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Evaluating hydroxytyrosol as a healthier alternative in no-sulfites-added red wine: Impacts on chemical stability and sensory profiles during bottle aging

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ABSTRACT

Hydroxytyrosol (HT), a natural phenolic antioxidant, was evaluated as a potential alternative to sulfur dioxide (SO_2) in no-sulfites-added (NSA) organic Sangiovese red wine. The wine was enriched with HT at three concentrations (30, 60, and 120 mg/750 mL) and compared to a control over 12 months of storage at room temperature. Parallel model wine studies were conducted to investigate HT-SO_2 interactions and better understand their independent antioxidant behaviour. HT-enriched wine demonstrated dynamic chemical evolution, with HT levels increasing during early storage, declining between six and nine months, likely due to autoxidation and transformation, and stabilizing thereafter. Higher HT concentrations enhanced polyphenol content, reduced dissolved oxygen, and positively influenced sensory attributes such as "red fruit," "dried fruit," and "vegetative" notes, especially in early stages. Although sensory and chemical differences among samples diminished after nine months, HT-enriched wine retained improved colour stability (a*, C*) and preserved mineral content (Mg, K, Ca) throughout storage. In model wine, HT degradation was concentration-dependent, and its stability was unaffected by SO_2, confirming independent mechanisms of action. HT slightly enhanced SO_2 retention but did not interfere with its antioxidant role. Sensory analysis showed increased overall preference for wine with 60 and 120 mg/750 mL HT during specific periods. These findings highlight HT's potential as a viable, natural additive to improve oxidative stability and sensory complexity in NSA red wine, though further studies are needed to refine its application and assess regulatory implications in wine.

1. Introduction

Sulfur dioxide (SO_2) is still the most used preservative in winemaking for its ability to protect wine from oxidation, microbial spoilage, and enzymatic browning. However, it is also a known allergen that can provoke adverse reactions in sensitive individuals, especially asthmatics, such as bronchoconstriction, skin irritation, or digestive discomfort. Excessive intake, often due to the cumulative consumption of sulfite-containing foods and beverages, has been associated with potential damage to vital organs (Vally et al., 2009). According to the World Health Organization, the daily limit of SO_2 ingestion is 0.7 mg/kg of body weight (WHO, 2009). The maximum limit allowed by the EU is

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150 mg/L for dry red wines and 200 mg/L for dry white wines (Kontaxakis et al., 2020). Due to the high demand for healthy and organic products by consumers, the food and beverage industry has a keen interest in reducing the use of chemical additives or replace it with natural compounds (Costanigro et al., 2014). In this regard, scientists and winemakers are developing new strategies to reduce or replace SO_2 with natural compounds such as resveratrol (Kontaxakis et al., 2020), chitosan (Castro Marin, 2019; Picariello et al., 2020; Tika & Puspaningrat, 2022), chitosan-genipin film (Nunes et al., 2016; Rodrigues et al., 2022), hydroxytyrosol (Rafaela Raposo et al., 2016; Raposo et al., 2016), chitooligosaccharides (COS) obtained from hydrolyses of chitosan oligomers (Hao et al., 2020), limonene (Chen et al., 2021), α-pinene (Hou et al., 2020), plant extracts such as grape stem extract (Esparza et al., 2020; Nogueira et al., 2023), grape extracts (Fia et al., 2023) and stilbene extracts (Gutiérrez-Escobar et al., 2023).

Nogueira et al. (2023) recently performed a comparative study with Tempranillo wine enriched with SO_2, and SO_2 substituted with Mazuelo grape stem extract. Their results showed similar antioxidant capacity as well as concentration of total phenolic and flavonoids. Also, the triangle test performed on the samples did not show any significant difference in sensory attributes. Instead, a significant difference was observed in the total anthocyanin contents, showing a high concentration in wines enriched with SO_2. They suggested that Mazuelo stem extract could be used as an alternative for SO_2 during winemaking, but further research is still necessary to confirm its potential as a wine preservative. The same research group performed a partial substitution of SO_2 in Tempranillo wine with the same Mazuelo grape stem extract and "Vinetan", a commercial vine wood extract (Esparza et al., 2020). The control wine (without extract addition) exhibited slightly higher levels of total polyphenols, anthocyanins, and flavonoids compared to the wines enriched with the extracts. However, this marginal increase was not considered significant, as no notable differences were observed in antioxidant activity. Furthermore, sensory analysis indicated that the use of both extracts as partial substitutes for SO_2 resulted in wines with organoleptic qualities comparable to, or in some cases exceeding, those of wines enriched with SO_2.

Chen et al. (2021) used limonene in grape must as a substitute for SO_2 during winemaking, and results were compared to must enriched with SO_2. Similar trends in ethanol content and pH value were observed for both treatments, and more particularly, the must treated with limonene showed the lowest content of total bacteria, showing that limonene essential oil from citrus fruits could be a potential substitute for SO_2 in the winery. In a recent study, Fia et al. (2023) demonstrated that the use of unripe grape extract, alone or in combination with chitosan, in Sangiovese wines could be a potential alternative to SO_2. This approach contributed to colour stabilisation by promoting the formation of polymeric pigments. Also, the sensory quality was positively accepted by the consumers compared to the control samples (15 mg/L free SO_2 addition). α-pinene, a member of a natural monoterpene class (Allenspach & Steuer, 2021), was also proposed as a natural alternative to SO_2 in red wine in a study conducted by Hou et al. (2020). Among the different tested concentrations, 0.125% α-pinene emerged as the optimal dose, providing antimicrobial activity comparable to SO_2 while preserving wine fermentation efficiency, unlike higher concentrations of 0.25% and 0.5% that impaired ethanol production and sensory properties. Additionally, this concentration significantly improved wine clarity, colour attributes, and free radical scavenging activity, indicating its potential as a functional and sensory-compatible alternative to SO_2 in red winemaking. A similar application of α-pinene also reported in white wine, demonstrating effective microbial stability, colour clarity, and sensory quality without impairing fermentation at low concentrations (0.03125–0.0625 g/100 mL) (Shih et al., 2020).

Hydroxytyrosol (HT) is a naturally occurring phenolic compound primarily derived from olive oil and olive leaves, recognized for its potent antioxidant, anti-inflammatory, and antimicrobial properties (Bertelli et al., 2020). HT was approved by the European Food Safety Authority (EFSA) in 2024, indicating a level of hydroxytyrosol up to 215 mg/kg in vegetable oils and 175 mg/kg in margarine safe for consumption (Ceci et al., 2024).

Wine is another source of naturally occurring HT and was detected first in Italian wine by Di Tommaso et al. (1998) with subsequent confirmation by Boselli et al. (2006) in two white wines produced in the Marche region (Italy). Although present in relatively low concentrations, HT levels in wine have been reported to range from approximately 1.98 to 3.89 mg/L, with red wines generally exhibiting higher concentrations than white wines (R. Raposo et al., 2016). Our recent study (Ceci et al., 2024) demonstrated that the addition of HT to an organic red wine (85% Sangiovese and 15% Cabernet Sauvignon), already containing SO_2 at levels permitted for organic wines, significantly enhanced its oxidative stability and sensory properties. HT proved to be a promising natural alternative or complement to SO_2, aligning with the increasing demand for healthier, high-quality wines with reduced sulfite content.

However, the potential of HT as a standalone preservative in the complete absence of SO_2 remains largely unexplored. The novelty of the present study therefore, lies in evaluating HT as a natural antioxidant for the total replacement of added sulfites in an organic Sangiovese wine under real bottle-aging conditions. Different concentration (30, 60, and 120 mg/750 mL) of HT was added to Sangiovese wine just before bottling, and different parameters were evaluated during 1-year of storage. These were general oenological parameters, pH, dissolved oxygen, elements, and sensory properties. In addition, a model wine approach was also used to evaluate the interaction between hydroxytyrosol and sulfur dioxide under controlled conditions, aiming to investigate factors that could impact its stability over time.

2. Materials and methods

2.1. Chemical and reagents

All solvents, reagents, and organic modifiers employed for the HPLC-MS analysis were of mass spectrometry (MS) grade and sourced from Merck Life Science S.r.l. (Milan, Italy). Ultrapure water used to prepare mobile phases and sample dilutions was produced in-house using an Arium Mini purification system (Sartorius Italy S.r.l., Varedo, Monza Brianza, Italy), ensuring high analytical purity throughout the study. The hydroxytyrosol (HT) used in current study was a food-grade preparation (Polyphenol-HT1®), supplied by Nova Mentis Ltd (Dublin, Ireland) which consists of a concentrated aqueous solution containing hydroxytyrosol (≥98% pure), with a relative density of 1.15 kg/m^3 (65% (w/v) HT sol.) and was obtained through a proprietary biotechnological process developed by the manufacturer.

