomics, machine learning, and physicochemical analyses can provide accurate strategies to unravel these complex mechanisms (Basile et al., 2021; Jiménez-Carvelo et al., 2019; Lemos Junior et al., 2018). These novel methods give researchers new insights into how biological agents, chemical reactions, and environmental factors interact, particularly in terms of color changes. This knowledge can help us better understand the microbial connections that influence the final color outcomes. Grasping these changes is essential for creating sustainable food systems that depend on natural fermentation processes to enhance product quality and stability.

This study aims to develop a novel combined approach to investigate the biological mechanisms underlying colour changes in sustainable food systems. In particular, it focuses on white wine colouration influenced by *S. bacillaris* as co-inoculum in an alcoholic fermentation with *S. cerevisiae*. We divided this study into four key steps: (1) evaluate the yeasts' interaction and their impact on colour, (2) identify interfering compounds, considering the effect of clarification methods, (3) analyse relevant genes at the genomic level, and (4) understand performance fermentationand their organic acid production. The overall objective is to clarify the role of *S. bacillaris* in white wine colouration during natural fermentation and provide new insights regarding sustainable practices in the food industry.

2. Materials and methods

To evaluate how yeast can alter colour differences, we divided the process into the following four steps:

Step 1 (Initial Yeast Evaluation)

Firstly, we evaluated whether the studied yeast truly influences the colour based on traditional clarification methods used for white wine production taking into account food engineering processes as possible interferences for false positive results.

Step 2 (Interference of Compounds)

Secondly, we checked what are the potential compounds that could interfere with the colour change, such as total phenol content, and the presence of antioxidants.

Step 3 (Genomic Analysis)

In the third step, we aimed to evaluate genes correlated with the metabolism of bioactive compounds at the genomic level using a strategy that combines: (1) identification of orthologous genes, (2) five machine learning models, and (3) analyses of Single Nucleotide Polymorphisms (SNPs) to identify high-impact variants correlated with phenotypic outcomes.

Step 4 (Fermentation Performance)

Finally, we conducted performance fermentationtests to understand how the most representative strains behave regarding nitrogen consumption and organic acid production, which can also be correlated with wine colour.

2.1. Yeast strain

Seven strains of *Starmerella bacillaris* (FRI751, PAS13, PAS66, PAS92, PAS103, PAS151, and PAS173) were isolated from fermenting musts of the dried Raboso Piave grape variety, as detailed by Lemos Junior et al. (2016). A commercial strain of Saccharomyces cerevisiae (EC1118®, Lallemand Inc., Montreal, Canada) was used as a reference strain. Starter cultures were prepared by inoculating 5 mL of YPD broth (yeast extract 10 g/L, peptone 10 g/L, dextrose 20 g/L) in 15 mL tubes using colonies from YPD agar plates. After incubating at 30 °C for 24 h, a stationary phase culture was achieved with cell concentrations ranging between 7 × 10^7 and 3 × 10^8 cells/mL as determined by optical density (OD) measurements at 600 nm and confirmed by colony-forming unit (CFU/mL) counts using the serial dilution method.

2.2. Fermentation trials

2.2.1. Synthetic and natural must

During the sequential fermentation, both S. bacillaris and S. cerevisiae EC1118 were inoculated at 1 × 10^6 cells/mL. The strain EC1118 was added to the must 48 h after the inoculation with S. bacillaris. The synthetic must MS300 (100 g/L glucose, 100 g/L fructose, and 6 g/L malic acid, pH 3.3) was used in all assays as described by Bely et al. (1990).

2.3. Clarification methods used in step 1

Post-fermentation, the clarification methods of centrifugation and filtration, were employed to assess the impact of the yeast strains on the wine's colour. Non-clarified fermentation served as control.

2.3.1. Nitrogen assimilation and bioreactor assay in step 4

2.3.1.1. Nitrogen assimilation assay. EC1118 was added to the must 24 h after the inoculation with S. bacillaris following the Kjeldahl method performed according to Carlson et al. (2018).

2.3.1.2. Bioreactor assay. EC1118 was added to the must 40 h after the inoculation with S. bacillaris.

Fermentations in bioreactors were performed in 1-l Multifors bioreactors (Infors HT, Basel, Switzerland). These instruments are equipped with sensors to monitor temperature and pH and with a flow meter red-y mod. GSM-A95A-BN00 to determine CO_2 outflow (Infors HT) (range 1–20 mL/min).

2.4. White natural must (step 2)

The base white wines were produced at the experimental winery of the Oenology School in Conegliano (Treviso, Italy) using Incrocio Manzoni grapes. The grape must, containing 160 g/L of reducing sugars and with a pH of 3.5, was used for fermentation. Following yeast inoculation, 120 mL bottles were incubated at 20 °C. Each bottle, containing 100 mL of must, was equipped with a closure that allowed carbon dioxide to escape. The fermentation progress was monitored by recording the weight loss of each culture twice per day. Fermentation was deemed complete when the daily weight loss dropped below 0.05 g. All experiments were conducted in triplicate.

2.5. Chemical analysis of synthetic and white natural must

2.5.1. HPLC analysis

At the end of the fermentation, individual samples were filtered through a 0.22 μm WWR filter. Subsequently, 10 μl of each sample was analysed using an HPLC system (Shimadzu, Japan) equipped with a refractive index detector. The detector was set at a wavelength of 600 nm for measuring concentrations of glucose, glycerol, and ethanol. For acetic acid quantification, a UV detector was utilized. Calibration curves for glucose, fructose, glycerol, acetic acid, and ethanol were established before injecting the samples into the HPLC. Chromatographic analysis was performed using a ROA-Organic Acid H + column (Phenomenex, USA), maintained at 65 °C. The mobile phase was 5 mM H_2SO_4, with a flow rate of 0.5 mL/min.

2.5.2. Colour parameters

The colour of the wine produced with synthetic and natural must was analysed by a tristimulus colourimeter to quantify colour changes using a Chroma Meter CR-410 (Konica-Minolta, Milan, Italy) in the CIE 1976 (L^*, a^*, b^*) colour space. Before measurements, the instrument was standardized against a white tile to ensure accuracy and consistency. For white wines samples colour differences (ΔE) were calculated as the Euclidean distance between two points in the three-dimensional colour space defined by L*, a^*, and b^* coordinates (Boasiako et al., 2024b; Boasiako et al., 2024c).

$$\%DPPH^* - SA = (1 - \frac{A_{Sample}}{A_{control}}) \times 100 \quad (1)$$

absorbance of the sample (A_{Sample}) and is the of the control absorbance ($A_{control}$).

Additionally, parameters such as C, a/b, and H were determined to provide a comprehensive assessment of colour changes. The experiment was conducted following established protocols and guidelines for colour analysis in food research as described elsewhere (Lante et al., 2016; Zhang et al., 2019).

2.5.3. Determination of Total polyphenol content (TPC)

The total polyphenol content was measured according to a modified Folin–Ciocalteu method (Stratil et al., 2008). The measurement was performed by mixing 780 μL of distilled water with 50.0 μL of Folin–Ciocalteu reagent and 20.0 μL of the appropriately diluted sample. After exactly 1 min, 150 μL of 20 % (w/v) Na_2CO_3 was added. The mixture was stored for 1 h in the dark at room temperature and the absorbance was read at 750 nm. Gallic acid was used to obtain a calibration curve with standard solutions within the range of 0.050–0.70 mg/mL. The results were expressed as mg/L gallic acid equivalent (mg GAE/L).

