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Application of NMR for the assessment of physiological and sensorial performances of *Saccharomyces cerevisiae* strains as candidate starters for the production of sparkling wine

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ABSTRACT

The autolysis of yeasts cells in sparkling wines production has a key role for foam formation, wine stability and aroma composition. The present research was focused on the application of NMR spectroscopy on a group of isolated *Saccharomyces cerevisiae* strains to compare their autolysis patterns and select the most promising candidates as sparkling wine starters.

The strains clustered in three groups with different autolysis profiles, comprising compounds reported to play significant roles in foam quality, aroma, mouthfeel, taste and cell stress protection, such as nucleotides, amino acids and cyto-protective molecules like myo-inositol and choline. Seven strains were then selected and characterized by sensory analysis. Correlation analysis linked autolysis metabolites to sensory traits, including odor and in-mouth descriptors, as well as foam properties. The strains were grouped in four couples with differing technological potential, showing varying potential for foam stabilization (higher levels of choline and myo-inositol), odor complexity and fineness (higher levels of nucleotides), in mouth persistence (related to myo-inositol), odor intensity (correlated with the levels of uridine and tyrosine). NMR analysis showed to be an application to monitor autolysis markers being a powerful tool to select starter strains for in sparkling wines production.

1. Introduction

Yeast plays a crucial role in the fermentation process of sparkling wine production. The primary species involved in this process is *Saccharomyces cerevisiae*, commonly known as the 'wine yeast'. For wine production, specific strains of yeast, known as starters, are carefully selected based on their ideal physiological, biochemical, and oenological characteristics. These selected strains should possess the ability to carry out the alcoholic fermentation process with predictable and controllable outcomes, in order to meet the technological request of the process (Vigentini et al., 2017). In the case of the secondary fermentation in sparkling wines, above all those obtained by the *méthode Champenoise* with long-term fining on lees, the desired technological performance involves ethanol, pressure and temperature resistance, as well as flocculation and autolysis capacity (Prokes et al., 2022).

Nowadays, selected yeast strains are commercially available for sparkling wine production, making the fermentation reproducible for the winemakers, thus avoiding drawbacks and potential deviations in the final product. However, relying solely on particular commercially available dry active yeast strains can have some limitations, as there are only a few strains commonly used by winemakers worldwide. This might lead to homogenization of the microbial agent, reducing the diversity of yeast strains present in the vineyard environment (Grieco et al., 2019; Tristezza et al., 2013; Tufariello et al., 2014). Moreover, while commercially available wine starters from foreign wine-producing regions may have undeniable oenological value, they often fail to fully develop the authentic flavours and aromas of a specific wine (Tufariello et al., 2019). To address this, microbiologists, wine producers, and sales managers advocate for using locally chosen starter ecotypes that align with the unique characteristics of the local product. This approach aims to enhance the overall quality and distinctiveness of the wine produced, offering a more genuine representation of the regional terroir (Capozzi

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et al., 2015; Lappa et al., 2020; Sidari et al., 2021; Tristezza et al., 2013; Vigentini et al., 2017).

The secondary fermentation, or "prise de mousse", is a pivotal process in sparkling wine production, responsible for the development of effervescence and the unique sensory characteristics of the final product. The selection of appropriate yeast starter strains for this phase is crucial, as these microorganisms must possess specific oenological and technological features to ensure a successful fermentation and desired wine quality: robust fermentation kinetics; alcohol and sulfur dioxide tolerance; autolytic capacity, thus releasing intracellular compounds into the wine; aroma and flavor contribution; resistance to stress conditions (Capozzi et al., 2022; Garofalo et al., 2016).

There are numerous studies in the literature about the selection of indigenous microflora to be used in grape must fermentation. Recently, several studies have deepened the identification of yeast starter cultures for sparkling winemaking (Capozzi et al., 2022). In fact, novel protocols are available in literature proposing different technological and qualitative main criteria to select yeast strains for the secondary fermentation of base wine such as autolytic ability (Perpetuini et al., 2016), increased latency period and a higher cultivability (Vigentini et al., 2017), increased aroma molecules production (Di Gianvito et al., 2018), polyphasic genotypic and technological screening (Garofalo et al., 2018), enhancement of regional character (Tofalo et al., 2022).

However, when determining whether a strain of *S. cerevisiae* is suitable for use as a starter in the production of sparkling wine, the assessment of the metabolite profile released during autolysis is an important step (Sidari et al., 2021). Profiling autolysis metabolites allows to evaluate the trend, speed, and the yeast capacity to release compounds of significant importance for wine odor, flavour, in mouth perception, and foam properties (Alexandre & Guilloux-Benatier, 2006; Charpentier et al., 2005).

Together with traditional analytical techniques as liquid/gas chromatography and mass spectrometry, Proton Nuclear Magnetic Resonance (^{1}H NMR), coupled with chemometric method, is widely used in control quality, geographical origins assessment, and traceability foodstuff analysis (Hatzakis, 2019). Thanks to its ability to detect a wide range of metabolites with high specificity and reproducibility, NMR provide a qualitative and quantitative snapshot of all the molecular components (amino and organic acids, alcohols, sugars, and phenolic compounds) present in a complex matrix (Le Mao, Da Costa, Bautista, et al., 2023; Le Mao, Da Costa, & Richard, 2023; Vion et al., 2024). The NMR-based metabolomics approach was successfully in wine authentication and for classification studies (Le Mao, Da Costa, & Richard, 2023; Solovyev et al., 2021), as well as in assessing the effects of geographical conditions (Gougeon et al., 2018) and anthropic factors agricultural practices and production processes (Scutarasu et al., 2021). To date, few studies reported NMR-based metabolomics analysis of sparkling wines and focused on sparkling wines classification based on quality, geographical origin or aging in combination with chromatographic methods and mass spectrometry (Charnock et al., 2024; Jagatić Korenika et al., 2024; Le Mao, Da Costa, Bautista, et al., 2023; Serra-Cayuela et al., 2013).

The objective of this work was to apply, for the first time, the ^{1}H NMR spectroscopy-based metabolomics to support the screening protocol, for identifying promising strains of *S. cerevisiae* strains, with technological potential for carrying out secondary fermentation in sparkling wines (traditional method), with a special focus on the cell autolysis process and its sensory outcomes.

2. Materials and methods

2.1. Yeast isolation, identification and characterization

Primitivo (*Vitis vinifera* L.) grapes (80 kg) were harvested in the Acquaviva delle Fonti (Bari, Apulia) and spontaneous fermentation was carried out in 100 L sterile steel tanks in an experimental cellar with a temperature ranging between 18 and 20 °C. Fermentation was daily monitored by measuring the Babo grade (°Ba; 1°Ba = 10 g L^{-1} of fermentable sugars in the must). The oenological selection was carried out as previously described (Tristezza et al., 2012). Briefly, yeast populations were sampled at the end of alcoholic fermentation (0°Ba; 12.9 % v/v ethanol) and from the residual lees. Yeast isolates were firstly screened for their ability to not produce hydrogen sulphide (Grieco et al., 2011). One hundred colonies were selected according to their phenotype that indicated the absence of hydrogen sulphide production. Yeast total genomic DNA was prepared according to De Benedictis et al. (2011). The isolates belonging to the *Saccharomyces* genus (*sensu stricto*) were identified according to the length of the rDNA region spanning the 5.8 S rRNA gene and flanking the internal transcribed spacers 1 and 2. PCR assays were carried out as previously described (Tufariello et al., 2019). Thirty-seven *S. cerevisiae* isolates were selected at random and characterized by interdelta typing (Supplementary Fig. S1) (Tristezza et al., 2009). The obtained molecular fingerprinting enabled the clustering of the *S. cerevisiae* populations in different strains.