2.2. Sampling

2.2.1. Production of NSA Sangiovese wine and HT enrichment

The harvest of Sangiovese grapes took place in September 2022 in the region of Tuscany (Italy). After that, the grapes underwent destemming and crushing at Il Borro Wines (S. Giustino V.no, Italy), followed by a passage through a temperature exchanger, then transferred into cement tanks at approximately 15 °C. On the same evening, yeast strains were added, and alcoholic fermentation started and lasted for about 10 days within a temperature range of 25 to 28 °C. During the fermentation (with maceration) in a tank, pumping-over and plunging were carried out daily. Aeration was done in the grape must on the first day to stimulate the beginning of fermentation, and after 10 days, the mass was racked. The wine derived from the initial pressing was kept distinct from that obtained from pressing the grape marc. The gently pressed wine was then subjected to fermentation with malolactic bacteria. Then the decantation started, and after some days of stabilization, the wine was separated from the lees. The wine was then transferred to concrete tanks for 6 months before bottling. At bottling, 2-(3,4-

dihydroxyphenyl) ethanol, also known as hydroxytyrosol (HT), was added to 750-mL wine bottles in three different concentrations (30, 60, 120 mg/750 mL). These wines were compared with control wine sample that received no addition of HT nor sulfite. The codes used to describe each wine category in the present study are C = control; A = 30 mg/750 mL; B = 60 mg/750 mL and D = 120 mg/750 mL.

2.2.2. Model wine sampling

A wine model solution was prepared by dissolving 2.5 g/L potassium bitartrate in 12% ethanol (v/v) with distilled water, resulting in a total volume of 4 L (0.48 L ethanol, 3.52 L distilled water, and 10 g potassium bitartrate). This model wine solution was used to prepare 16 different model wine samples in triplicate spiked with HT and potassium metabisulfite ($K_2S_2O_5$), with concentrations calculated to deliver 100 mg/L molecular SO_2 was added: a control sample containing 100 mg/L SO_2 without HT (control), a low-dose HT sample 60 mg/L with 100 mg/L SO_2 (low HT), a high-dose HT sample 120 mg/L with 100 mg/L SO_2 (high HT), and another high-dose HT sample 120 mg/L without SO_2 (high HT without SO_2). The samples were stored in bottles at room temperature and were analyzed at four intervals in triplicate: baseline (T0), one month (T1), three months (T3), and six months (T6).

2.3. Analytical and statistical methods

2.3.1. General oenological parameters

An automatic multi-parametric wine analyzer MIURA One (Exacta-&Optech Labcenter S.p.A., San Prospero, Modena, Italy) was used to evaluate the basic oenological parameters of wine. Before the analysis, a calibration curve against reference standards was recorded. All samples were analyzed after filtration on 0.22 μm syringe filters without any specific sample preparation. Wine samples were analyzed for the following parameters: acetic acid; lactic acid; malic acid; tartaric acid; glucose-fructose; total polyphenol content; free and total SO_2, including total SO_2 for model wine. The pH was measured on an XS pH 60 VioLab benchtop pH meter (XS Instruments, Carpi, Italy) previously calibrated at pH 7.0 and 4.0. Dissolved oxygen was determined on an ORBISPHERE 3650 Portable Analyzer equipped with a 29,971–72 separated piercing head with ~ 1.5 bar N_2 flow and a GA2 × 00 O2 EC sensor.

2.3.2. Determination of elements in wine

The determination of elements (S4) in the wine (Na, Mg, Al, Si, P, S, K, Ca, Mn, Fe, Cu, Zn) was based on a published report (Fu & Shi, 2019). The wine samples were diluted 1:25 with 2% HNO_3 and directly analyzed by Inductively Coupled Plasma Mass Spectrometry using a 7800 ICP-MS from Agilent. Compound quantitation was performed using La at 1 mg/L as internal standard (S4).

2.3.3. CIELab colourimetric analysis

Colour parameters: L* lightness, a* red/green coordinate, b* yellow/blue coordinate, C* chroma (CIE, 1986) were recorded using a reflectance spectrophotometer Minolta CR-400 Chroma Meter (Minolta Corp., Osaka, Japan). The instrument was calibrated over the provided white ceramic plate.

2.3.4. LC-MS/MS analysis of HT

The LC-MS analysis of HT was conducted using a UHPLC-QqQ/MS instrument (Agilent LC/TQ 6465 system) equipped with a 1260 Infinity II UHPLC with a quaternary pump system, a 1260 Infinity II WR PDA detector, in series to an AJS ESI QqQ mass analyzer. The method was adapted according to research done by Poggesi et al. (2022) with a slight modification. Briefly, the separation of hydroxytyrosol from other phenolic compounds was performed using a Poroshell 120, SB-C18 2.1 mm × 100 mm × 2.7 μm column (Agilent Technologies Italia, Milan, Italy) kept at 30 °C at 0.35 mL/min flow rate. The mobile phase consisted of A) 0.1% formic acid in degassed ultrapure water and B) 0.1% formic acid in acetonitrile LC-MS analytical grade. The gradient separation program was: 0–1 min (5% B), 1–10 min (15% B), 10–15 min (25% B), 15–18 min (40% B), 18–21 min (95% B), 21–24 min (95% B), 24–25 min (5%), 25–28 min (5%). The injection volume was 5 μL. The PDA detector was set to record the absorbance in the 200–700 nm wavelength range using a 4 s response time (1.25 Hz) and 4 nm slit width, with 1 nm spectrum steps. The MS detection was performed in ESI- ionization mode, with the following parameters: mass range = m/z 200–750, scan time = 500 ms, step size = 0.1 amu, fragmentor potential = 135 V, cell acceleration = 5 V, N_2 gas temperature = 340 °C, N_2 gas flow = 13 L/min, nebulizer pressure = 50 psi, sheath gas heater = 350 °C, sheath gas flow = 12 L/min, capillary voltage = −3500 V, nozzle voltage = −500 V. Hydroxytyrosol (HT) added to the NSA red wine and to the model wine were quantified using the analytical standard of HT (3-hydroxytyrosol, CAS RN: 10,597-60–1, purity > 98.0%) provided by TCI EUROPE. The calibration curves were built at each storage time to quantify the added HT in the NSA wine and model wine (0.5 – 200 mg/L), and the quantification was done using a PDA detector (wavelength = 280 nm). The results are expressed as regression curves built on an artificial wine at T1 – T12 (control and 12 months, respectively).

2.3.5. Sensory analysis

2.3.5.1. Panel composition and training. A panel of twelve subjects (5 females and 7 males, ages 25 to 39) was recruited among students and staff from the Free University of Bozen-Bolzano. After a full explanation of the aim of the experiment, informed consent was signed by suitable candidates who agreed to participate in the subsequent training and sensory analysis sessions, without any monetary reward. No ethics procedure was conducted, resulting in the study being low risk, because the samples were commercially produced, and the panellists were instructed to spit the samples. Moreover, the quantity of alcohol for each session was under the tolerance threshold (23.4 mL). The panel received rapid and specific training on how to evaluate the wine based on CATA (Check-All-That-Apply) combined with Projective Mapping methods (Moss et al., 2022). The training took place one week before the sessions for each storage point and was divided into two days of one-hour each. On the first day, recognition and classification of aroma standard solutions were performed by asking subjects to identify and recognize different aromas such as “fresh wood”, “coffee”, “cloves”, “black pepper”, “liquorice”, “cherry”, “strawberry”, “green bell pepper” and “strawberry jam” prepared as described in ***Supplementary Material Table S1***. The following day, the recognition and classification of standard samples were performed by asking subjects to identify the taste and to order the standard solutions according to the perceived intensity of each descriptor. The subjects were then instructed to use an intensity horizontal scale to rate the perceived intensity for taste and/or tactile sensation including bitterness (0; 1; 2 g/L caffeine), sourness (1 g/L lactic acid; 1 g/L malic acid; 1 g/L tartaric acid), saltiness (0; 0.5; 1 g/L sodium chloride) and astringency (0.5; 1; 2 g/L alum). All the standard solutions were prepared in water, and all the reagents were food-grade. Also, the flavour descriptors such as “woody”, “red fruit”, “black pepper”, “liquorice”, and “green bell pepper” were evaluated on the same day. Descriptors specific to the Sangiovese variety found in various literatures were used (Canuti et al., 2020; Pagliarini et al., 2013; Rinaldi et al., 2020). During the training session, the subjects were provided with plain crackers with low salt and water and could rinse their mouth between samples. Training sessions were performed with Cysensy, an SQL binding web software developed in collaboration with the Computer Science Faculty of the Free University of Bozen/Bolzano.