2.5.4. Evaluation of the antioxidant activity by Ferric-Reducing antioxidant power (FRAP) assay

The FRAP assay was performed according to the original method defined by Stratil et al., (2008). For the FRAP reagent, three solutions were used: solution A contained 0.30 M acetate buffer prepared by dissolving 1.90 g/L of sodium acetate in distilled water and adjusted to pH 3.6 with 16.0 mL/L glacial acetic acid, solution B was prepared by dissolving 0.0100 M TPTZ (2, 4, 6-tripyridyl-s-triazine) in 0.040 M HCl and solution C contained 0.0200 M $FeCl_3 \cdot 6H_2O$ in dH_2O. Prior to the determination, 25.0 mL of solution A, 2.50 mL of solution B, and 2.50 mL of solution C were mixed and the final FRAP reagent was incubated at 37 °C in a water bath for 4 min before use. After incubation, 0.150 mL of the diluted sample was left to react with 2.85 mL of the FRAP reagent for 30 min in the dark and the absorbance was measured at 593 nm. The results were expressed as mmol/L trolox equivalent (mmol TE/L).

2.5.5. Evaluation of the antioxidant activity by DPPH (2,2-Diphenyl-1-Picrylhydrazyl) assay

The DPPH assay was carried by measuring thorough spectrometer the radical scavenging activity, using 1,1-diphenyl-2-picryl-hydrazyl free radical as described by Stratil et al. (2008). The DPPH solution (100 μM) was prepared by dissolving 3.60 mg of DPPH in 100 mL of EtOH. An aliquot of 25.0 μL of the sample was mixed with 975 μL of DPPH solution and left for 30 min in the dark before measuring the

absorbance at 515 nm. A blank sample was also prepared by using EtOH instead of wine. The results were expressed in mmol/L trolox equivalent (mmol TE/L).

2.6. Genome comparison and Orthology analysis

Two strains of *S. bacillaris* were selected for their colour-changing capabilities, their performance fermentationand for the bioactive compounds produced based on previous studies (Lemos Junior et al., 2017; Lemos Junior et al., 2019a).

To analyze protein sequences among *S. bacillaris* FRI751, PAS13, and *S. cerevisiae* S228c, we employed the SPOCS (Species Paralogy and Orthology Clique Solver) web tool, as previously described (Curtis et al., 2013; Lemos Junior et al., 2018). Using machine learning in R version 3.6 (packages caret), statistical models sorted the protein sequences into three groups based on their similarities, thus identifying orthologous relationships in three groups as i) between *S. cerevisiae* S228c and *S. bacillaris* strains (FRI751 and PAS13), ii) as well as within the strains themselves, iii) FRI751 and PAS13 in comparison, o *S. cerevisiae* S228c.

Techniques such as Linear Discriminant Analysis (LDA), Classification and Regression Trees (CART), k-Nearest Neighbors (kNN), Support Vector Machines (SVM) with a linear kernel, and Random Forest (RF) were applied to identify the best model to represent SNPs dataset. The selection of the most effective model was aimed at discerning variations in crucial technological aspects like carbon metabolism and amino acid biosynthesis.

After this comparative analysis, we focused on the specific genes that clustered together between *S. bacillaris* strains FRI751 and PAS13, particularly looking at variations at SNPs levels (small, medium and high impact). Small variants were identified as follows: reads were filtered with Trimmomatic software (Bolger et al., 2014), and high-quality reads of *S. bacillaris* FRI751 were aligned to the PAS13 genome using Bowtie2 software (version 2.2.4) (Langmead & Salzberg, 2012). The resulting SAM files were then processed to BAM format and sorted with SAMtools (Li et al., 2009), followed by variant extraction using the mpileup tool. The generated Binary Call Format (BCF) files were converted to Variant Call Format (VCF) using BCFtools (https://github.com/samtools/BCFtools). Finally, the SnpEff program (version 4.3 g) was utilized to analyze the VCF files, using the genes of *S. bacillaris* PAS13 as a reference in order to predict the impact of variants according to Cingolani et al., (2012).

2.7. Statistical analysis

Each analysis was performed on three biological replicates. Data from physicochemical and biochemical analyses were subjected to one-way ANOVA with Tukey's test ($P < 0.05$) using R software (version 3.4.4). The stacked area plot, PCA, clustering high dimensional analysis were carried out by ggplot 2, Spearman's rank correlation matrix, P values, and false discovery rate correction (FDR) were generated by cor. test and visualized by corrplot package. The GInaFit was used to understand the kinetics of nitrogen consumption considering the models Log-linear regression, Weibull, Geeraerd, Weibull + tail, Mixed Weibull.

3. Results and discussion

3.1. Evaluation and validation of colour-produced S. bacillaris strains with S. cerevisiae

3.1.1. Effect of colour evaluation S. bacillaris by food engineering approach

The sequential fermentation performances of *S. bacillaris* with *S. cerevisiae* strains were determined in synthetic must. The must composition had high sugar content (200 g/L glucose), yeast assimilable nitrogen (YAN) content (300 mg/l) and pH 3.2. The range of CO_2 production was determined after 26 days. The fermentation performance, sugar and organic compound analysis (data do not show) were carried out as reported elsewhere (Lemos Junior et al., 2016, 2018; Lemos Junior, Nadai, et al., 2020, 2020).

To understand the influence of *S. bacillaris* on wine colour, we examined the end-of-performance fermentationalongside *S. cerevisiae* in synthetic must using two clarification methods: filtration and centrifugation. These two clarification methods as the principal techniques used in the wine industry to clarify and stabilize wine, ultimately enhancing its quality and shelf life (Boasiako et al., 2024a). It is essential to determine if the colour changes can be associated with these processes, therefore we focused on strategies to comprehend the real impact of this yeast on wine colour. As shown in Table 1, finer filtration (0.22 μm) reduced significantly these colour parameters. Statistical analysis indicated that the control, centrifugation, and filtration presented 5, 4, and 3 classes of colour differences, respectively. The first method use the centrifugal force to separate suspended solids and particles such as yeast cells, bacteria, and grape solids, resulting in a clearer and visually appealing wine (Vernhet, 2019).

In filtration, the wine goes through a porous membrane to selectively remove suspended particles and colloids (Reis et al., 2019). These results confirmed that smaller particles and impurities that may have escaped centrifugation can derive in the false positive results, as it was certified based on the numbers of groups produced in Table 1.

Filtration used membranes with 0.22 μm, therefore not only clarifies the wine but also helps stabilize it by removing potential sources of haze formation and microbial contamination (Reis et al., 2019). In Table 1, a focus on the filtration parameters was done, showing 3 *S. bacillaris* strains (PAS66, PAS151 and FRI751) had significant differences for green scale colour. Regarding the *b* parameter, PAS 13, PAS 92, PAS 151,

Table 1
Colour evaluation after the fermentation of strains in the synthetic must. The lightness (L*), red–greenness (a*), and yellow–blueness (b*).