A single representative for each of the fourteen identified strain was deposited in the ISPA Collection (http://www.ispa.cnr.it/Collection/) and then they were tested in micro-fermentation assays, in order to evaluate strain-specific technological and oenological properties. Fermentation experiments were performed in triplicate using grape juice (Primitivo, pH 3.3, 205 g L^{-1} of sugars) at 20 °C. Flasks containing 200 mL of pasteurized grape juice (added with 50 m g L^{-1} of free SO_2) were inoculated under aseptic conditions with the above yeast strains with an initial population of 2×10^6 CFU/mL. The fermentation process was monitored by measuring the loss of weight because of CO_2 production.

2.2. Sparkling wine production

Sparkling wine production was carried out at Tenute Chiaromonte (Acquaviva delle Fonti, Apulia, Italy). The base wine was obtained from Primitivo grape must (16.90 °Babo, pH 3.3; assimilable nitrogen 151 g L^{-1}) by adding the yeast inoculum (1.5×10^6 CFU mL^{-1}) to 500 L of must. The alcoholic fermentation proceeded at 20 °C, with its progress monitored daily by measuring the concentration of residual sugars. The obtained wine [alcohol 11.2 % (v/v), pH 3.25, volatile acidity 0.22 g L^{-1}, total SO_2 70 mg L^{-1}, total acidity 5.81 g L^{-1}] was used for prise de mousse. Production of sparkling wines was performed using the traditional method, following the protocol reported by Garofalo et al. (2018). The procedure consisted of four steps: i) a single yeast colony was cultured in YPD (20 g L^{-1} bacteriological peptone, 20 g L^{-1} glucose, 10 g L^{-1} yeast extract; Sigma Aldrich, Milan, Italy), ii) activation in base wine/water (base wine 16 mL, water 16 mL, sugar 4 g, yeast extract 0.1 g), iii) acclimation in base wine/water (base wine 120 mL, water 40 mL, sugar 10 g, yeast extract 0.1 g); iv) inoculation of the acclimated yeast suspension in base wine added with 30 g L^{-1} sucrose g L^{-1} and 0.3 g L^{-1} Nutriferm Start (Esseco, Trecate, Novara Italy). The second fermentation was carried out in 750 mL bottle for sparkling wine closed with crown cap and the *bidule* at 15 °C by horizontally keeping the bottles and the internal pressure in the bottles was measured using an aphrometers (Oenoitalia, Erbusco, Brescia, Italy). The commercial strain *of S. cerevisiae* DV10 (Lallemand, Petaluma, CA, USA) was employed. Bottles were disgorged after 10 months aging on lees at 15 °C.

2.3. Chemical analyses

General grape juice and wine parameters (alcohol content, residual sugars, pH, titratable and volatile acidity, tartaric, citric, lactic acid, malic acid, glycerol, and total sulfur dioxide) were determined using WineScan FT120 (Foss, Hillerød, Denmark) instrument. The analyses were performed in triplicate on sparkling wine samples degassed after incubation in an ultrasound bath for example, for 15 min.

2.4. Sensory analysis

A panel of twenty trained panellists, aged between 35 and 60 years and experienced in the sensory evaluation of wine, was recruited to conduct a sensory evaluation on sparkling wines (see Fig. S4). Participation was voluntary, with all panellists providing informed consent; no compensation was offered. Subjects with known allergies or health conditions were excluded from the study. A strict privacy protocol was implemented, which included online data collection and guaranteed anonymity. Given the inherently safe nature of sparkling wines, ethical approval was not required.

The sensory evaluations were carried out in a controlled environment designed to minimize extraneous influences. The evaluation room was free of noise and odours and contained eight individual booths, maintained at a constant temperature of 23 °C and illuminated with white light. Each assessor was served 50 mL of sparkling in wine testing glasses (ISO 3591:1977), with the wine maintained at 5 °C. The tasting sessions were conducted in triplicate, with all samples evaluated during each session and a 30-min break provided between sessions.

The sensory parameters were selected based on a review of the literature as well as panel-generated descriptors to ensure a comprehensive and non-redundant evaluation. Drinking water was provided for palate cleansing between samples description and avoid overlapping. Drinking water was used for mouth rinsing between sample Sensory evaluation was performed using a 4-point scale. Panellists rated visual attributes (clarity, bubble size, foam stability, bubble rate, hue, color intensity), four olfactory attributes (complexity, intensity, fineness, equilibrium), and four gustatory-olfactory elements (complexity, intensity, equilibrium, persistence). Additionally, the panellists provided a comprehensive and overall score for the sensory quality of each evaluated sample.

2.5. NMR analysis

The analysis was performed at the General and Inorganic Chemistry Laboratory of Department of Biological and Environmental Sciences and Technologies (DiSTeBA), University of Salento.

2.5.1. Samples preparation

Three bottles for each of the 15 employed strains (14 autochthonous and 1 control) were used to sample the sparkling wine produced. For each sample, 900 μL of sparkling wine were mixed with 100 μL of phosphate buffer (1 M KH_2PO_4 in deuterated water D_2O) at pH 2.9 containing 0.1 % TSP (trimethylsilylpropanoic acid) as internal standard, and NaN_3 (sodium azide) to prevent microbial contamination. Then, 600 μL of the resulting solution was transferred into a 5 mm NMR tube for spectral acquisition.

2.5.2. NMR spectra acquisition and processing

All the NMR analyses were performed on a Bruker Avance III 600 MHz Nuclear Magnetic Resonance spectrometer (Bruker, Germany) with a z axis gradient coil and an automatic tuning-matching unit (ATM). All the NMR spectra were acquired at a constant temperature of 300 K, after 5 min for thermal equilibration, by a Bruker Automatic Sample Changer, interfaced with the software IconNMR Version 5 (Bruker). For each sample, a NMR spectrum was collected by using a water signal suppression pulse program (zgcppr) to avoid distortion of water close spectrum regions. The following acquisition parameters were used: 128 scans (with 16 dummy scans), time domain (TD) of 64 k data points, w relaxation delay (RD) of 5.0 s. A spectral width (SW) of 12,019.230 Hz (20.0276 ppm) and an acquisition time (AQ) of 2.7262144 s were used. The resulting Free Induction Decay (FID) was multiplied by an exponential weighting function corresponding to a line broadening of 0.3 Hz before Fourier transformation, phase adjustment, and baseline correction. All spectra were referenced to the trimethyl silyl propionate standard (TSP) signal at 0.00 ppm. Spectra were processed by using TopSpin 3.5 pl 7 (Bruker). Wine metabolites were identified and assigned on the basis of homo- and hetero-correlated bidimensional NMR spectra (2D ^{1}H Jres, ^{1}H COSY, [^{1}H,^{13}C]-HSQC, and [^{1}H,^{13}C]-HMBC) acquired with standard pulse sequences. Assignments were confirmed by comparison with literature data (Le Mao, Da Costa, Bautista, et al., 2023; Mascellani et al., 2021).