2.3.5.2. CATA (Check-All-That-Apply) and projective mapping. The panelists performed a sensory analysis using the Projective Mapping (also known as Napping®) method to differentiate, based on similarity, the samples. Also, each sample was evaluated using the CATA (Check-All-That-Apply) methodology (Ceci et al., 2024). During the sensory

test, panelists evaluated a total of 12 samples. Each type of wine (control, 30, 60, and 120 mg of HT in 750 mL wine) was prepared in triplicate. The wine samples were evaluated in two sessions of one hour each, and six bottles per session were analyzed. The wine bottles stored at control room temperature were opened just before the analysis and the wine was served in a Latin Square complete balanced design to the panelists in ISO glasses (30 mL/glass), codified with 3-digit numbers. The trained panel was asked to place the wine samples according to their perceived sensory affinity on a GeoGebra Classic sheet (https://geogebra.org/classic) on which a rectangle was drawn (projective mapping paper, size 53 × 35 cm). After testing each sample, panelists marked the position of each sample on the paper with the sample code. The 'X' and 'Y' coordinates of each sample on the GeoGebra Classic sheet were collected. Qualitative terms from CATA were recorded for each sample and written as a frequency table. The list of CATA descriptors with their respective definition is given in Table 1. An "overall preference" was evaluated by asking the panelists to rank the samples according to their personal preferences. The preferences were expressed as a ranking list (the three most preferred samples) from each panelist and written as a frequency table. Furthermore, the panels were also asked to write down the off-odours and off-flavours for each sample, if they were perceived. Water and plain crackers with low-added salt were also provided to the participants during the evaluation sessions to cleanse their palates between samples.

2.3.6. Statistical analysis

Statistical analysis was performed using XLStat Lumivero (2023) with statistical significance determined using an alpha value of 0.05. ANOVA (analysis of variance) followed by Tukey HSD (honestly significant difference) for post-hoc mean separation was used to identify the sensory and chemical variables that were significantly influenced by the study factors. Also, Multiple Factor Analysis (MFA) was applied to explore the main trends differentiating the samples and the experimental variables with a set of continuous variables: basic oenological parameters, dissolved oxygen (D.O. mg/L), colour parameters (CIELAB), pH, measured HT, total polyphenolic content, minerals and sensory attributes for each time of storage (T1, T3, T6, T9, and T12 months). Additional Cochran's Q test and differential expression test using XLStat Lumivero (2023) were performed to understand significant attributes mostly contributing to the differences in the wine samples and have been reported in the Supplementary Material 1 (S1 to S10). In the model wine analysis, Origin Pro SR1 (2025) was employed to graphically illustrate the storage effects on HT and total SO_2 over six months.

Table 1
Sensory descriptors used to evaluate wine samples with their respective definition.

Sensory descriptors	Descriptors	Definition
AROMA		
Red fruit	Strawberry, blackberry, cherry, blackcurrant	Fruit with red or black skin and wild fruit from the forest
Dried fruit	Fig, prune, raisin, strawberry jam	Jam from strawberries or other red fruits. Dried raisin, prune, or fig odour
Vegetative	Green bell pepper, tomato stem, green grass	Aroma of cut green bell pepper, tomato stem, green grass
Spicy	Liquorice, black pepper, clove, star anise	Aroma from liquorice, black pepper, cloves, and star anise
Woody	Burned, oak, coffee, smoked	Oak resinous odour, coffee toasted, smoked odour
TASTE		
Warmness	Alcohol	Burning sensation in the mouth (chemesthetic sensation)
Astringency	Alum/tannin	Puckering mouthfeel caused by the tannins, precipitation of saliva, and dryness in the mouth
Sourness	Acids (citric, malic, lactic, tartaric)	Having an acidic taste, resembling that of vinegar or lemon juice
Bitterness	Caffeine	Bitter taste typical of a coffee beverage
Saltiness	Salt or glutamate	Having a salty/savoury taste
FLAVOUR		
Red fruit	Strawberry, blackberry, raspberry, cherry, blackcurrant	Having flavour from red fruit such as strawberry, blackberry, raspberry, cherry, blackcurrant
Woody	Fresh wood and toasted tannins	Flavour typical of toasted barrique
Vegetative	Green bell pepper	Flavour typical of fresh vegetable
Spicy	Cloves, nutmeg, liquorice	Spicy flavour typical of cloves, liquorice, and nutmeg

3. Results and discussion

3.1. Multiple factor analysis

The impact of HT addition at varying concentrations on wine was assessed using MFA as an exploratory approach. This method integrated multiple datasets, including basic oenological parameters, dissolved oxygen (D.O. mg/L), colour metrics (CIELAB), pH, measured HT concentration, total polyphenolic content, elemental composition, and sensory attributes across storage intervals (T1, T3, T6, T9, T12 months). The overall preference was included in the MFA as a separate supplementary variable and should not be interpreted as a measure for consumer analysis as in this study, a semi-trained panel conducted the evaluation, and the hedonic responses may reflect expert familiarity with the product category rather than general consumer liking. An identification of true drivers for liking would require a separate consumer analysis with a larger panel number (Nguyen et al., 2020). A unified multivariate representation highlighted correlations among variables and clustering trends across observations. Fig. 1 presents the score plot and loading projections for each storage point, with the first two partial axes explaining 35–38% of the total variance. Moreover, all wine samples were analyzed in triplicate.

After one month of storage (Fig. 1**A, B, C**), control wine samples (C) without HT addition were distinctly separated from the HT-enriched wine along F1, primarily driven by differences in elemental profile, glucose-fructose content, pH, and total polyphenols. However, no separation was observed among wine samples enriched with varying HT concentrations. Total SO_2, originating from alcoholic fermentation, and total polyphenols exhibited an inverse correlation with tartaric, lactic, and acetic acid. Similarly, dissolved oxygen and pH were anti-correlated. HT content positively correlated with colour parameters, dissolved oxygen, and the organic acids (acetic, lactic, and tartaric). Regarding the sensory profile (Fig. 1**C**), the "overall preference" was primarily associated with attributes such as "astringency", "bitterness" and "vegetative" aroma and flavour, which were more pronounced in wine samples with a low HT addition (A, 30 mg/750 mL; B, 60 mg/750), although no definitive separation among these wine samples was achieved. Conversely, control wine samples were characterized by "woody" flavour and aroma, "sourness," and "red fruit" aroma, which was the only significantly different attribute. Notably, "astringency" demonstrated an inverse correlation with "saltiness", "warmness", "dried fruit" aroma, and "spicy" flavour.

After three months of storage (Fig. 1**D, E, F**), the wine enriched with higher concentrations of HT (60 and 120 mg/750 mL) clustered together and were separated from both the control and 30 mg/750 mL wine samples along F1. This separation was predominantly influenced by tartaric acid, lactic acid, and elements (and the high content of HT, as expected). In contrast, control wine samples and those with 30 mg/750 mL of added HT were not distinguishable and were associated with free and total SO_2, dissolved oxygen, and sodium (Na). HT exhibited an inverse correlation with colour parameters, dissolved oxygen, free and total SO_2. In terms of the sensory profile (Fig. 1**F**), "sourness", "saltiness", and "bitterness" were inversely correlated with "overall preference", while "red fruit" flavour, "woody" aroma, "warmness" and