Control – without clarification method

Samples	a*	b*	L*
MS300N	-7.454 ± 0.008^{B}	3.156 ± 0.009^{A}	79.748 ± 0.58^{D}
EC1118N	-7.366 ± 0.020^{A}	3.06 ± 0.010^{DE}	79.64 ± 0.142^{E}
PAS13N	-7.454 ± 0.005^{E}	3.048 ± 0.011^{EF}	79.996 ± 0.64^{ABC}
PAS66N	-7.48 ± 0.007^{F}	3.092 ± 0.008^{B}	80.088 ± 0.040^{A}
PAS92N	-7.44 ± 0.001^{D}	3.09 ± 0.010^{BC}	79.982 ± 0.036^{BC}
PAS103N	-7.464 ± 0.00^{5E}	3.036 ± 0.015^{F}	80.064 ± 0.097^{AB}
PAS151N	-7.44 ± 0.010^{D}	3.074 ± 0.015^{CD}	79.962 ± 0.082^{C}
PAS173N	-7.416 ± 0.011^{C}	3.068 ± 0.016^{D}	79.838 ± 0.076^{D}
FRI751	-7.462 ± 0.008^{E}	3.07 ± 0.016^{D}	80.092 ± 0.063^{A}

Centrifugation

Samples	a*	b*	L*
MS300N	-7.398 ± 0.008^{BC}	3.156 ± 0.09^{AB}	79.748 ± 0.58^{B}
EC1118N	-7.366 ± 0.009^{A}	3.114 ± 0.022^{E}	79.574 ± 0.051^{C}
PAS13N	-7.396 ± 0.009^{B}	3.128 ± 0.013^{DE}	79.758 ± 0.068^{AB}
PAS66N	-7.408 ± 0.008^{D}	3.160 ± 0.012^{A}	79.82 ± 0.048^{A}
PAS92N	-7.408 ± 0.004^{D}	3.134 ± 0.009^{CD}	79.804 ± 0.050^{AB}
PAS103N	-7.406 ± 0.005^{CD}	3.138 ± 0.013^{CD}	79.788 ± 0.056^{AB}
PAS151N	$-7.4 \pm 0.000B^{CD}$	3.124 ± 0.009^{DE}	79.762 ± 0.060^{AB}
PAS173N	-7.392 ± 0.004^{B}	3.14 ± 0.012^{BCD}	79.764 ± 0.046^{AB}
FRI751	-7.408 ± 0.004^{D}	3.15 ± 0.010^{ABC}	79.806 ± 0.060^{AB}

Filtration

Samples	a*	b*	L*
MS300N	-7.398 ± 0.008^{A}	3.156 ± 0.009^{A}	$79.748 \pm 0\text{-}058^{D}$
EC1118N	-7.396 ± 0.015^{A}	3.152 ± 0.013^{AB}	79.794 ± 0.053^{DC}
PAS13N	-7.402 ± 0.01^{ABC}	3.138 ± 0.013^{CD}	79.788 ± 0.082^{DC}
PAS66N	-7.41 ± 0.007^{BC}	3.158 ± 0.08^{A}	79.832 ± 0.044^{ABC}
PAS92N	-7.406 ± 0.009^{ABC}	3.136 ± 0.005^{DC}	79.846 ± 0.48^{ABC}
PAS103	-7.406 ± 0.005^{ABC}	3.132 ± 0.008^{D}	79.818 ± 0.054^{ABCD}
PAS151	-7.412 ± 0.011^{C}	3.142 ± 0.011^{BCD}	79.898 ± 0.069^{A}
PAS173	-7.4 ± 0.001^{AB}	3.132 ± 0.011^{D}	79.804 ± 0.056^{BCD}
FRI751	-7.41 ± 0.007^{BC}	3.148 ± 0.011^{ABC}	79.884 ± 0.085^{AB}

and PAS 173 showed higher intensity towards blue colour compared to the other strains. Additionally, four strains—PAS 66, PAS 92, PAS 151, and FRI 751—conferred greater brilliance to the fermented synthetic must than the other strains (Table 1).

Both clarification methods removed not only undesirable particles but also beneficial components such as aromatic compounds, phenolics, and proteins that contribute to wine complexity and mouthfeel (Gaspar et al., 2019).

The presence of the solids or cells are responsible for a false positive colour evaluation, according to Vernhet (2019), that evidenced the real colour of water after the filtration.

3.1.2. *Effect of S. bacillaris on colour evaluation in white wines*

The results in natural must using sequential fermentations of *S. bacillaris* and *S. cerevisiae* EC1118 were evaluated in the same conditions used for the synthetic must. The EC1118 fermentation was finished after 12 days and sequential fermentations in 16 days, when CO_2 production had widest range and weight loss was lower than 0.05 g. Information about fermentation performance, and sugar and organic compound contents is available elsewhere (Lemos Junior et al., 2016, 2018; Lemos Junior, Nadai, et al., 2020, 2020).

Significant differences were noted in the *a* values, with EC1118 (-7.314 ± 0.01) showing enhanced red tones compared to FRI751 (-7.34 ± 0.01) and PAS173 (-7.33 ± 0.01). These findings suggest that EC1118 might reduce greenish tones more effectively than some other strains, enhancing the red hues that are often sought after in white wines to achieve a more appealing golden hue.

The analysis revealed significant differences in luminosity (*L*) among yeast strains, with EC1118 showing a consistently higher *L* value compared to other strains such as FRI751, PAS103, PAS13, PAS173, PAS66, and PAS92 (Table 2). The mean *L* value for EC1118 was 76.99 ± 0.05, indicating a lighter colouration of the wine, which could be beneficial for styles where a paler hue is preferred. These differences are statistically significant and suggest that EC1118 could influence the phenolic content differently, thus affecting the wine's light reflectance (Zhang et al., 2019).

The ΔE values, which represent the overall colour change, were significantly different ($p < 0.05$) when comparing EC1118 against all other strains. The mean ΔE for EC1118 was 77.43 ± 0.06. This indicates a pronounced impact on wine colouration, highlighting the strain's potential to alter the visual appeal significantly. The differences found confirm that *S. bacillaris* strains can be used for adjusting the visual aspects of wine to enhance marketability according with consumers requestion.

Chroma values showed variability, with significant differences particularly noted between strains like PAS103 (8.22 ± 0.01) and PAS173 (8.26 ± 0.01), and between EC1118 and PAS173. This variability in chroma reflects differences in how these strains impact colour saturation, potentially making the wine appear more vibrant and visually appealing.

Table 2
Colour parameters in white wine.

	Model	Mean Difference	p-adj	Lower	Upper	Significant
L*	EC1118 vs. FRI751	0.222	0.0003	0.0816	0.3624	Yes
	EC1118 vs. PAS103	0.186	0.0034	0.0456	0.3264	Yes
	EC1118 vs. PAS13	0.164	0.0132	0.0236	0.3044	Yes
	EC1118 vs. PAS173	0.218	0.0004	0.0776	0.3584	Yes
	EC1118 vs. PAS66	0.214	0.0006	0.0736	0.3544	Yes
	EC1118 vs. PAS92	0.232	0.0002	0.0916	0.3724	Yes
a*	EC1118 vs. FRI751	−0.03	0.0002	−0.0483	−0.0117	Yes
	EC1118 vs. PAS173	−0.024	0.0039	−0.0423	−0.0057	Yes
	FRI751 vs. PAS103	0.036	0	0.0177	0.0543	Yes
	FRI751 vs. PAS13	0.024	0.0039	0.0057	0.0423	Yes
	FRI751 vs. PAS151	0.026	0.0015	0.0077	0.0443	Yes
	PAS103 vs. PAS173	−0.03	0.0002	−0.0483	−0.0117	Yes
	PAS103 vs. PAS66	−0.022	0.01	−0.0403	−0.0037	Yes
	PAS103 vs. PAS92	−0.02	0.0246	−0.0383	−0.0017	Yes
	PAS151 vs. PAS173	−0.02	0.0246	−0.0383	−0.0017	Yes
ΔE	EC1118 vs. FRI751	0.224	0.0004	0.0816	0.3657	Yes
	EC1118 vs. PAS103	0.184	0.0044	0.042	0.326	Yes
	EC1118 vs. PAS13	0.163	0.0152	0.0214	0.3055	Yes
	EC1118 vs. PAS173	0.22	0.0004	0.0781	0.3621	Yes
	EC1118 vs. PAS66	0.215	0.0006	0.0729	0.3569	Yes
	EC1118 vs. PAS92	0.233	0.0002	0.091	0.375	Yes
C	EC1118 vs. PAS173	0.031	0.0311	0.0018	0.061	Yes
	FRI751 vs. PAS103	−0.037	0.0074	−0.0662	−0.007	Yes
	PAS103 vs. PAS173	0.04	0.0024	0.0108	0.07	Yes
	PAS103 vs. PAS92	0.031	0.0382	0.001	0.0602	Yes

3.2. *Polyphenolic content and antioxidant activities*

The quantification of total polyphenols and their antioxidant capacities using FRAP and DPPH assays in wine. Polyphenols in white wines, particularly tannins, can change colour and the overall sensory profile.