2.6. Statistical analysis

The ^{1}H NMR spectra were segmented into areas or histograms (buckets) of the same size (0.04 ppm width), excluding the regions corresponding to the residual non-deuterated water (4.9–4.75 ppm) and ethanol signals, by using Bruker Amix 3.9.15 (Analysis of Mixture, Bruker BioSpin GmbH, Rheinstetten, Germany) software.

In order to minimize possible differences in metabolite concentrations due to sample preparation and/or acquisition conditions, the total sum normalization was applied (Van den Berg et al., 2006). Subsequently, the data matrices (buckets) were subjected to the Pareto scaling method. The data table obtained from all aligned buckets row reduced spectra was used for further multivariate data analysis. using the software Metaboanalyst, version 5.0 (Xia et al., 2009). The normalized dataset was subjected to exploratory data analysis. Hierarchical clustering analysis (HCA) method was utilized to obtain an overview of features of interest (metabolites) that are potentially significant in discriminating the studied group (yeast strains). The resulting dendrogram was calculated using the Euclidean distance algorithm and the clustering results were showed in the form of heat-map developed by cluster aggregation based on the average method, and the heat-map reported only group average. Moreover, a correlation analysis was applied to highlight the metabolites co-varying positively or negatively with the sensory mean scores.

3. Results and discussion

Primitivo, an indigenous Apulian cultivar, is a cornerstone of Apulia's industrial wine chain, driving both regional identity and global market appeal (Alba et al., 2024; Noviello et al., 2024; Paradiso et al., 2024). It was chosen to produce base wine for regional sparkling wine due to its rich, bold flavour profile and natural acidity. Primitivo grapes, with their deep fruitiness and structure, could provide a robust foundation for sparkling wines, balancing freshness and complexity. This results in unique regional expressions, highlighting the terroir, enhancing the wine's character, and aging potential.

As the first step of the selection of oenological autochthonous yeasts associated with natural fermentations of Primitivo grape must, serial dilutions of must and lees collected at the end of spontaneous fermentation were spread on selective solid medium, thus allowing the isolation of 100 yeast colonies no or low H_2S producers. Thirty-six isolated were randomly selected and identified as *Saccharomyces cerevisiae* by the molecular analysis of yeast rDNA and then characterized at strain level using a PCR-based assay, relying on the amplification of interdelta regions (Fig. S1). The obtained molecular fingerprinting enabled the clustering of the *S. cerevisiae* population in 14 different strains. The *S. cerevisiae* population showed an elevated polymorphism, calculated as the ratio between the number of molecular patterns and the number of isolates (40 %).

All the identified strains were deposited in the ISPA Collection (http://www.ispa.cnr.it/Collection/). They were firstly tested for base wine production, in order to assess the strain-specific technological and oenological properties. The main chemical compounds present in musts fermented by each one of them are shown in Supplementary materials (Table S1). The production of ethanol fell in the range 10.13–11.99 % (vol.), while residual sugars ranged from 0.27 to 0.59 g L^{-1}. The fourteen strains produced satisfactory amount of glycerol, with values of up to 7.25 g L^{-1} (SC15).

The kinetic of *prise de mousse* during the secondary fermentation was

Table 1
Enological parameters (means ± S.D., n = 3) of the sparkling wines obtained with the *Saccharomyces cerevisiae* strains.

ID	Ethanol (% v/v)	Reducing sugars (g/L)	Titratable acidity (g/L tartaric acid)	Volatile acidity (g/L acetic acid)	pH	Malic Acid (g/L)	Lactic Acid (g/L)	Tartaric Acid (g/L)	Glycerol (g/L)	Methanol (g/L)
3SC	12.92 ±0.03	n.d.	5.31 ±0.027	0.66 ±0.009	3.47 0	1.49 ±0.087	0.65 ±0.044	2.62 ±0.048	6.99 ±0.13	0.11 ±0.003
5SC	12.87 ±0.01	n.d.	5.09 ±0.003	0.27 ±0.002	3.41 0	1.77 ±0.039	0.15 ±0.007	2.7 ±0.027	7.00 ±0.067	0.11 ±0.002
6SC	12.85 ±0.01	n.d.	5.14 ±0.045	0.26 ±0.002	3.39 ±0.002	1.76 ±0.02	0.19 ±0.056	2.63 ±0.034	6.88 ±0.046	0.11 ±0.002
7SC	12.73 ±0.01	±0.16 ±0.113	5.01 ±0.021	0.65 ±0.006	3.48 ±0.002	1.67 ±0.019	0.11 ±0.018	2.4 ±0.119	6.63 ±0.1	0.11 ±0.002
9SC	12.93 ±0.01	n.d.	5.1 ±0.037	0.76 ±0.006	3.48 ±0.002	1.55 ±0.052	0.26 ±0.016	2.46 ±0.043	6.65 ±0.046	0.11 0
10SC	12.94 ±0.018	n.d.	5.05 ±0.01	0.60 ±0.009	3.46 ±0.003	1.67 ±0.072	0 ±0.031	2.51 ±0.006	6.9 ±0.054	0.11 ±0.001
15SC	12.93 ±0.008	n.d.	5.06 ±0.002	0.26 ±0.001	3.46 ±0.001	1.7 ±0.002	0.04 0 ± 0.018	2.56 ±0.004	7.05 ±0.01	0.11 ±0.003
21SC	12.90 ±0.022	n.d.	5.08 ±0.02	0.71 ±0.005	3.40 ±0.003	1.68 ±0.02	0.14 ±0.007	2.57 ±0.09	6.8 ±0.044	0.11 ±0.003
22SC	12.82 ±0.010	n.d.	5.04 ±0.019	0.25 ±0.003	3.41 ±0.012	1.69 0.036	0.12 0.009	2.66 0.106	6.88 0.12	0.11 0.001
24SC	12.86 ±0.039	n.d.	5.21 ±0.024	0.26 ±0.002	3.42 ±0.012	1.55 ±0.052	0.38 ±0.051	2.62 ±0.067	6.91 ±0.107	0.11 ±0.003
25SC	12.84 ±0.012	n.d.	5.09 ±0.008	0.25 ±0.005	3.41 ±0.005	1.65 ±0.012	0.09 ±0.018	2.57 ±0.009	6.86 ±0.062	0.11 ±0.002
26SC	12.85 ±0.021	±0.22 ±0.031	5.08 ±0.02	0.70 ±0.001	3.48 ±0.003	1.77 ±0.036	0.11 ±0.015	2.47 ±0.015	6.93 ±0.044	0.11 ±0.001
28SC	12.99 ±0.009	n.d.	5.01 ±0.036	0.61 ±0.01	3.46 ±0.006	1.69 ±0.044	0.02 ±0.011	2.52 ±0.036	6.85 ±0.088	0.1 ±0.001
36SC	12.80 ±0.019	n.d.	5.11 ±0.025	0.27 ±0.004	3.42 ±0.002	1.72 ±0.02	0.14 ±0.05	2.54 ±0.014	6.78 ±0.018	0.11 0
CTRL	12.73 ±0.006	n.d.	5.26 ±0.011	0.36 ±0.004	3.46 ±0.006	1.27 ±0.006	0.81 ±0.03	2.39 ±0.093	6.59 ±0.046	0.11 0

described by a sigmoid curve and all the strains tested concluded the secondary fermentation and produced high-pressure values in the bottles (about 7 bar, Supplementary Fig. S2).