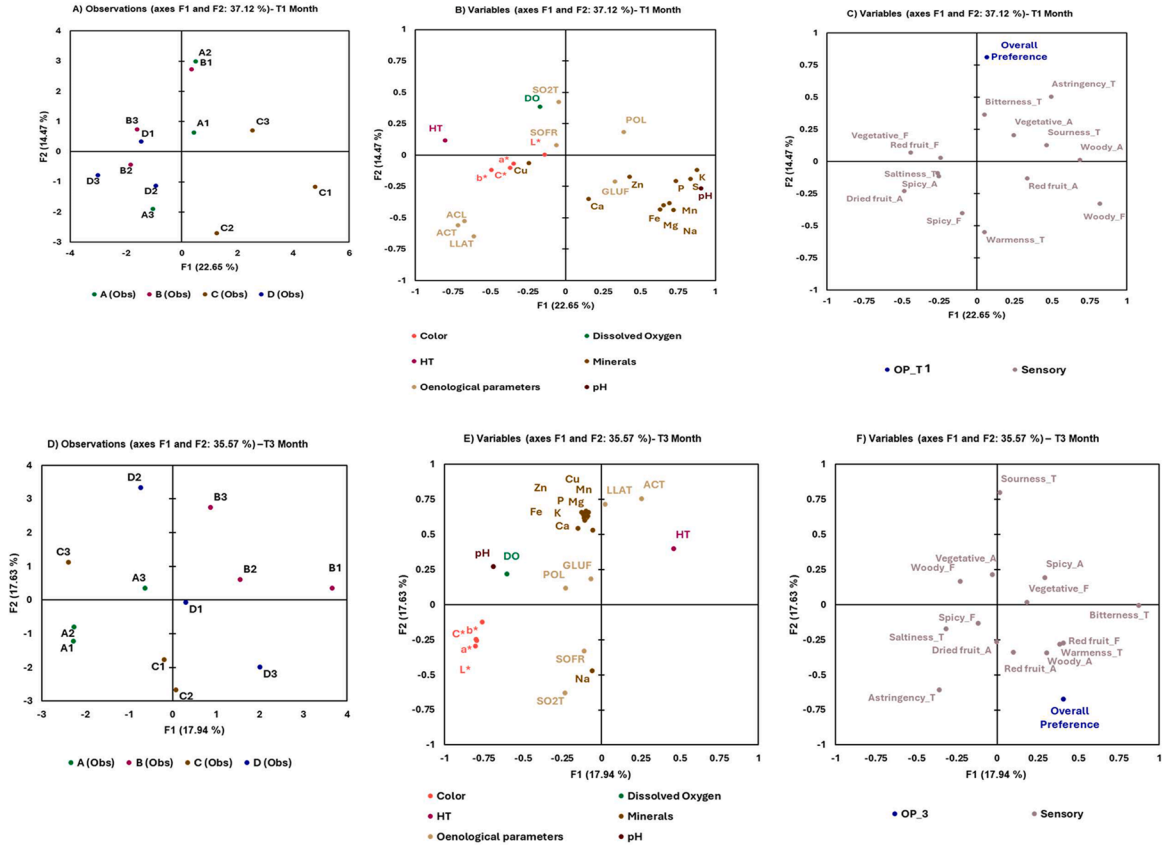

Fig. 1. represents the MFA of wines obtained from the first 2 components with the different datasets used in the computation. (A, B, C) represent the observation and loadings plots respectively at one month of bottled storage; (D, E, F) at three months; (G, H, I) at six months; (J, K, L) at nine months; (M, N, O) at twelve months; ACL: acetic acid (g/L); LLAT: lactic acid (g/L); LMAL: malic acid (g/L); ACT: tartaric acid (g/L); SOFR: free sulfur dioxide (mg/L); SO2T: total sulfur dioxide (mg/L); GLUF: glucose-fructose (g/L) and POL: total polyphenol (mg/L); L*: lightness; a*: redness; b*: yellowness; C*: Chroma; pH: pH; DO = dissolved oxygen (mg/L); Na, Mn, F, Cu, Zn (µgm/L), Mg, P, S, K, Ca (mg/L) = minerals quantified using a calibration curve. In the variables' graphs of sensory, A = Aroma; F = Flavour, and T = Taste. The wine samples are presented in triplicate C1,2,3: control 0mg/750 mL; A1,2,3: 30mg/750 mL; B1,2,3: 60mg/750 mL; D1,2,3: 120mg/750 mL. Overall preference is projected as a separate supplementary variable.

"vegetative" flavour contributed positively to the overall preference of the wine samples. The first dimension showed a negative association with "astringency", which was significantly different, "saltiness", "spicy" flavour, "red fruit" and "dried fruit" aromas, effectively distinguishing control and 30 mg/750 mL wine samples from those enriched with higher HT concentrations. Meanwhile, attributes such as "spicy" aroma, "warmness", "red fruit" flavour, "vegetative" flavour and aroma, and "bitterness" were key to differentiating the 60 and 120 mg/750 mL wine samples.

By six months of storage (Fig. 1**G, H, I**), the wine samples showed minimal differentiation across HT concentrations, including the control, indicating a convergence of sensory and chemical characteristics over time. Lightness (L*) was inversely correlated with chroma (C*), as well as the red-green (a*) and blue-yellow (b*) colour parameters, consistent with findings by Darnal et al. (2023). Dissolved oxygen displayed an inverse correlation with total polyphenols, free and total SO_2, while pH and lactic acid were positively correlated, and both were inversely related to tartaric acid. HT levels were positively associated with total polyphenols (as expected) and inversely correlated with other studied variables. Regarding the sensory profile (Fig. 1**I**) at six months, the wine samples with 60 and 120 mg/750 mL HT emerged as the most preferred. These wine samples were characterized by "dried fruit" aroma (significantly different), "vegetative" flavour, and "saltiness", suggesting that the higher HT concentrations contributed positively to sensory attributes over extended storage.

At nine months of storage (Fig. 1**J, K, L**), dissolved oxygen, HT, and several oenological parameters, including glucose-fructose, total polyphenols, and lactic, acetic, and tartaric acids, positively characterized the enriched samples (30, 60, and 120 mg/750 mL). These samples formed a distinct cluster, separating them from the control samples. Additionally, pH showed a strong positive correlation with elements and colour parameters and was anti-correlated with HT, dissolved oxygen, and oenological parameters. The measured HT showed a positive correlation with the total polyphenols, acetic, and tartaric acids, as well as the dissolved oxygen. The control sample, as illustrated in Fig. 1**L**, was characterized by "astringency" and "red fruit" flavour. In contrast, the enriched wine was mainly distinguished by "red fruit" (significantly different), "dried fruit" aromas, "warmness", "bitterness", "sourness", and "woody" flavour.

By the twelfth month (Fig. 1**M, N, O**), the oenological and sensory profiles continued to evolve. Elements such as copper, sodium, and zinc were strongly associated with colour parameters and helped to distinguish control and low-concentration wine from the more concentrated HT samples. Dissolved oxygen and pH maintained their positive correlation, consistent with trends observed at T3 and T6. Basic oenological parameters and most elements showed strong correlations and were instrumental in samples with elevated added HT (60 and 120 mg/750 mL) along F1.

Sensory results (Fig. 1**O**) showed that control wine samples were predominantly associated with "red fruit" aroma, which was significantly different, while the 120 mg/750 mL wine samples were positively correlated with "spicy" aroma. Meanwhile, low-enriched wine samples

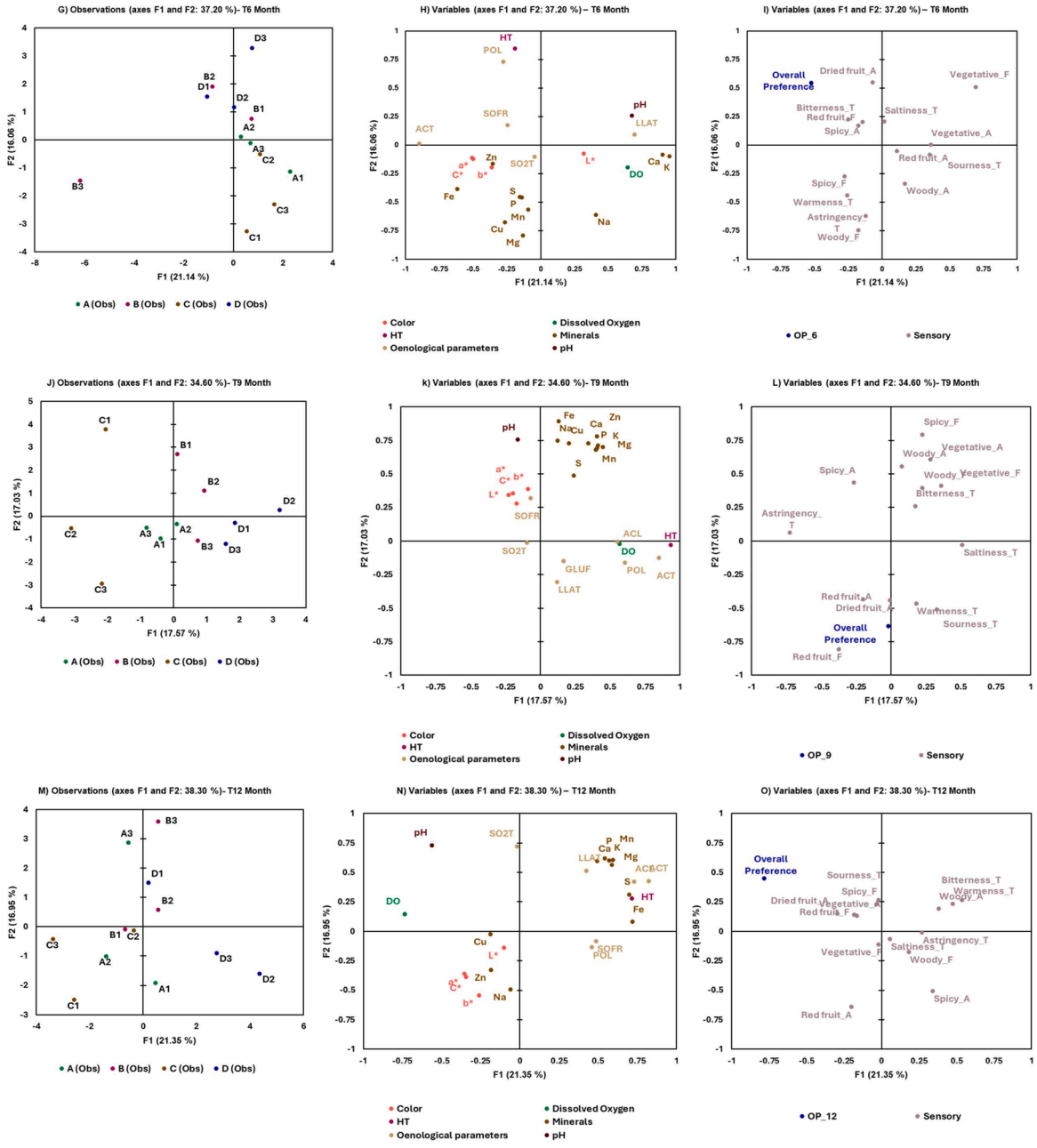

Fig. 1. (*continued*).