These compounds are responsible for the hue and the potential to withstand oxidative changes over time. The FRAP assay provides insight into the reducing power of wine, which influences its ability to maintain vibrant colour in the face of oxidation, while the DPPH assay assesses the capacity of wine polyphenols to neutralize damaging free radicals, thus preserving colour intensity and clarity.

The Fig. 1A was used to visualize the percentage of variance explained by each principal component within PCA. In this case, the first dimension (67 %) accounts for a significant majority of the variance, suggesting it is the most informative for distinguishing between samples. Subsequent dimensions contribute progressively less 27 %, 6 % respectively.

The Fig. 1B illustrates the contribution of each variable (Total Polyphenol, DPPH, FRAP) to the first two principal components. The closer a variable vector is to the circle, the better it is represented by the two principal components. Here, Total Polyphenol shows a high positive correlation with Dimension 1, while DPPH and FRAP show a smaller, yet still considerable, contribution. The angles between the vectors suggest that while DPPH and FRAP are somewhat positively correlated, Total Polyphenol is less related to the other two variables.

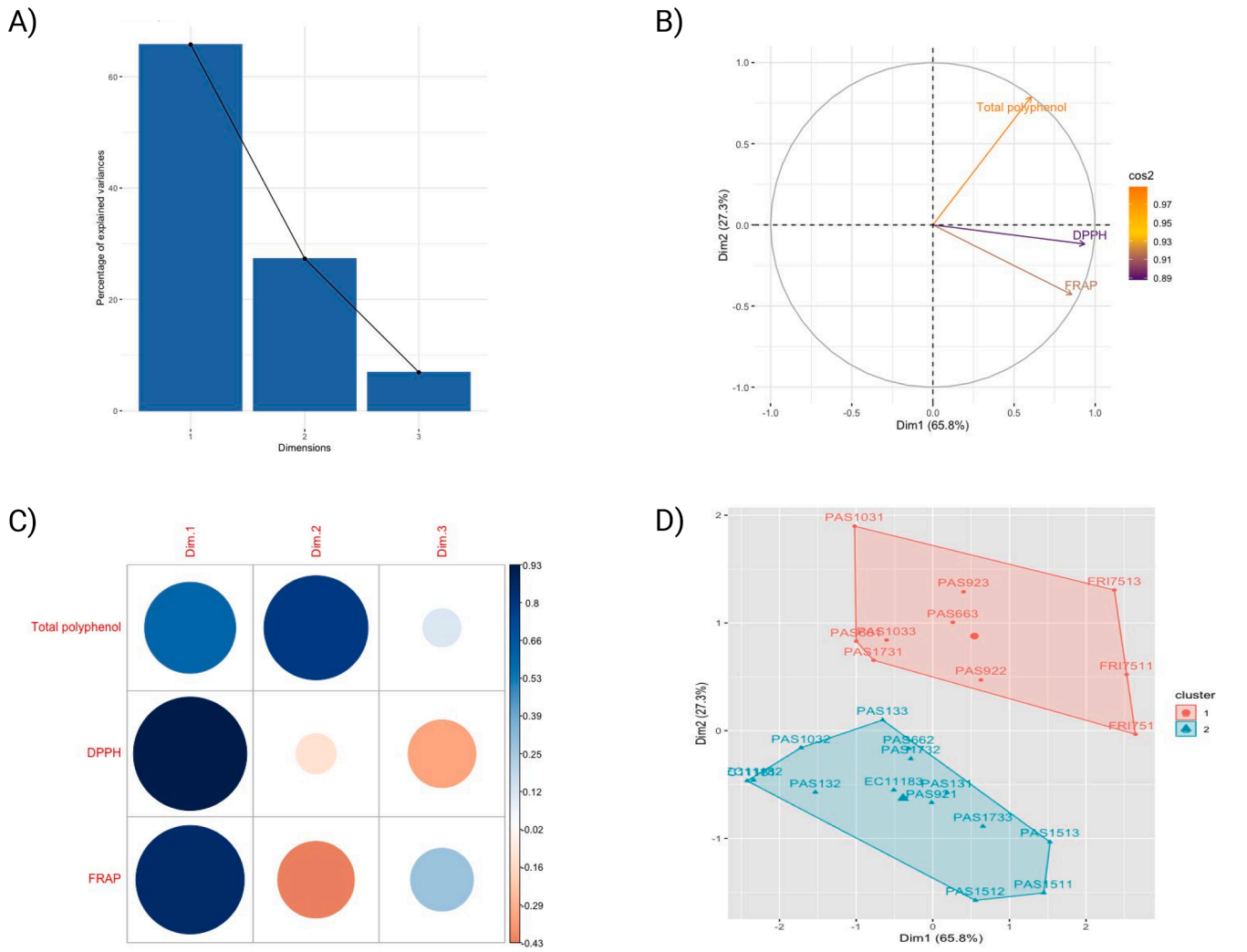

Fig. 1. Barplot representing percentage of explained variances for 3 dimensions summarizing 100% **(A)**, direction of contribution of each variable validated by the cos2 values **(B)**, corplot based on Pearson correlations **(C)**, PCA plot representation integrated with a clustering algorithm **(D)**. In this analysis all replicates were used, therefore the last number refers to individual replica. This figure was made merge R software output and improved in Biorender software.

To confirm this results, Fig. 1C indicates the pairwise Pearson correlation coefficients between the variables. The size and colour of the circles reflect the strength and direction of the correlations. Total Polyphenol and composition 1 exhibit a positive correlation. DPPH and FRAP exhibit a strong positive correlation with composition 1, while Total Polyphenol composition 1 have strong positive correlation. This suggests differing roles or responses of the measured antioxidants in the samples.

After this validation statistical model used, Fig. 1D showed a distribution of samples and their correlation coloured by 2 clusters, which could be determined by a clustering algorithm (k-means). In this biplot, the first principal component (Dim1) separates the samples along the horizontal axis, with potential groupings or similarities among the samples suggested by their proximity to each other and to the vectors representing the variables. To confirm the results and our hypothesis, a *t*-test was used to compare cluster 1 and cluster 2. The results showed significant differences in Total Polyphenol, DPPH, and FRAP ($P < 0.05$).

Bedides that, a factor analysis results was done by the combination of the means and standard deviations for Total Polyphenol (mg GAE/L), DPPH (mmol TE/L), FRAP (mmol TE/L) (Supplementary Table 2 and Fig. 2), reveal distinct characteristics for the strains FRI751, PAS13, and EC1118. FRI751, with a mean Total Polyphenol of 299.33 ± 3.51, DPPH of 1.09 ± 0.01, and FRAP of 0.95 ± 0.02, shows strong clustering in the upper-right quadrant of the factor analysis plot. This indicates that FRI751 consistently exhibits high antioxidant activity and polyphenol content than the other strains.