The main chemical compounds present in sparkling wine fermented by the *S. cerevisiae* strains are reported in Table 1. The average alcoholic strength in white sparkling wines was over 12 % v/v, with values ranging from 12.73 to 12.94 % vol. Total acidity mean was >5 g L^{-1}, and volatile acidity resulted in most cases higher than 0.6 g L^{-1}, with the exception of 5SC, 6SC, 15SC, 22SC, 24SC, 25SC and 36SC strains. The main differences among the strains regarded the contents of lactic acid and glycerol.

The yeast strain isolated and characterized demonstrated that they could be optimal starter cultures for sparkling wine production, because they were able to grow in a base wine containing at least 10–12 % v/v ethanol, a low pH (2.9–3.3), tolerate low temperatures (10–15 °C), total SO_2 concentration of 50–80 mg L^{-1}, low volatile acidity (0.2–0.4 g L^{-1}), high pressure (5–6 bars), and glycerol content of 5–20 g L^{-1} (Borrull et al., 2015).

3.1. NMR spectra profiling and strain clusterization

A representative sparkling wine sample ^{1}H NMR spectrum is showed as relative expansions in Fig. 1a and 1b, while Supplementary Fig. S3 reports he whole spectra dataset. The identified metabolites and the corresponding chemical shifts are reported in Supplementary Table S2. The ethanol resonances represent the dominant signals in the spectrum. Moreover, compounds from different families were observed: other alcohols (isobutanol, isopentanol, methanol, myo-inositol, isoamyl alcohol, phenetyl-alcohol); amino acids (alanine, proline, threonine, tyrosine), nucleotide derivatives. A targeted approach was chosen to exploit the potential of ^{1}H NMR spectroscopy, focusing on non-volatile metabolites related to cell autolysis.

In particular, the signals considered for the NMR-assisted selection were related to cell autolysis and included amino acids (proline, tyrosine, alanine, threonine), membrane-derived compounds (myo-inositol, choline), nucleic acid-derived compounds (uridine and other nucleotides derivatives).

Proline is one of the most abundant amino acids present in wine (Gnoinski et al., 2021; Martínez-Rodríguez et al., 2002). It was found in high concentrations in both must and wine because yeast does not utilize this amino acid. Nevertheless, proline has a pivotal role as a stress protectant in yeast cells towards various abiotic stress factors including ethanol, and is therefore involved in cellular synthesis and accumulation, being cytosolic levels regulated by exchanges with the vacuole (Mukai et al., 2019; Takagi, 2008; Thomas et al., 1994). Consequently, grape variety, base wine composition and lees aging time (Hidalgo et al., 2004; Moreno-Arribas et al., 1998; Pozo-Bayón et al., 2010) influence the concentration of proline found in sparkling wines. Specifically, when comparing the average amino acid content of base wines obtained from different Champagne grape varieties, a much higher content of proline was observed in Chardonnay than in Pinot Noir (Desportes et al., 2000). It can be assumed that the different concentrations of proline found in the various samples, considering they all belong to the same grape variety, are indicative of different yeast cell degradation times related to each sample. In other words, where the proline concentration is lower, we would expect yeast cells to require a longer time for autolysis and to release this metabolite into the medium (Sartor et al., 2021). Proline enhances foam properties by positively impacting bubble persistence (Martínez-Lapuente et al., 2015; Moreno-Arribas et al., 2000).

Unlike proline, alanine is involved in yeast metabolism, so its concentration varies not only based on the grape variety but also on the yeast strain (Bozdogan & Canbas, 2011). This compound derives from pyruvic acid, either through the decarboxylation of aspartic acid, transamination reactions, or through the intervention of ammonium nitrogen. The low values observed for some samples may be related to subsequent transformations through decarboxylation, deamination,