(30 and 60 mg/750 mL) showed the highest correlation with most of the sensory attributes, such as "astringency", "saltiness", "vegetative" flavour and aroma, and "woody" flavour. Notably, "overall preference" showed a strong association with "red fruit" flavour and "dried fruit" aroma, highlighting the interplay between sensory attributes and HT concentration over extended storage.

The evolution of overall preference over the 12 months of bottle storage is illustrated in Fig. 2, which presents the panel's preferences across 12 wine samples evaluated at 5 time points. The bars in the figure indicate the overall preference with which each sample was most preferred by the assessors and showed distinct trends over the storage period, influenced by the concentration of HT and time. At T1, all the samples received a relatively low preference score compared to the other storage times, suggesting that the wine samples, regardless of the treatments, were still at an early development stage. By T3, however, there was an improvement in the preference across all treatments, indicating that short-term ageing enhanced the wine's sensory appeal. Notably, the samples containing 60 mg/750 mL of HT continued to increase in preference at the later storage points (T6 and T9), which suggests that a moderate amount of HT addition may contribute positively to the wine sample's sensory quality over time. On the contrary, control and 30 mg/750 mL samples peaked at T3 storage time and later showed a gradual decline, while the highest concentration (120 mg/750 mL) consistently showed a lower preference, implying that excessive amounts of HT may negatively impact the sensory attributes as the wine ages. By T12, the samples containing 60 mg/750 mL of HT still received the highest preference, while the others received lower preference, further supporting the positive influence of the moderate amount of HT levels on the sensory quality of the wine.

3.2. *Non-added free and total SO_2 over 12 months of storage in wine samples*

The evolution of naturally occurring SO_2, originating mostly from

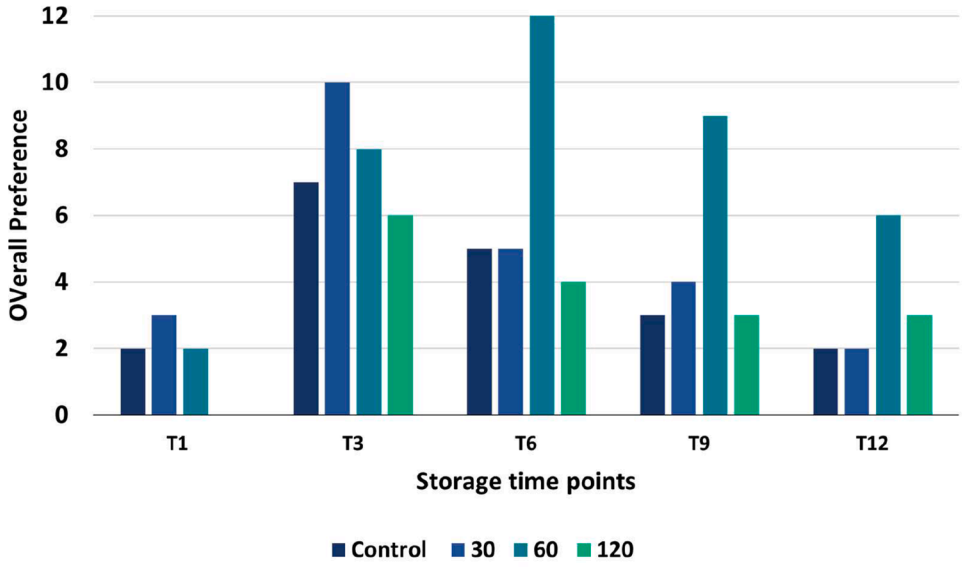

Fig. 2. Frequency table representing the overall preference expressed by the assessors on 12 wine samples across 5 time points. The bars represent the number of times a specific sample was most preferred by the panel.

alcoholic fermentation, was evaluated using ANOVA, considering storage time, HT concentration, and their interaction effects as factors. Regarding the concentration factor, Fig. 3**A** and **B** show the One-way ANOVA of the effect of HT concentration on free and total SO_2, respectively. No statistically significant differences were observed in either free or total SO_2 levels across wine enriched with 30, 60, or 120 mg/750 mL HT, indicating that HT addition did not directly influence the naturally occurring SO_2 content. Regarding the time factor, Fig. 3**C** and **D** show the time evolution of free and total SO_2, respectively. At three months, the concentration of free SO_2 (Fig. 3**C**) reached its lowest value (~8.7 mg/L), significantly lower than at other storage periods, where no substantial differences were observed. Total SO_2 levels (Fig. 3**D**) were highest at T1, followed by T3, with both differing from samples stored for six to twelve months, which showed lower and stabilized total SO_2 levels.

However, considering the interaction between storage time and HT concentration, significant differences emerged: at T12 (twelve months), the wine enriched with 60 and 120 mg/750 mL of HT showed significantly higher free SO_2 levels compared to the T3 wine enriched with 30, 60, and 120 mg/750 mL HT (Fig. 3**E**). For total SO_2 (Fig. 3**F**), the highest levels were observed in T1 samples across all HT concentrations, followed by T3, while samples stored for six months or longer exhibited lower and statistically similar levels regardless of HT concentration. These results suggest that naturally occurring SO_2 levels are primarily influenced by storage time, with minimal contribution from HT concentration, reflecting a stabilization process throughout wine ageing.

To investigate and better characterize the behaviour of SO_2 in the absence of complex matrix effects, a model wine solution was employed. This simplified system enabled a more controlled evaluation of SO_2 stability and its interaction with HT. Total SO_2 concentration was selected as the reference parameter, as the lack of typical wine-binding compounds could influence free SO_2 measurements. SO_2 loss can be attributed to a gradual consumption due to oxidative reactions (Grogan, 2015; Mlček et al., 2023). The presence of HT appeared to modestly improve SO_2 retention over time, with the high HT + SO_2 treatment maintaining higher SO_2 levels compared to the control (see detailed results in the Supplementary Material 1, paragraph "**SO_2 evolution in model wine samples**"). The effect was most apparent after 6 months, where the high HT treatment retained ~6.3 mg/L more SO_2 than the control and ~1.3 mg/L more than the low HT group (Fig. 4). These findings suggest that HT may contribute to slowing SO_2 depletion, potentially due to its radical-scavenging capacity (Davalos et al., 2018), which reduces the oxidative demand placed on SO_2. Overall, the finding suggests that hydroxytyrosol does not interfere negatively with SO_2 and may complement its antioxidant role in model wine by preserving SO_2 from premature oxidation or binding. While SO_2 levels declined over time as expected, the slower rate of decrease in HT-containing samples indicates that HT and SO_2 each contribute to oxidative stability via different mechanisms: SO_2 as an oxygen scavenger and carbonyl-binding agent (Abramovič et al., 2015), and HT as a direct radical scavenger (Davalos et al., 2018; Sun et al., 2018). The findings also provide support for the concept of using HT as a partial substitute or functional complement to SO_2 in wine and wine-like systems, like the use of glutathione (GSH) as a partial substitute for SO_2 reported by Panero et al. (2015) in their study.

3.3. *Evolution of HT in wine over 12 months of storage*

The evolution of HT content in wine samples over twelve months of storage was quantified, and the results are presented in Fig. 5. The curves represent the average values obtained from triplicate analysis, and ANOVA was performed for each HT concentration (0, 30, 60, 120 mg/750 mL), considering "time" as a factor. Tukey's test ($p < 0.05$) was applied, with means followed by different letters indicating statistically significant differences for the same concentration.