PAS13, with a mean Total Polyphenol of 285.67 ± 2.52, DPPH of 1.03 ± 0.03, and FRAP of 0.86 ± 0.02, forms a distinct cluster with moderate scores on Factor 1 and lower scores on Factor 2. This strain provides a balanced profile of antioxidant activity and polyphenol content, influencing sensory attributes like taste and colour stability. The consistency in measurements is indicated by the low standard deviations. In contrast, EC1118, with mean values of 281.67 ± 2.08 for Total Polyphenol, 1.01 ± 0.03 for DPPH, and 0.82 ± 0.03 for FRAP, shows lower values on both factors, suggesting a balanced but less pronounced antioxidant activity compared to FRI751 and PAS13. EC1118′s consistent performance with moderate antioxidant properties makes it a versatile choice for producing stable wines. These insights help in selecting the appropriate strain based on desired wine attributes, with FRI751 excelling in robust antioxidant properties, PAS13 offering a balanced profile, and EC1118 providing reliable but moderate antioxidant characteristics (Fig. 3). It was confirmed by ANOVA, significant differences were found among strains ($p = 0.0019$), $p = 0.0081$, and $p < 0.0001$ respectively for Total Polyphenol averages, DPPH and FRAP values.

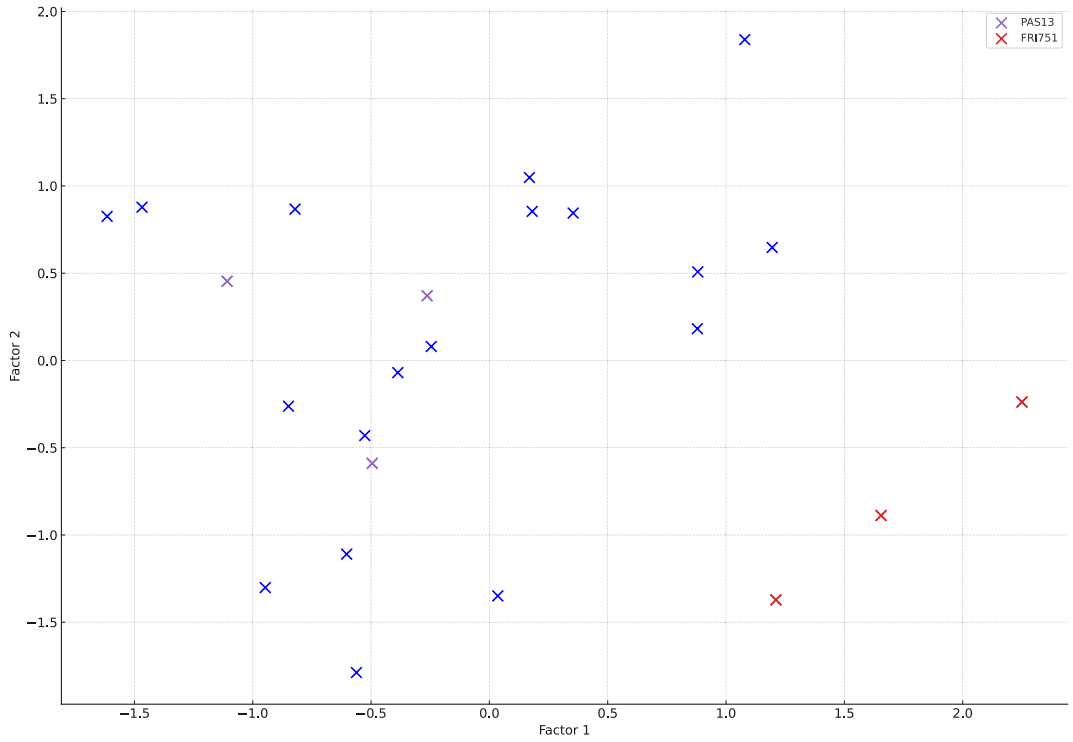

Fig. 2. Factor Analysis Results of Total Polyphenols, DPPH, and FRAP across different yeast strains. The scatter plot displays the factor scores for each strain, with Factor 1 and Factor 2 representing the underlying dimensions that capture the variability in the data. The strains PAS13 and FRI751 are highlighted to show their distinct profiles in relation to the other strains.

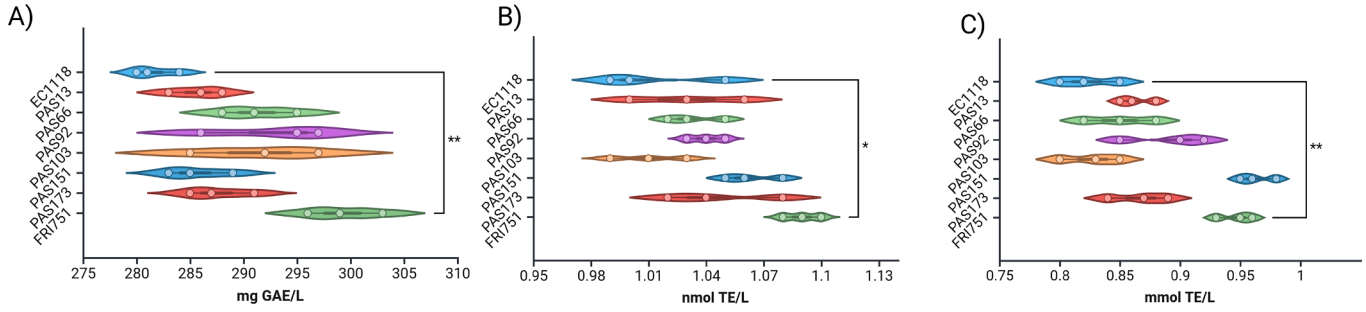

Fig. 3. Comparative analysis of Total Polyphenols, DPPH, and FRAP across different yeast strains. The figure displays the violin distributions and variations of these parameters: **(A)**Total Polyphenol content; **(B)** DPPH radical scavenging activity; **(C)** FRAP values. The results highlight significant differences in these parameters among the strains, reinforcing the findings from the *t* test statistical analysis (* = $p < 0.05$) and (** = p ⟨001), image generated by Biorender software.

3.3. Analysis of mutations on genes with technological interests: A genomics approach

After conducting the SPOCS analysis, 8,601 protein sequences were selected, 5,923 associated with S228c, 5,742 with PAS 13, and 5,735 with FRI751. To determine whether there are significant differences between functional genes association such as carbon metabolism and amino acid biosynthesis, statistical models were utilized, considering the protein sequences from SPOCS and incorporating data normalization.

The data from SPOCS and incorporating data normalization were categorized into three groups based on similarities (genes common alignment) to facilitate the identification of orthologous relationships from SPOCS data between genes in *S. cerevisiae* S228c and the *S. bacillaris* strains (FRI751 and PAS13), referred to as "Category 1". "Category 2" included common genes within the FRI751 and PAS13 strains themselves. "Category 3" comprised the genes in S228c that did not align with PAS13 and FRI751.

Machine learning methods using SVM and RF were the most effective statistical models in supporting our hypothesis (if there are genes that got a mutation correlated direct or indirect with changes colour in the white wine), each achieving an accuracy of 0.999. This was in agreement with Iqbal et al., (2014) which reported that these models can be utilized to manage complex and high-dimensional data, including protein sequences and their features for alignments and mutations from proteins sequences (Dara et al., 2022).

Consequently, this allowed to define a strategy for the identification of unique protein sequences in the two *S. bacillaris* strains through SNP analysis. Specifically, these sequences did not align with selected protein clusters shared by FRI751 and PAS13 strains orthologs. Subsequent analyses were directed towards elucidating the roles of specific genes involved in the biosynthesis of pigments that might influence the colouration of wine, as well as in polyphenol metabolism. A comparative genomic approach identified nineteen genes exhibiting mutations with a predicted high impact between PAS13 and FRI751 strains. These genes were screened and subsequently categorized based, and the impact of

classified mutation on their putative functions and potential impact on polyphenols interaction in musts (Fig. 4).

The substantial genetic variations observed between strain PAS13 and strain FR751 could underlie the phenotypic differences, including colour, in white wine production. For instance, the GRR1 gene shows a significant number of missense variants (N = 21) and synonymous variants (N = 41), suggesting changes in protein structure and function that might alter metabolic pathways or cellular processes related to pigment production in yeast used during fermentation. Similarly, the high number of upstream gene variants in genes like SSK1 (N = 85) and HIP1R (N = 91) indicates potential regulatory changes affecting gene expression, contributing to different phenotypic traits observed in wine colour.