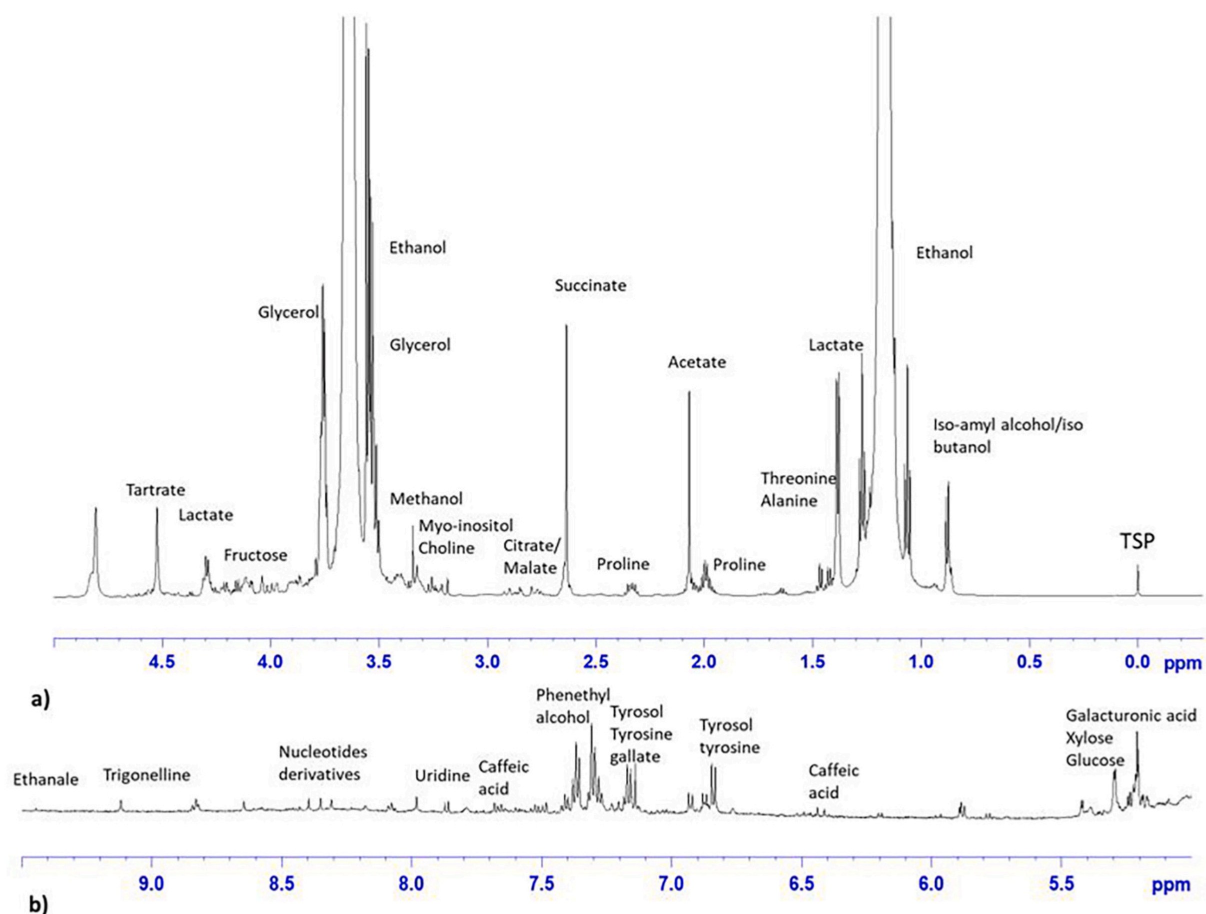

Fig. 1. Typical ^{1}H NMR (Nuclear Magnetic Resonance Spectroscopy) spectrum of a sparkling wine sample. Expansion of spectral areas in (a) (0.5–5 ppm), (5–10 ppm) region. The peaks of assigned metabolites are labelled. A stacked plot of the whole ^{1}H NMR spectra data set is reported in Supplementary Fig. S3

and/or synthesis reactions (Martínez-Lapuente et al., 2018). Moreover, alanine is strongly involved in Maillard reaction occurring during aging of sparkling wines (Charnock et al., 2022). Several studies have shown that alanine has a significantly positive impact on foam height (Martínez-Lapuente et al., 2015, 2018).

Together with proline, threonine was described to have a role in the perception of taste and aromatic properties of wines (Temerdashev et al., 2019).

It has been reported that the concentration of tyrosine increases after secondary fermentation (Prokes et al., 2022) as well as during in-bottle maturation, as observed after 365 days of maturation by Bozdogan and Canbas (2011). On the other hand, tyrosine was reported to be consumed by yeasts during secondary fermentation (Bozdogan & Canbas, 2011). This would explain its decrease in 9-months aged sparkling wines, compared to the base wine, reported by Pérez-Magariño et al. (Pérez-Magariño et al., 2013). Sartor et al. (2021) observed relevant increases of tyrosine only after 15 and 18 months of aging on lees, while Sun et al. (2024), who monitored the aging on lees of two sparkling wines for 10 months reported a certain decrease of tyrosine during aging. These apparently contrasting results suggest that this amino acid would probably characterize long-term aging of sparkling wines. From a quality point of view, tyrosine was significantly correlated with the main foam quality parameters (Condé et al., 2017; Sun et al., 2024).

Lipids deriving from cell membranes were suggested as markers of autolysis (Chen et al., 1980). However, phospholipids were not detected in an experiment of yeast autolysis in a model wine (Pueyo et al., 2000), while an experiment of accelerated autolysis in sparkling wines (Gnoinski et al., 2021) reported a decrease of phospholipids during 18 months aging on lees. This decrease could be related to acid-catalysed hydrolysis of these compounds. Therefore, we suggest that choline could be considered as a stable marker of lysis-deriving lipids. Previous studies applying ^{1}NMR on sparkling wines reported no significant effect of aging time (Charnock et al., 2024) on the content of choline, and significant differences of choline content between different sparkling wines typologies (Le Mao, Da Costa, & Richard, 2023). Another explanation for the presence of choline could be related to its role as osmolyte for cell volume regulation and cell protection towards environmental stresses (Wijayasinghe et al., 2017).

As regards myo-inositol, it is a precursor of inositol, a component of phospholipids, and has also been reported to play a function of cytoprotection (Michell, 2008; Yancey, 2005). This function is compatible with the chemical environment of base wines undergoing secondary fermentation, due to the osmolarity induced by sugar and dehydrating conditions related to ethanol. The protective function of myo-inositol could be confirmed by the results of Charnock et al. (2024), who found a significant effect of the added sugar on the levels of myo-inositol. Also this compound was reported with different levels in different sparkling wine typologies (Le Mao, Da Costa, & Richard, 2023).

As regards nucleotides, namely adenine, guanine, cytosine, thymine, and uracil, the variation in values among the samples can be explained by a slower degradation of DNA within the yeast cell. A loss of nucleic acids from yeast cells occurs mainly within the first 10–15 months of aging on lees, together with an increase in concentration in wine (Alexandre & Guilloux-Benatier, 2006; Charpentier et al., 2005). Nucleotides, particularly 5′-nucleotides such as 5′-GMP and 5′-IMP, are important flavoring agents found in many foods and beverages. These compounds have little or no taste or aroma on their own but can enhance the taste and mouthfeel of other compounds (Charpentier et al., 2005).