In control wine (0 mg/750 mL HT), naturally occurring HT was detected from T1 to T6, starting at 2.7 mg/L at T1, increasing to 3.2 mg/L at T3, peaking at 7.3 mg/L at T6, and then sharply declining to 0.7 mg/L at T9 and 0.00 mg/L at T12. These changes were statistically significant, highlighting the transient presence of HT in non-enriched wine, likely due to its natural formation in grape-derived polyphenols, as reported by Di Tommaso et al. (1998).

In HT-enriched wine (30, 60, and 120 mg/750 mL), a similar trend was observed, characterized by an initial increase during the early months of storage, followed by a rapid decline between T6 and T9, and stabilization thereafter. The wine enriched with 60 mg/750 mL of HT exhibited a steady increase from T0 to T3, followed by a slight decline at T6, a rapid drop at T9, and stabilization through T12. The wine enriched with 30 mg/750 mL of HT showed an initial increase in HT concentration, followed by a minor decline at T3. However, the levels remained relatively stable from T1 to T6, before decreasing in line with the trends observed at other concentration levels.

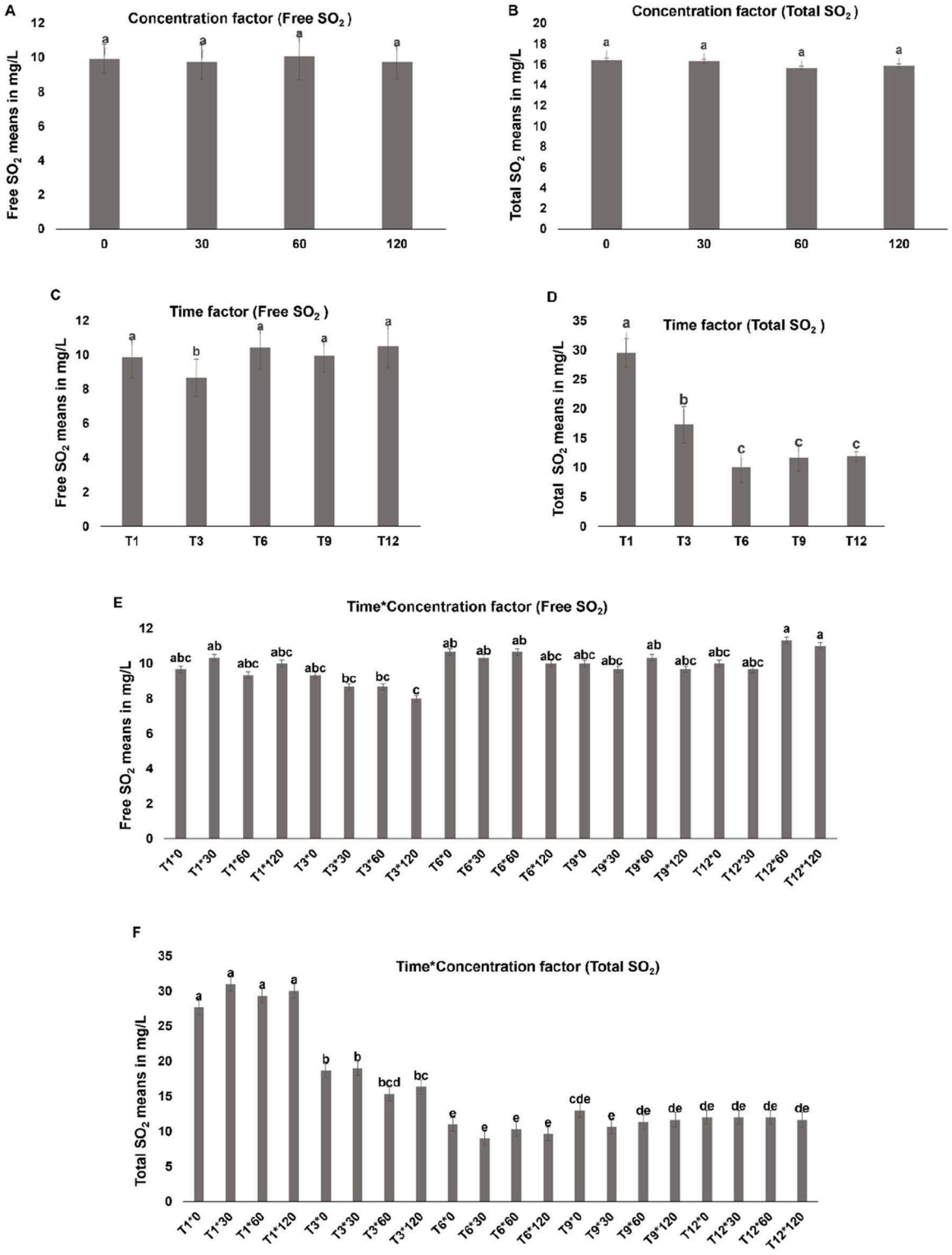

Fig. 3. represents the evolution of non-added free and total SO2 over 12 months of storage in bottles obtained from the ANOVA test. (A, B) Effect of HT concentration (30, 60, and 120 mg/750 mL) on free (A) and total (B) SO_2, assessed by one-way ANOVA. (C, D) Changes in free (C) and total (D) SO_2 during storage at 1, 3, 6, 9, and 12 months. (E, F) Interaction effects of storage time and HT concentration on free (E) and total (F) SO_2 levels, assessed by two-way ANOVA. (Means followed by different letters differ significantly by Tukey's test ($p < 0.05$). The bar represents the average of three replicates with the respective standard deviation. T1, T3, T6, T9, and T12 = one, three, six, nine, and twelve months, respectively. 0; 30; 60; 120 = 0mg/750 mL; 30mg/750 mL; 60mg/750 mL and 120mg/750 mL, respectively).

For the wine enriched with 120 mg/750 mL, the initial increase in HT was more pronounced, reaching its maximum at T6, after which a sharp decline occurred until T12. This suggests that higher HT concentrations may experience more rapid degradation over extended storage periods. Overall, the data indicate that while HT levels naturally fluctuate in control wine, enrichment with HT introduces a distinct pattern of early increase followed by decline and stabilization. These trends provide valuable insights into the behaviour of HT in wine matrices over

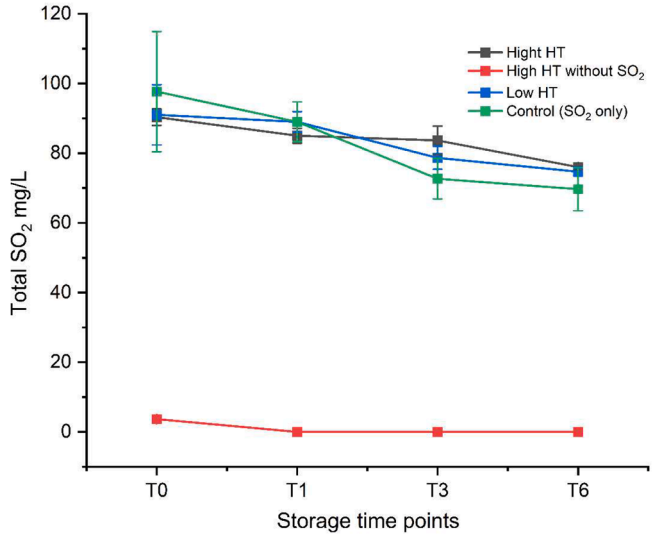

Fig. 4. shows changes in total SO_2 concentrations in model wine solutions over a six-month storage period under different HT treatment conditions. Treatments included: high HT with SO_2 (120 mg/L HT + 100 mg/L SO_2), high HT without SO_2 (120 mg/L HT), low HT with SO_2 (60 mg/L HT + 100 mg/L SO_2), and control (100 mg/L SO_2 only, no HT). Total SO_2 was measured at 0, 1, 3, and 6 months (T0, T1, T3, T6). Values represent the mean of triplicate samples (n = 3) ± standard deviation. A slower decline in total SO_2 was observed in HT-containing samples, suggesting a potential synergistic antioxidant effect between HT and SO_2.

time and highlight its sensitivity to storage conditions.

The evolution of polyphenols during bottle aging of wine has been extensively studied, but research on the specific behaviour of HT remains scarce. In a previous study (Ceci et al., 2024), SO_2 was added at the bottling stage in an organic red wine produced by the same winery as that used in the current study, and HT showed only a slight decrease over 12 months of storage, compared to the significant decline of endogenous HT in the control wine. Changes in phenolic composition during bottle ageing have been attributed to reactions, such as oxidation, condensation, and hydrolysis, which depend on grape variety and matrix composition (Monagas et al., 2005).