Colour differences in white wine can often be linked to variations in the expression of genes involved in pigment biosynthesis or regulation within the yeast strains used. The numerous upstream gene variants observed in strain PAS13 could lead to differential expression of these key genes compared to FR751. Regulatory changes in genes encoding enzymes or transporters involved in pigment synthesis pathways might result in variations in pigment production, influencing the final colour of the wine. Additionally, missense variants in structural or regulatory proteins, such as those found in POLRMT (N = 5) and RAD5 (N = 14), could alter their functions, further contributing to differences in wine colour.

The differences in genetic makeup between PAS13 and FR751 could also impact the yeast strains responses to stress during fermentation, influencing the final wine colour. Variations in stress response genes, such as those seen in GRR1 and SSK1, may affect how these strains cope with the environmental stresses of the fermentation process, leading to different metabolic outputs and pigment production.

The cellular response to environmental stresses is known to modulate metabolic pathways, such as those associated with the synthesis and modification of polyphenols (e.g., *SSK1*, *RAD5*, *DERL2_3*). It has been observed that stress conditions can increase the production of secondary metabolites, improve mitochondrial function, and influence gene expression related to stress and metabolism. For instance, polyphenols can activate nutrient-sensing pathways and help in maintaining a balance in metabolic processes under stress conditions (Ferreira et al., 2024;Shabbir et al., 2021). In yeast, the environmental stress response includes mechanisms to repair damaged cellular components and adjust metabolic activities to enhance survival. These responses involve a complex network of signaling pathways that can be influenced by the presence of polyphenols and other metabolic regulators (Ferreira et al., 2024).

Additionally, *TFIIS*, *SMARCC*, *SETD6* are implicated in the regulation of gene expression and chromatin architecture (Ring et al., 1998;Prather et al., 2005). The modulation of transcription and chromatin structure by these genes might indirectly influence the enzymatic landscape responsible for polyphenol biosynthesis and its subsequent modifications, thereby affecting polyphenol profiles in wine.

In the other hand, *POLRMT*, *pgsA*, *COQ9* are important to metabolic processes and cellular energy dynamics (Dinh & Bonnefoy, 2023; Johnson et al., 2005; Kelly & Greenberg, 1994). Alterations in these genes could indirectly modulate polyphenol dynamics by influencing the cell's metabolic capacity, including the biosynthesis of secondary metabolites critical for wine's sensory attributes.

Besides that, The *GRR1*, *DERL2_3* genes are associated with protein folding and function, with the protein quality control machinery, and are essential for the enzymatic activities involved in polyphenol biosynthesis and modification (Flick & Johnston, 1991). Variants on these genes associated with protein degradation and quality control mechanisms could, therefore, have downstream effects on polyphenol composition and concentration.

Intracellular transport and trafficking genes as *HIP1R*, *COG3* can affect the distribution and availability of substrates and enzymes integral to polyphenol metabolism (Toshima et al., 2007). Disruptions or

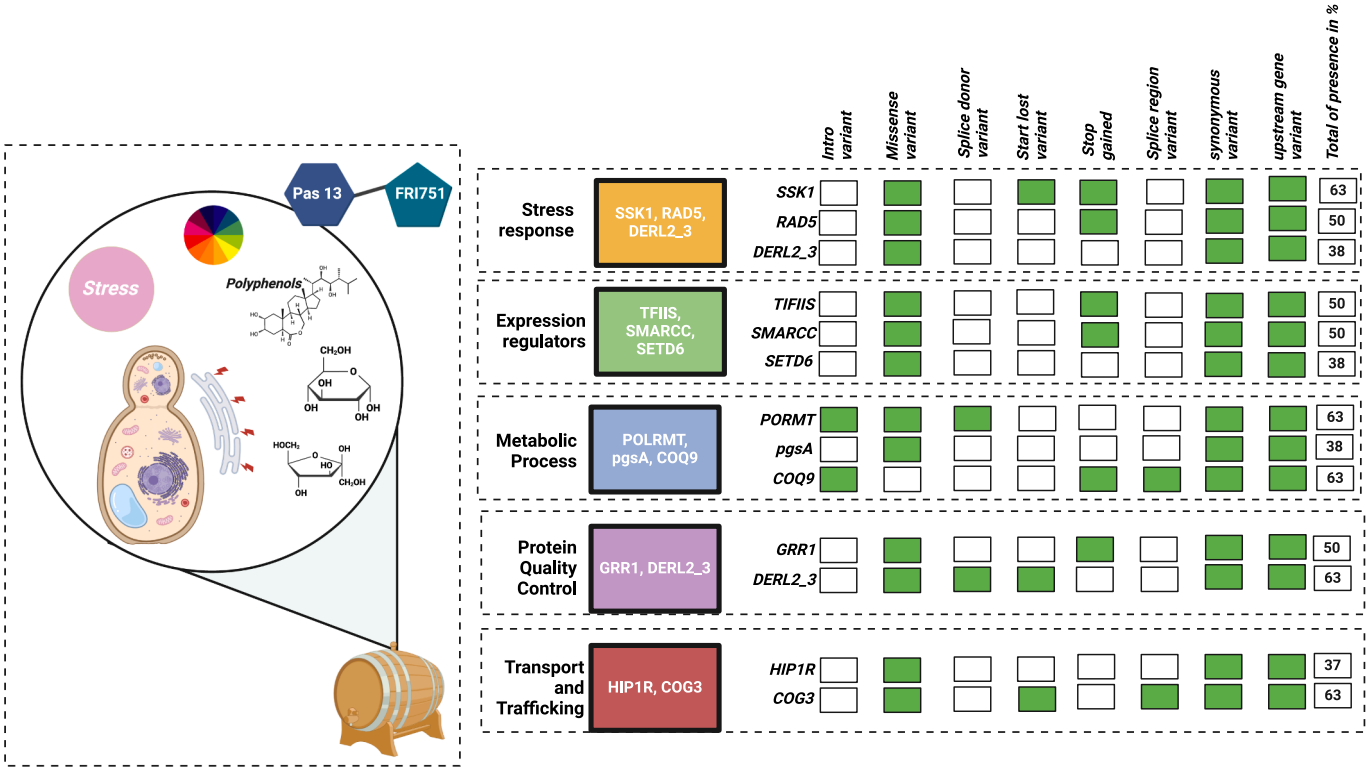

Fig. 4. Genes identified within the group exhibiting high mutation rate and their correlation with polyphenol profiles in wine. The boxes coloured in green highlight the presence of variants in the gene. This figure was made merge R software output and improved in Biorender software. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

modifications in these genes could lead to alterations in polyphenol biosynthesis pathways, with potential implications for wine colour and composition.

The cellular response to environmental stress intricately modulates metabolic pathways, particularly those involved in the synthesis and modification of polyphenols. Genes such as *SSK1*, *RAD5*, and *DERL2_3* play pivotal roles in these processes, acting as crucial defense mechanisms in yeast under stress conditions. Furthermore, transcription factors and chromatin remodelers as *TFIIS*, *SMARCC*, and *SETD6* significantly impact gene expression and chromatin architecture, indirectly influencing the enzymatic landscape for polyphenol biosynthesis.

Metabolic and energy-related genes such as *POLRMT*, *pgsA*, and *COQ9* further contribute by modulating the cell's metabolic capacity, thereby affecting the production and modification of polyphenols. Protein quality control genes like GRR1 and DERL2_3 ensure the proper folding and function of enzymes critical for polyphenol biosynthesis, while intracellular transport genes such as HIP1R and COG3 impact the distribution and availability of necessary substrates and enzymes.