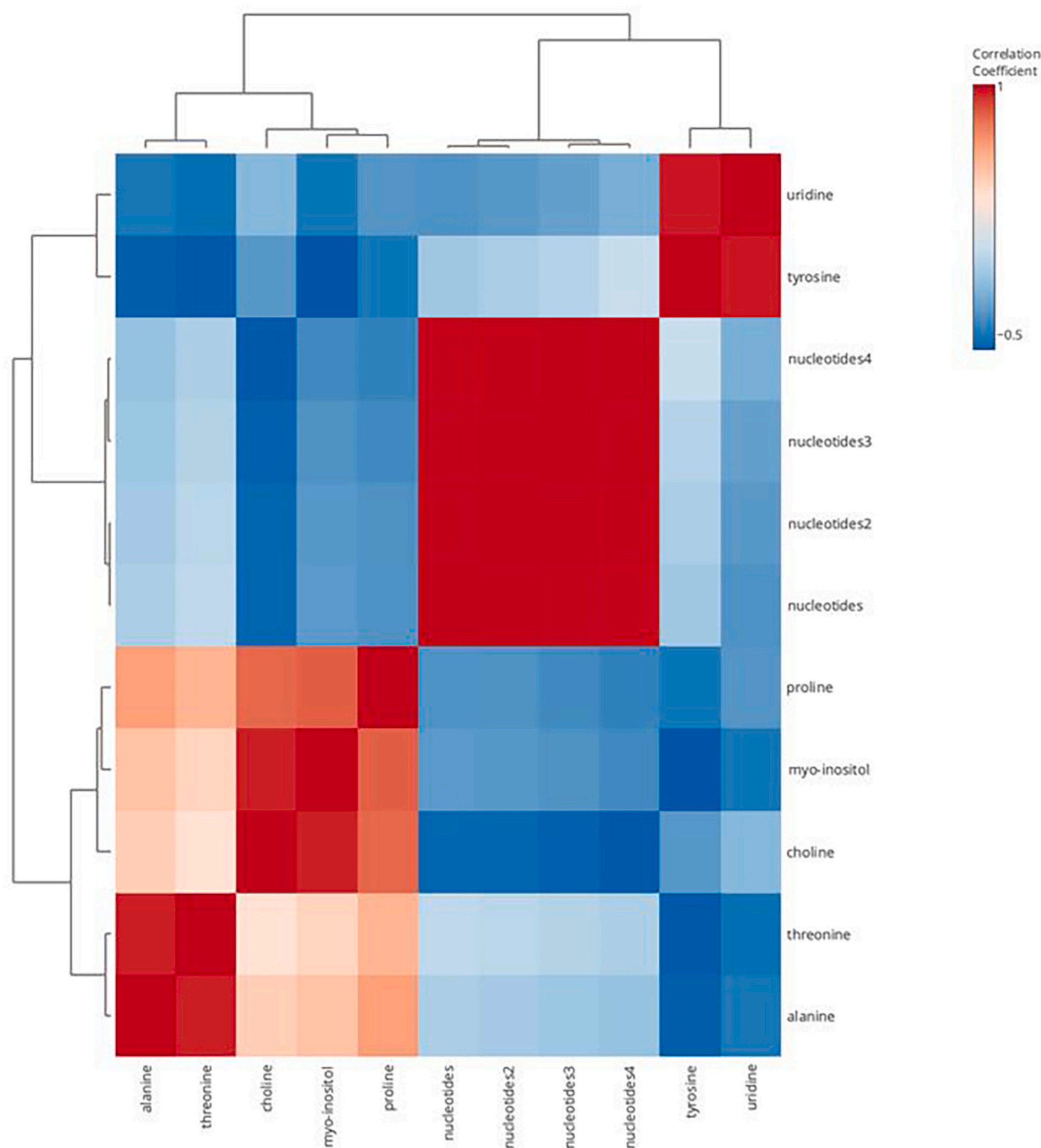

Fig. 2. Hierarchical clustering heat-map of Pearson correlation coefficients Heat-map of 11 metabolites of interest. The type (positive or negative) and strength (color intensity) of correlation are coded red and blue, respectively according to the bar on the right.

Tudela et al. reported uridine as the most abundant nucleoside in Cava sparkling wines (Tudela et al., 2013).

The ^{1}H NMR data related to the selected compounds are highlighted in bold characters in Supplementary Table S2. In this stage of the selection process of the candidate strains a correlation and clusterization approach was adopted, according to other similar researches, in order to individuate potential relations between the autolysis behaviour of the strains with their technological and sensory outcome (Capece et al., 2010).

Fig. 2 reports the hierarchical clustering heat-map of Pearson correlation coefficients heat-map of the signals of interest. Four main clusters of metabolites occurred. The first cluster included uridine and tyrosine, while the second cluster included all the other signals related to nucleic acid derivatives. Interestingly, the third cluster included the compounds that could share a cytoprotective role: proline, myo-inositol and choline. Finally, the fourth cluster, that showed a certain positive correlation with the third one, included the remaining two amino acids: alanine and threonine.

In order to highlight possible autolysis patterns, hierarchical cluster analysis (HCA) was applied to the selected metabolites. Sample strain means were used to group data according to their affinity in clusters of progressive dissimilarity. The resulting heat-map indicated the similarity among strains because of features of interest (Fig. 3). Yeast strains were clustered in three groups. The first cluster (from left to right in figure) included five strains (SC22, SC36, SC26, SC25, SC9). The sparkling wines fermented with these strains showed higher levels of tyrosine and nucleic acid derivatives. The second cluster included five other strains (SC6, SC3, SC5, SC10, and SC15). Their wines showed a richer

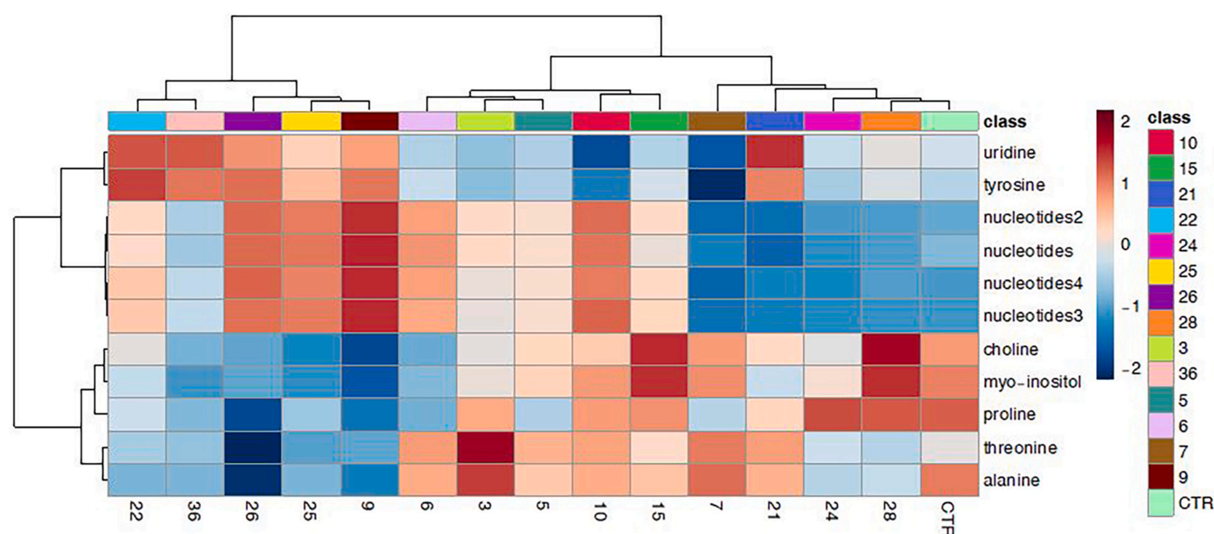

Fig. 3. Hierarchical clustering derived from ^{1}H NMR spectra of the selected fourteen strain samples. The heat-map is coloured according to the normalized intensity of the bins: blue: inferior; red: superior; grey: equal intensity. Clustering was performed on normalized and auto scaled binned-aligned ^{1}H NMR spectra using the Euclidean distance and cluster aggregation based on the Ward method. Groups were clustered using group means. Eleven different features of interest were used.