In the present study, HT concentration increased during the early storage stage and remained relatively stable up to six months, followed by a marked decrease between T6 and T9 and subsequent stabilization. As only HT itself was quantified, the underlying mechanisms cannot be directly demonstrated. However, the observed pattern is consistent with literature reports suggesting that HT may be released from more complex phenolic structures or bound forms under certain conditions (Boselli, 2015).

The subsequent decline of HT after six months may reflect oxidative or transformation processes of HT into other polyphenolic forms, such as dimers, as noted by Di Maio et al. (2011), although specific transformation products were not identified in this work.

Likewise, the stabilization of HT levels after nine months could hypothetically relate to equilibrium between degradation and regeneration pathways, including potential formation from precursors such as tyrosol reported in fermentation systems (Álvarez-Fernández et al., 2018; Fernandez-Bolanos et al., 2008).

Overall, the temporal evolution of HT observed during storage suggests that HT participates in dynamic reactions within the wine matrix, such as polymerization and depolymerization, release from complex precursors, chelation with metals or oxidation. However, this interpretation remains hypothetical because only HT concentration and total polyphenols were measured.

3.3.1. *Stability of HT in model wine solution*

Fig. 6 shows the decrease in HT concentration in model wine over a 6-month storage period as a percentage of their initial values (T0=100%), whereas the control with no HT, as expected, is showing 0% HT. After a month at T1, all high HT treatments retained >99% of their initial HT content, with negligible loss in the high HT treatments (0.08% loss with SO_2 and 0.6% loss without SO_2). The low HT group exhibited a more notable reduction of 9.7% loss, indicating an early and more pronounced vulnerability to stability at lower concentrations. By T3, the high HT group with SO_2 had declined to 92% of its original HT, while the high HT without SO_2 remained more stable (98.9%). The low HT treatment continued its downward trajectory, retaining only 88% of the initial HT. The greatest divergence appeared at T6, where both high HT treatments showed similar levels of drop, 71.2% remaining with SO_2 and 71.7% without SO_2 (approximately 28.5% loss). In contrast, the low HT sample retained only 47.8% of its original content, having lost over half of its HT (52.2%). The control sample, as expected, consistently showed 0% HT across all time points.

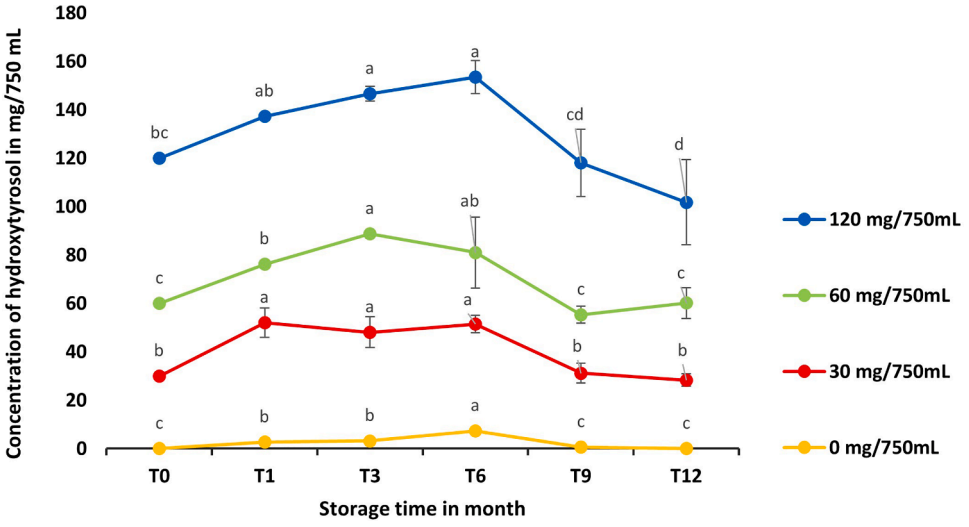

Fig. 5. shows the evolution of HT quantified over 12 months of storage in bottles. Means followed by different letters differed significantly by Tukey's test ($p < 0.05$) for the considered concentration. The curve represents the average of three replicates with the respective standard deviation. T0, T1, T3, T6, T9, and T12 = zero, one, three, six, nine, and twelve months, respectively. 0 ppm; 30 ppm; 60 ppm; 120 ppm = 0mg/750 mL; 30mg/750 mL; 60mg/750 mL, and 120mg/750 mL of HT/Sangiovese wine, respectively.

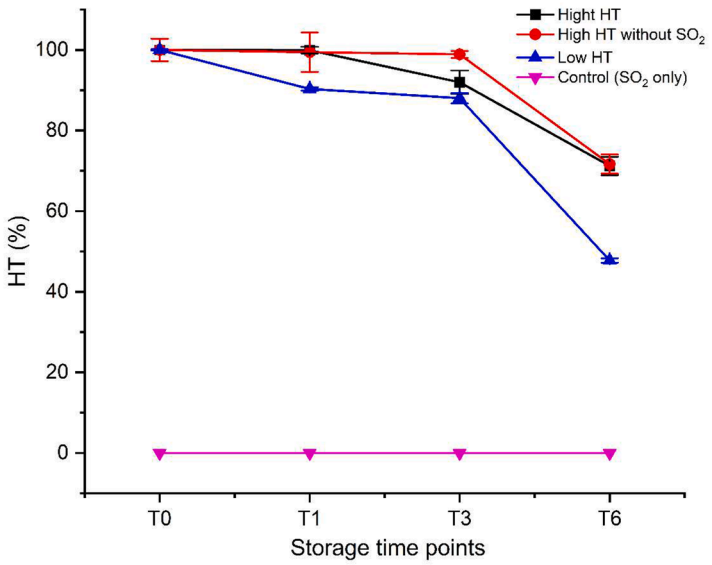

Fig. 6. shows the percentage of HT remaining over a six-month storage period in model wine solutions under different treatment conditions: high HT with SO_2 (120 mg/L HT + 100 mg/L SO_2), high HT without SO_2 (120 mg/L HT only), low HT with SO_2 (60 mg/L HT + 100 mg/L SO_2), and a control sample containing SO_2 only (100 mg/L, no HT). HT levels were normalized to 100% at T0. Data represent mean values (n = 3) ± standard deviation. Results indicate that HT degradation was more pronounced at lower concentrations and that the presence of SO_2 did not significantly enhance HT stability.

As seen in Fig. 6 the high HT treatment with and without SO_2 followed a nearly similar decreasing pattern, which suggests that SO_2 does not significantly influence the chemical stability of HT under model wine conditions. The absence of enhanced preservation in the SO_2-containing treatment supports previous findings (Ceci et al., 2024), indicating that SO_2, while effective at protecting wine constituents from oxidation, does not form protective adducts with HT or suppress its oxidative pathways. The low HT group (60 mg/L) showed significantly greater decline at every time point, losing nearly 10% by T1 and over 50% by T6. This concentration-dependent instability in HT may stem from reduced antioxidant redundancy, where lower availability of hydroxyl groups is available to neutralize radicals or scavenge oxygen, which limits the compound's ability to form stable phenoxy radicals and maintain antioxidant activity over time (Villaño et al., 2004). Moreover, lower concentrations of phenolic compounds are generally more prone to a reduction in stability in model wine systems due to the absence of synergistic interactions and matrix components typically found in real wine (Poklar Ulrih et al., 2020). This increased susceptibility of HT to a decrease in concentration under simplified conditions has also been observed by Zafra-Gómez et al. (2011), reinforcing the concentration-dependent instability. Whereas higher concentrations may allow self-regeneration mechanisms, where one antioxidant compound stabilizes or is regenerated from the previously reduced structure (Waterhouse & Laurie, 2006), contributing to the improved stability observed in the high HT treatments.

Interestingly, all HT-containing samples showed strong stability during the first month (T0–T1), with losses becoming more prominent between T3 and T6, which could be attributed to the slow autooxidation of HT (Di Maio et al., 2011). The most rapid decline in HT concentration occurred in the low HT group between T3 and T6. A similar loss was observed in HT added wine samples between T6 and T9, as shown in Fig. 5, although that decline appeared to be delayed, likely due to the presence of other antioxidant phenolic compounds in the wine matrix that might have offered additional protective effects.

These results highlight that HT alone is relatively stable under model wine conditions, particularly at higher concentrations, but that this stability is not significantly altered by the presence of SO_2. Therefore, while HT and SO_2 do not appear to interact negatively, their antioxidant roles may be functionally independent rather than synergistic in simple matrices. This indicates that HT can function as a standalone antioxidant in wine systems and may serve as a partial substitute for SO_2, particularly when added at higher doses.

3.4. Colours, elements, and oenological parameters of wine over 12 months of storage

The evolution of wine parameters over 12 months of storage was evaluated using ANOVA, considering HT concentration, time, and their interaction effects. While no significant differences were observed in the studied parameters based on HT concentrations alone (**Table S2a**), the time factor showed a significant impact on all parameters (**Table S2b**).