3.4. Performance fermentation Evaluation: Organic acid production and nitrogen consumption

3.4.1. Evolution of quantity of inoculum and nitrogen consumption

In these trials, we assessed the effect of *S. bacillaris* in co-inoculation with EC1118, considering two *S. bacillaris* (FRI751 and PAS13) strains and two initial inoculum concentrations of 10^6 and 10^7 CFU/mL, the EC1118 was inoculated after 24 h. The fermentation was conducted in MS300 medium at 23 °C for 192 h, reflecting the absence of nitrogen content present at the beginning of fermentation.

3.4.1.1. Performance fermentation. All data regarding performance fermentation and plate count are provided in Supplementary Table 3. Fermentation kinetics were monitored based on the density of the must and fermented must over a period of 192 h, reflecting the initial absence of nitrogen content. The results showed co-culture of FRI751-10^6 with EC1118 showcases elevated density values, peaking at a density of 1.0814 at the outset and modestly descending to 1.0205 by the end, portraying a vigorous and possibly more durable fermentation activity relative to the other studied combinations. Conversely, EC1118, when single, presents a more tempered density profile, commencing at a 1.0861 and tapering to 0.9962, suggestive of a more measured but steady fermentation course.

Shifting to pH trends, a gradual decline is observed over time, a hallmark of organic acid production during fermentation. The FRI751 10^6 and EC1118 amalgamation exhibits a tempered acidification trajectory, with initial pH levels of 3.34 marginally falling to 2.54. The EC1118 solo culture reflects a similar pattern, descending from an initial pH of 3.34 to a final pH of 2.55, indicating a controlled acid. However, comparatively, fermentations involving the EC1118 strain showed no significant differences in pH levels.

Within the CFU FRI751 and PAS13 in co-culture, the colony-forming units offer a quantifiable lens into *S. bacillaris* prevalence. Here, the union of PAS13 10^6 and EC1118 markedly burgeons, with the CFU count soaring from 2.70 x 10^6 to a zenith of 2.50 x10^8 at 72 h, thereafter, sustaining these heights until 192 h. This robust proliferation within the fermentative milieu could hint at an advantageous symbiosis or resilient survival strategy.

In other hand, the EC1118 exhibits a fluctuating CFU course, indicative of potential competitive dynamics or adaptive responses within the complex fermentation ecosystem, with counts oscillating from an initial 2. x10^6 up to 2.8 x10^7, considering a mean of FRI751 and PAS13 all conditions a mean of the 3.40 x10^8 at 24 h in single fermentation. These variations suggest that EC1118 dominance and vigour could be modulated by *S. bacillaris* presence as described (Binati et al., 2021; Lemos Junior, Binati, et al., 2020; Lemos Junior et al., 2018).

3.4.1.2. Nitrogen consumption. In a comparative analysis, we used Log-linear regression, Weibull, Geeraerd, Weibull + tail, and Mixed Weibull models to determine the best model for explaining nitrogen consumption in an interaction between *S. cerevisiae* and *S. bacillaris*. After these statistical analyses, the Weibull model (Table 3) was considered the best for explaining the modulation of nitrogen consumption. In this analysis, the single fermentation of EC1118 served as the reference for assessing the behaviour of various yeast strains or conditions.

The scale parameter delta for EC1118 is reported as 23.04 with a standard error of 2.63, indicative of moderate variability in event times. The p-value of 0.65 suggests the data do not significantly deviate from the expected Weibull distribution. The R-squared values of 0.999 and adjusted R-squared of 0.9962 for EC1118 underscore a model with an excellent fit, signifying that the Weibull model captures the underlying distribution of the data effectively for this strain. When contrasted with EC1118, FRI751 10^7 and FRI751 10^6 exhibit distinct variations. FRI751 107 presents with a lower delta value of 17.10 and a considerably high standard error, denoting less reliability in the parameter estimate.

A p-value closer to 0.5, coupled with a substantial standard error, indicates greater uncertainty in the shape of the distribution. Conversely, FRI751 106 shows a significantly higher delta value of 64.34, suggesting a broader spread of the time-to-event data. Its p-value exceeds 1 ($p = 2.10$), indicating an increasing failure rate over time, which may represent nitrogen consumption in the fermentation process and the interactions between the studied species.

In comparison, PAS13 10^7 and PAS 13 10^6 show closer alignment to EC1118 in terms of model fit, as reflected by their R-squared values. The delta and p-values for these groups suggest a decreasing failure rate over time, analogous to EC1118, with R-squared values above 0.95, evidencing a robust fit. However, it is critical to note the variability in standard errors across all strains, highlighting the inherent uncertainties in these estimates. Collectively, the Weibull model elucidates the different nitrogen consumptions characteristics between strains, with the consistent model fit of EC1118′serving as a reliable benchmark within this comparative framework.

The comparison between the strains PAS and FRI, as modelled by the Weibull distribution, revealed contrasting survival characteristics. PAS exhibited a more milder variation in event times, with a delta value of 22.76 to 39.98 and relatively lower standard errors, evidencing a more predictable and consistent behaviour. In contrast, FRI exhibits a significantly higher delta value, especially in FRI751 10^6 (64.34 ± 8.41), indicating a broader spread in the time to event, which may be interpreted as a higher variability in survival times or a different rate of occurrence for the time, it can be correlated the adaptation of EC1118. Additionally, the shape parameter p for FRI751 10^6 suggests an increasing failure rate over time ($p = 2.10$), diverging from the PAS's profile which leans towards a decreasing failure rate ($p < 1$). This results is supported as according to Lemos Junior et al., (2024), the Weibull model effectively captures the inactivation kinetics of various pathogens, including *Salmonella Typhimurium, Escherichia coli,* and *Staphylococcus aureus,* using fogging technology with peracetic acid and hydrogen peroxide.

3.4.1.3. Performance fermentation on bioreactors. Based on the results obtained from Weibull Model, we assessed the effect of *S. bacillaris* FRI751 in single, co-inoculation with EC1118, considering FRI751

Table 3
Weibull Model parameters delta, p, R-Square and R-Square adjusted.

Strains	delta	p	R-Square	R-Square adjusted
EC1118	23.04	0.65	0.9981	0.9962
FRi751 10^6	64.34	2.10	0.9752	0.9505
FRi751 10^7	17.10	0.48	0.9252	0.8504
Pas 13 10^6	39.98	0.68	0.9587	0.9173
PAs13 10^7	22.76	0.64	0.9843	0.9685

strain, the EC1118 was inoculated after 40 h. The fermentation was conducted in MS300 medium at 20 °C and was stopped when CO_2 outflow reached 0, reflecting the absence of CO_2 production, therefore, the end of fermentation.

Fig. 5A illustrates the CO_2 emission over the course of several days, serving as an indicator of yeast biomass concentration during fermentation. This graph shows that the EC1118 strain exhibits an intial sharp increase in growth initially, which then levels off, whereas the FRI751 strain demonstrates a more gradual and consistent rise in CO_2 production. The overlay of the EC1118 and FRI751 curves indicates potential interactions between the strains, suggesting either a symbiotic relationship or competitive dynamics that influence their growth patterns.

Fig. 5B depicts the acetic acid concentrations produced by different yeast strains at several time points (16, 24, and 40 h), as well as at the end of fermentation (denoted as 'F'). Acetic acid, a byproduct of yeast metabolism, is generally considered undesirable in high quantities as it contributes to the wine's volatile acidity. Statistically significant differences in acetic acid production between the strains at certain time points are indicated by double asterisks, highlighting results from ANOVA tests with $P < 0.05$. Notably, the FRI751 strain produced a higher concentration of acetic acid, which could potentially impact wine quality negatively if it surpasses the sensory threshold as described by Binati et al., (2021).