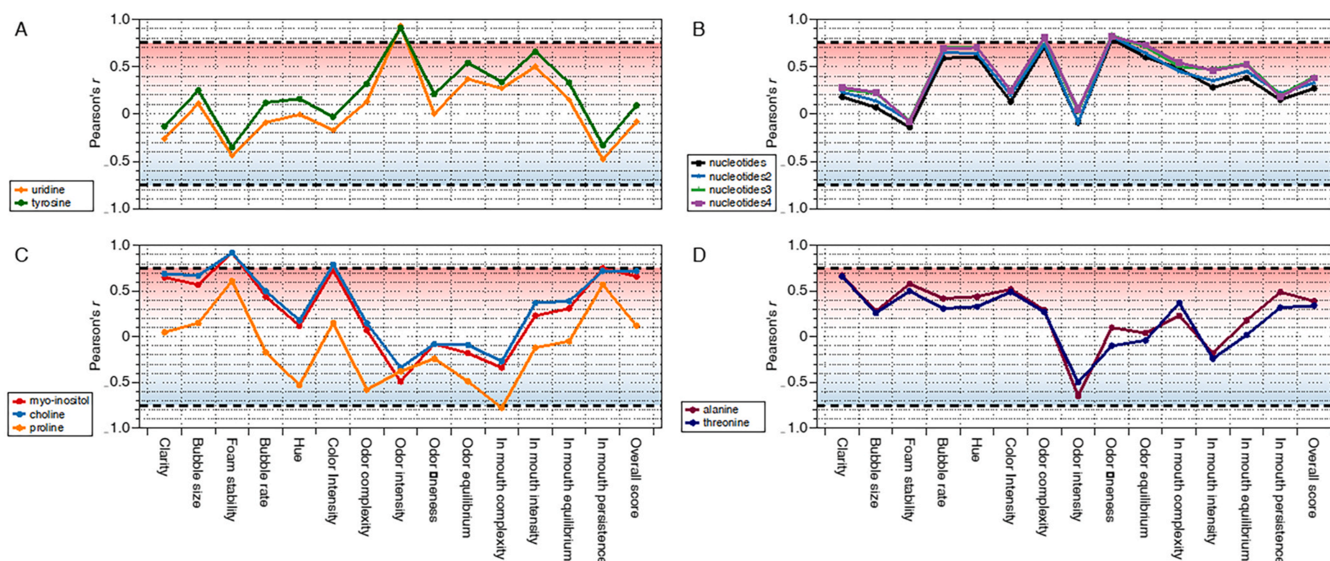

Fig. 4. Correlation profiles obtained from the correlation analysis of the sensory descriptors with the metabolites grouped in the four clusters. A, cluster I (tyrosine and uridine); B, cluster II (nucleotide-derived metabolites); C, cluster III (myo-inositol, choline, proline); D, cluster IV (alanine, threonine). The horizontal dashed lines indicate the threshold of statistical significance of correlations (p = 0.05).

profile, with quite high levels of nucleotide derivatives as well as of cytoprotective compounds (choline, myo-inositol and proline) and other amino acids (threonine and alanine). The third cluster of strains, including the control yeast and four other strains (SC7, SC21, SC24, SC28) showed a shifted autolysis profile, poorer of nucleic acid derivatives.

3.2. Sensory analysis

The following oenological and technological parameters were used as discriminants in choosing which of the 14 sparkling wines produced should be evaluated by sensory analysis: acetic acid concentration <0.6 g L^{-1}; concentration of residual sugars varying from 0 to 3 g L^{-1}. The primary screening indicated that the selected yeast strains 5SC, 6SC, 15SC, 22SC, 24SC, 25SC and 36SC satisfied the above requested parameters. Indeed, the optimal value for volatile acidity in sparkling wine is generally around 0.4–0.6 g L^{-1}, since values higher that those is indicative of the presence of defects, such as unpleasant notes of acetic acid and lack of freshness in the final product (Borrull et al., 2015.)

The mean scores and standard deviations obtained for the different wines are reported in the Supplementary Table S3, while the sensory profiles are reported in Supplementary Fig. S4.

A correlation analysis was then performed, to relate the different autolysis profiles with their sensory outcome. Fig. 4 reports the correlation profiles obtained from the correlation analysis of the sensory descriptors with the metabolites grouped in the four clusters previously described (see Fig. 3).

As expected, metabolites belonging to the same cluster showed similar correlation profiles with the sensory descriptors. Tyrosine and uridine (cluster I, Fig. 4A) showed no relevant correlation with visual descriptors, regarding clarity, color and foam properties. However, the values of the Pearson's *r* coefficient increased in correspondence of

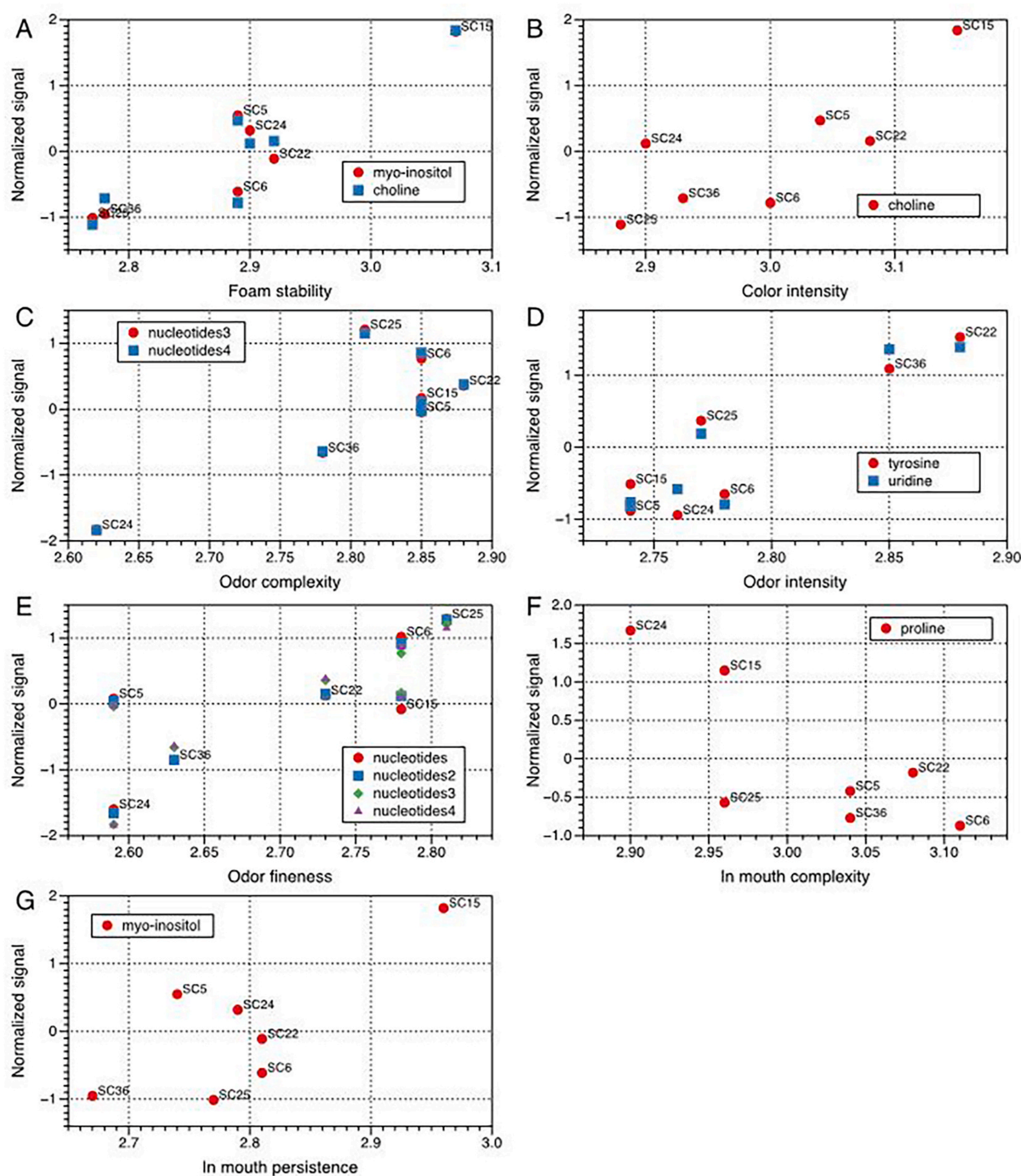

Fig. 5. Scatter plots of significantly correlated couples of descriptors and ^{1}H NMR signals. Signals were normalized in order to be plotted in the same panel.

descriptors related to olfactory and in mouth properties. In particular, they were significantly correlated with the odor intensity of wines. Even though the direct relation of these two compounds with odor intensity in sparkling wine has not been previously reported, we suggest that these metabolites could be indicators of advanced autolysis (Sartor et al., 2021) and therefore of the advanced state of aroma forming reactions occurring during aging on lees.