Among oenological parameters, tartaric acid levels were highest in the T3 samples, followed by T1, and progressively decreased during longer storage times (T6, T9, T12). Conversely, lactic acid showed an inverse trend, with the highest levels observed at T12 and T9, followed by T6, T3, and T1, reflecting the gradual malolactic fermentation process. Dissolved oxygen was significantly higher in the early storage phase (T1) compared to later time points (T3, T6, T9, and T12), where no statistical differences were noted. Total polyphenol content peaked at T9, followed by T6, T12, and T3, with the lowest concentration observed at T1. Acetic acid content increased significantly by T12, followed by T1 and T9, while the lowest concentrations were observed at T3 and T6.

Regarding colour parameters, a* (redness) values were highest in T1 samples, followed by T6, T3, T12, and T9. Chroma (C*) showed a similar trend, with the highest values recorded at T1, followed by T6, T3, T12, and T9, which could be attributed to the antioxidant potency of the HT during the early months of storage stabilizing anthocyanins or other red pigments. Lightness (L*) values were highest at T12, followed by T1, T6, T9, and T3. The b* parameter (yellowness) was highest at T12, T1, and T9, followed by T6, with the lowest values observed in T3 wine samples. Although no clear trend in colour change was observed over time, the increase in b* values is a common indicator of oxidative browning. This observation is consistent with findings by Rinaldi et al. (2021), which reported an increase in yellowness (b*) and a decrease in redness (a*) during wine ageing, attributed to oxidation processes. Similar changes have also been documented in other studies on aged red wine (Monagas et al., 2006; Zhang et al., 2021).

Mineral content also exhibited significant temporal variation. Magnesium (Mg), potassium (K), calcium (Ca), and manganese (Mn) were significantly higher in T12 and T3 wine samples, followed by T6, with

lower concentrations detected in T1 and T9. Sodium (Na) levels peaked at T3, while phosphorus (P) and iron (Fe) were highest at T3, followed by T12, with lower levels at T6. Copper (Cu) concentrations were highest at T1, followed by T3, and decreased in long-term storage samples (T6, T9, T12), which may be due to HT-induced chelation (Pietrelli et al., 2017) or reduction of Cu^{2+} to less soluble forms, promoting precipitation (Briante et al., 2003).

The interaction between storage time and HT addition (**Table S2c**) significantly affected most parameters except free SO_2 and L* (lightness). At T6, T9, and T12, both control and enriched samples (30 mg/750 mL, 60 mg/750 mL, and 120 mg/750 mL) exhibited higher lactic acid content compared to early storage samples (T1 and T3). Acetic acid levels were highest across all T12 samples, irrespective of HT concentration. Total polyphenol content was significantly higher in all T9 samples, while the lowest levels were observed at T1. Tartaric acid content was highest in T1 and T3 samples, particularly in the wine enriched with 30 mg/750 mL and 120 mg/750 mL at T1. Dissolved oxygen levels were highest in the T1 control wine and in samples enriched with 30 mg/750 mL and 60 mg/750 mL, differing significantly from other storage points. These findings demonstrate that while HT concentration had limited direct effects, storage time and its interaction with HT addition influenced the evolution of wine parameters, highlighting the complex dynamics of ageing in wine.

4. Conclusions

This study provides a detailed assessment of the stability and impact of HT in red wine and model wine systems over extended storage. HT enrichment at varying concentrations (30, 60, and 120 mg/750 mL) influenced the chemical, sensory, and antioxidant profile of wine throughout 12 months of bottle ageing. In the real wine matrix, HT-enriched samples initially diverged from control wine in both sensory and compositional characteristics, particularly at three and six months of storage, with elevated HT levels correlating positively with polyphenol content and sensory attributes such as “dried fruit” aroma and “vegetative” flavour. However, these distinctions diminished over time, suggesting convergence in wine profiles beyond six to nine months, possibly due to shared oxidative and matrix-mediated transformation pathways.

The evolution of HT during storage revealed a consistent trend across all enriched samples: initial stabilization or slight increase, followed by a sharp decline between six and nine months, then stabilization at lower levels. The decline is likely linked to autoxidation, dimerization, or transformation into other phenolic structures, rather than direct interaction with sulfur dioxide. Importantly, HT remained detectable at significant levels after 12 months, particularly in higher-dose treatments, demonstrating its moderate persistence and potential as a long-term antioxidant. In model wine, HT stability was primarily concentration-dependent, with high-dose HT showing significantly greater retention than lower doses, regardless of SO_2 presence. The addition of SO_2 had no significant protective effect on HT, and their co-presence did not indicate any synergistic interaction. Instead, HT acted independently, suggesting it could function as a complementary or partial substitute for SO_2 in simplified systems, particularly when used at higher concentrations. Moreover, SO_2 levels were primarily influenced by storage time rather than HT concentration. In the real wine, a significant decline in naturally produced SO_2 occurred by three months, with stabilization thereafter. In model wine, the presence of HT at high concentration modestly slowed SO_2 depletion, reinforcing the view that HT may contribute indirectly to oxidative stability.

Finally, this study highlights HT’s potential as a natural antioxidant for wine preservation, capable of enhancing polyphenol stability and contributing positively to sensory quality, especially in the early to mid-stages of bottle ageing. Its effectiveness is closely linked to concentration and storage duration, with optimal sensory and chemical benefits observed at 60 and 120 mg/750 mL within the first six months of storage. These findings provide a strong basis for further exploration of HT as an alternative or supplement to sulfur-based preservatives in winemaking, with implications for cleaner labelling and improved oxidative management.

Ethical statement – studies in humans

The study was conducted following the Declaration of Helsinki (World Medical Association, 2013). There was no cost to participate, refusal did not imply any penalties or loss of benefits, and participants were permitted to withdraw at any time without giving any reason. The study was evaluated as low risk for the researchers involved. Therefore, ethical committee approval was not sought for the following reasons: i) participants (panelists) were instructed to expectorate the wine samples after analysis; ii) the samples and additives used were commercially available products, and iii) hydroxytyrosol (HT) was certified as food-grade. The panelists were recruited voluntarily and were asked to sign an informed consent form, which told them about the presence of non-added sulphite in the wine samples to avoid any related issues. An affirmative reply was required to enter the survey. The wines contained approximately 13% v/v ethanol (3.9 g of ethanol per 30 mL sample), and the maximum quantity of wine per glass per session was 30 mL. Participants were informed that an organic Tuscany wine from the commerce, containing around 13% v/v ethyl alcohol, was enriched by a food-grade HT product (grape wines naturally contain a concentration of around 1.98 – 3.89 mg of HT mg/L). The European Food Safety Authority (EFSA) has declared the levels of hydroxytyrosol up to 215 mg/kg in vegetable oils and 175 mg/kg in margarine safe for consumption. In this study, the concentration used is under the safety level given by the EFSA (Panel on Dietetic Products et al., 2017).

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AI/Assisted writing statement

During the preparation of this work, the authors used ChatGPT (OpenAI) to support English language editing and improve the readability of selected text passages. After using this tool, the authors carefully reviewed and edited the content as needed and take full responsibility for the scientific accuracy and integrity of the published article.

Data availability

The data presented in this study are available on request from the corresponding author.

CRediT authorship contribution statement

Wasim Akhtar: Writing – review & editing, Writing – original draft, Visualization, Validation, Software, Data curation. **Prudence Fleur Tchouakeu Betnga:** Writing – original draft, Visualization, Validation, Software, Methodology, Investigation, Formal analysis, Data curation. **Aakriti Darnal:** Writing – review & editing, Validation, Methodology, Investigation, Formal analysis, Data curation. **Adriana Teresa Ceci:** Writing – review & editing, Validation, Methodology, Investigation,

Formal analysis, Data curation. **Edoardo Longo:** Writing – review & editing, Validation, Methodology, Investigation, Formal analysis. **Simone Poggesi:** Writing – review & editing, Validation, Methodology, Investigation, Formal analysis. **Fabio Trevisan:** Writing – review & editing, Formal analysis. **Renzo Nicolodi:** Writing – review & editing, Resources. **Reeta Davis:** Writing – review & editing, Supervision, Resources. **Meg Walsh:** Writing – review & editing, Supervision, Resources. **Kevin E. O'Connor:** Writing – review & editing, Supervision, Resources. **Enrico Angelo Altieri:** Writing – review & editing, Resources. **Tanja Mimmo:** Writing – review & editing, Resources. **Emanuele Boselli:** Writing – review & editing, Supervision, Resources, Funding acquisition, 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. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

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Supplementary materials

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.afres.2026.102027.

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