Glycerol is another bioactive compound produced during the fermentation and contributes to the body and mouthfeel of wine (Lemos Junior, De Oliveira, et al., 2021). Fig. 5C shows a significant increase in glycerol production, particularly in the combination of EC1118 and FRI751 as according to Lemos Junior, Nadai, et al., (2020).

The data indicates that the EC1118 strain produced the highest ethanol concentration, a feature particularly evident at the 24-hour mark (Fig. 5D). The combined strain fermentation does not reach the same ethanol levels as EC1118 alone, suggesting a possible inhibitory effect of FRI751 on ethanol production as according to (Lemos Junior et al., 2019b).

4. Conclusion

This study provides an in-depth evaluation of the biological mechanisms underlying colour changes in white wine influenced by different strains of the yeast *Starmerella bacillaris*. By employing a combination of food engineering, genomic analysis, and biostatistics, we investigated the interactions between *S. bacillaris* and *S. cerevisiae* during alcoholic fermentation. Our findings underscore the significant impact of *S. bacillaris* on wine colouration, total polyphenol content, and pigment stability, revealing its potential as a sustainable alternative to chemical additives in winemaking.

Key findings include the adaptability and performance of *S. bacillaris* strains in co-inoculation with *S. cerevisiae*, which demonstrated distinct colour profiles and fermentation kinetics. The genomic analysis identified nineteen genes in *S. bacillaris* with a high number of variants, twelve of which have a potential impact on the phenotypic properties associated with wine colour. These genes are implicated in metabolic pathways that influence the synthesis and breakdown of colour-affecting compounds, highlighting the strain's potential for tailored fermentation strategies.

The effective application of the Weibull model to capture the intricate dynamics of nitrogen consumption provided a reliable benchmark for assessing yeast performance. We observed a significant relationship

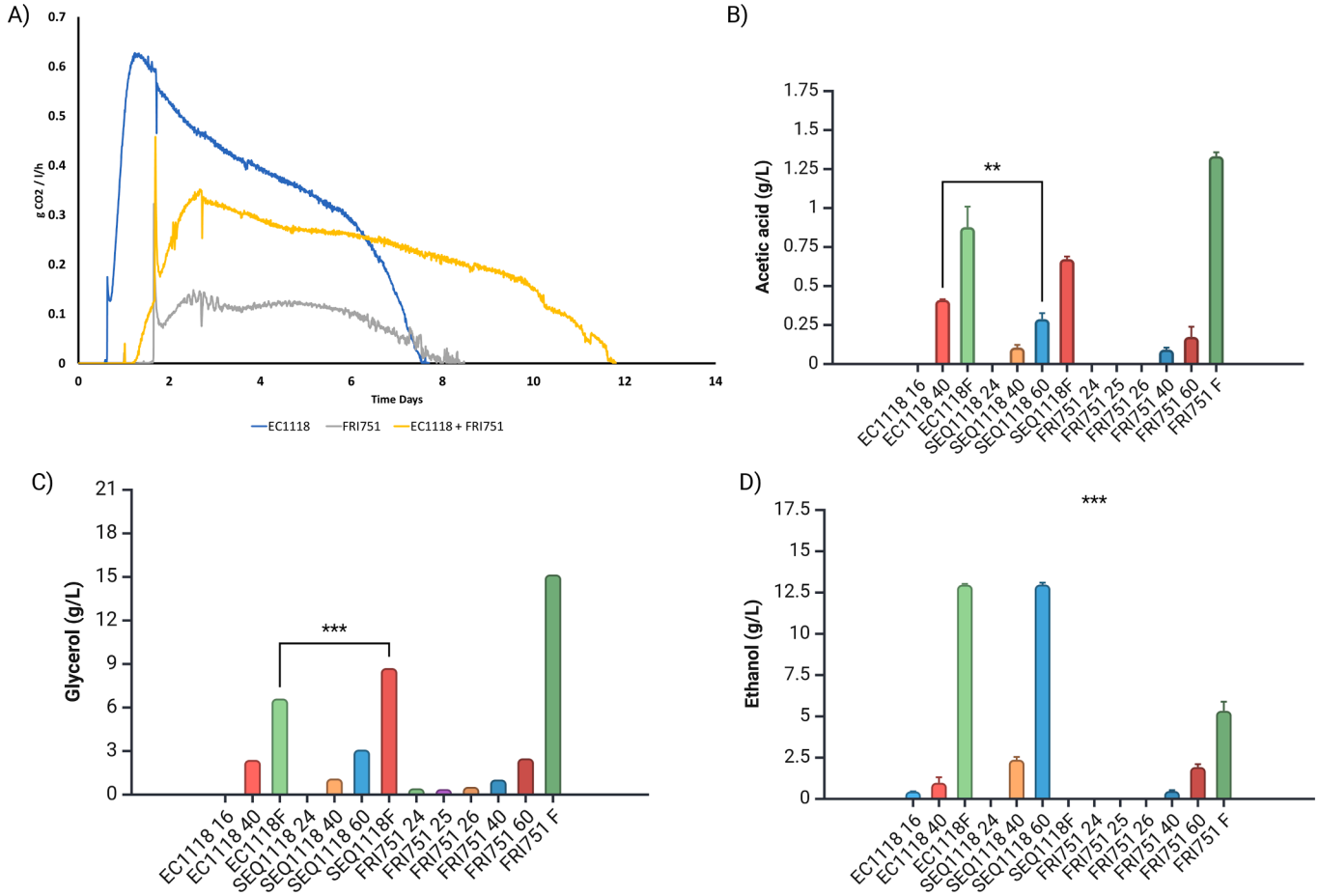

Fig. 5. Fermentation parameters in bioreactors, Single fermentation EC1118 and FRI751, sequential fermentation of EC118 + FRI751 **(A)** kinetics fermentation curves **(B)** acetic acids content **(C)** glycerol content and **(D)** ethanol content.

between nitrogen availability and organic acid production, which impacts the sensory attributes and stability of the wine. These results align with our aim to explore how different yeast strains affect fermentation outcomes and emphasize the potential of *S. bacillaris* to enhance wine quality through natural colouration processes.

Future research should explore the genetic basis of nitrogen consumption and organic acid production in yeast strains through advanced genomic and transcriptomic analyses. Investigating new yeast strains capable of modifying food colour and sensory properties can lead to innovative applications in fermented food production. Additionally, developing machine learning models to predict fermentation outcomes based on genetic and environmental data will enhance precision in fermentation control. Multidisciplinary studies integrating food engineering, microbiology, genomics, and biostatistics will be essential. Expanding omics analyses to include proteomics and metabolomics will offer a comprehensive understanding of fermentation dynamics.

Our study advances the understanding of yeast fermentation dynamics and opens new avenues for enhancing food quality through innovative fermentation strategies. By embracing multidisciplinary approaches, we can drive further innovation, improve food production processes, and develop new applications for yeast strains in the food industry. These findings pave the way for new trends in identifying and applying yeast strains that can change the colour of food, helping to screen and understand the colour change mechanisms in food matrices.

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CRediT authorship contribution statement

Vinicius da Silva Duarte: Formal analysis, Writing – review & editing. **Laura Treu:** Conceptualization, Writing – review & editing. **Stefano Campanaro:** Conceptualization, Formal analysis, Writing – review & editing. **Andre Fiorante Guerra:** Methodology. **Alessio Giacomini:** Methodology. **Albert Mas:** Methodology. **Viviana Corich:** Funding acquisition, Methodology, Visualization, Project administration, Validation. **Wilson José Fernandes Lemos Junior:** Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Supervision, Writing – original draft, 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

No data was used for the research described in the article.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodres.2024.114862.

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