The four signals related to nucleotides showed almost overlapping correlation profiles (cluster II, Fig. 4B). The contribution of these compounds to the visual properties of the wines showed no significant relation, even though the correlation of two signals (8.34 and 8.38 ppm) showed a significance of the positive correlation with foam stability close to the conventional threshold (p = 0.08 in both cases). Similar significance could be observed with the hue of wines.

However, significant positive correlations were found for nucleotides with odor descriptors, namely complexity and fineness. Their role as flavoring agents has been previously reported (Charpentier, 2010). Sparkling wines spiked with nucleotides showed different flavour profiles compared to control wines, even if spiked amounts were lower than perception thresholds, probably due to synergism phenomena (Charpentier et al., 2005). These results suggest that their impact rather than increasing odor intensity, improves its perceived complexity.

In the same manner, myo-inositol and choline showed very similar correlation profiles (cluster III, Fig. 4C). In addition, proline presented analogous trends, even though the values of Pearson's r were systematically lower. Myo-inositol and proline levels showed a significant correlation with the perceived foam stability. This could either be linked to their direct contribution to bubble stability, as suggested by both Moreno-Arribas et al. (2000) and Martínez-Lapuente et al. (2015) who also found positive correlations of instrumentally measured foam properties with proline and other amino acids, or be indirectly related to the release of cytosolic content (Michell, 2008), including surface-active molecules. This could explain also the correlation of both molecules with the color intensity. Interestingly, the myo-inositol signal was also positively correlated with the perceived in mouth persistence. In a previous work on perceived body in still white wines, Skogerson et al. (2009) found significantly higher signals of myo-inositol in medium-body wines compared to low- and high-body groups. Moreover,

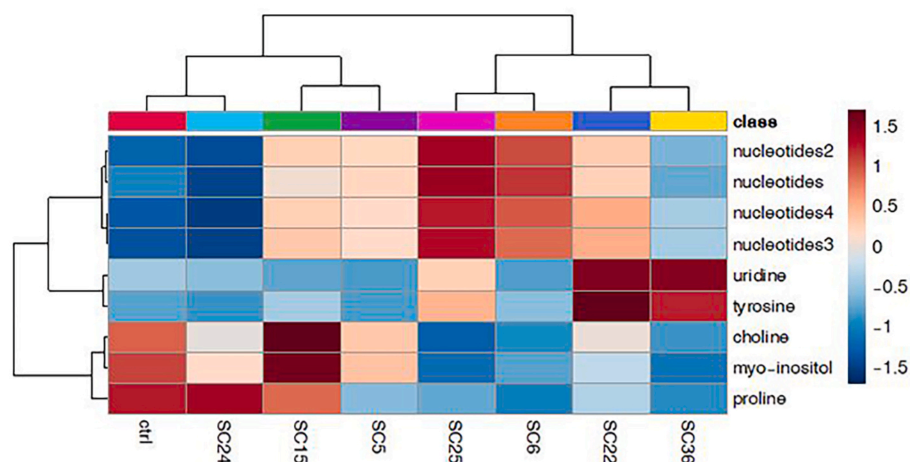

Fig. 6. Hierarchical clustering derived from ^{1}H NMR spectra of the seven candidate strains together with the control strain, based on the features correlated with sensory descriptors. The heat-map is coloured according to the normalized intensity of the bins: blue: inferior; red: superior; grey: equal intensity. Clustering was performed on normalized and auto scaled binned-aligned ^{1}H NMR spectra using the Euclidean distance and cluster aggregation based on the Ward method. Groups were clustered using group means.

an unexpected negative correlation of the signal of proline with the in mouth complexity was observed and requires further investigation about either a direct role or, more probably, and indirect relation of proline release with other autolysis features that could be involved in the perception of in mouth complexity. However, the possible role of proline as indirect marker of this sensory feature could be investigated.

Finally, alanine and threonine (cluster IV, Fig. 4D) presented overlapping correlation profiles, though no significant correlation was found.

The scatter plots of significantly correlated couples of descriptors and signals was reported in Fig. 5. Signals were normalized in order to allow plotting in the same panel.

Fig. 6 reports the heat map obtained from the hierarchical clustering derived from ^{1}H NMR spectra of the seven candidate strains together with the control strain, based on the features correlated with sensory descriptors. According to the results of hierarchical clustering, the eight strains were grouped in four couples with differing technological potential. From left to right, the first cluster included the control yeast and the strain SC24, having similar autolysis profile. The second cluster included the strains SC15 and SC5. Based on the present results, these strains showed promising potential for foam stabilization (higher levels of choline and myo-inositol) as well as odor complexity and fineness (higher levels of nucleotides) and in mouth persistence (related to myo-inositol). The third cluster, including the strains SC25 and SC6 was particularly characterized by the signals of nucleotides and could therefore show promising odor complexity and fineness, showing on the other hand lower values of the features related to the in mouth descriptors. Finally, the strains SC22 and SC36, included in the fourth cluster, showed the highest levels of uridine and tyrosine, and could be considered as potential yeasts to increase odor intensity.

The seven final candidate strains exhibited different technological potentials related to their autolysis profile. Nucleotides, amino acids, and cyto-protective molecules could therefore be evaluated as useful markers for screening the sensory and technological potential of candidate strains.

The scalability in the employment of a novel starter strain for secondary fermentation in sparkling wine production detains several pros (such as enhanced wine quality and tailored fermentation) and cons (such as scale-up and stress tolerance issues) (Di Gianvito et al., 2019). In fact, while novel yeast strains offer quality and innovation benefits, their scalability demands extensive adaptation trials to ensure fermentation efficiency, sensory consistency, and economic viability at the industrial level.

4. Conclusions

In conclusion, the ^{1}H NMR technique was for the first time applied as a supportive tool for the oenological selection of yeast starter strains. NMR analysis provided a comprehensive view of their technological potential. The information gleaned from this analysis offers valuable guidance in screening, selecting, and optimizing yeast strains for specific applications, such as the production of sparkling wines.

CRediT authorship contribution statement

Chiara Roberta Girelli: Writing – original draft, Investigation, Data curation. **Ilaria Prezioso:** Visualization, Data curation. **Gabriele Fioschi:** Writing – review & editing, Visualization, Data curation. **Ana Nita:** Writing – original draft. **Francesco Paolo Fanizzi:** Writing – review & editing, Supervision, Resources. **Maria Tufariello:** Methodology, Investigation, Data curation. **Francesco Grieco:** Writing – original draft, Visualization, Supervision, Conceptualization. **Vito Michele Paradiso:** Writing – original draft, Conceptualization.

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.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fbio.2025.106563.

Supplementary data to this article can be found online at.

Data availability

Data will be made available on request.

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