Applied Food Research 4 (2024) 100513

Contents lists available at ScienceDirect

Applied Food Research

journal homepage: www.elsevier.com/locate/afres

Enhancing the stability of a red organic wine through hydroxytyrosol supplementation at bottling: A time-dependent analysis

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ARTICLE INFO

Keywords:
Organic red wine
Sensory quality
Wine phenolics
Aroma compounds
Hydroxytyrosol

ABSTRACT

The effect of the addition of hydroxytyrosol (HT) in a red organic wine (Sangiovese 85 % and Cabernet Sauvignon 15 %) was evaluated. After six months of storage in a concrete tank, the wines were spiked with HT in three different amounts (30, 60, 120 mg/750 mL in wine) and compared with a control bottled wine (no HT addition). The bottled wines were stored at room temperature and were opened after a period of one, three, six, nine and twelve months to perform chemical and sensory analyses (T1, T3, T6, T9, and T12, respectively). Storage time was the factor that most influenced all wines parameters with respect to the different treatments. However, wine added with 60 and 120 mg/750 mL HT had a lower amount of acetic acid than the control at T3 and T6. At T6, the measured HT was negatively correlated with acetic acid ($R^2 = 0.81$), thus suggesting that HT preserved the wine from oxidation. Later, this trend was not confirmed. The content of HT spiked was stable over twelve months of bottle storage; only the endogenous HT, which is naturally present in wine, showed a decrease of 44 % after twelve months of bottle storage. Yellowness (b*) was higher in the 30 mg/750 mL at T9 compared to the other samples. Lightness (L*) showed a decrement of 11 %, while a* (redness) increased by 21 % from T1 to T12. After T6, the wine fortified with 120 mg/750 mL was preferred by the sensory panel; however, it was the only sample to be clustered by the panel at T9 and "red fruits," "spicy", and "astringency" sensory attributes played an important role in this separation.

1. Introduction

Sulfites are incorporated into the must or grape juice during the winemaking process to safeguard against oxidation and microbial contamination, thereby preventing the formation of undesirable flavors in the wine. Before bottling, sulfur dioxide (SO_2) is strategically used to enhance the wine's shelf life. However, SO_2 content must be monitored, as excessive amounts can adversely affect the sensory attributes of wine, such as aroma and taste (Ruiz-Moreno et al., 2015). In addition, some individuals may be sensitive to sulfites, causing allergic reactions or other medical issues such as headaches, bronchospasm, bradycardia, gastrointestinal symptoms, urticaria, angioedema, and hypotension (Lester, 1995). Wine producers closely monitor and control the use of SO_2 to strike a balance between its beneficial effects and potential drawbacks, ensuring that the wine remains of high quality, meeting safety standards, and consumer preferences. The World Health Organization (WHO)) reports a maximum acceptable daily intake (ADI) of 0.7 mg of SO_2 per kg of body weight (Joint FAO/WHO Expert Committee on Food Additives., 2009; Lisanti et al., 2019). Numerous studies are actively exploring alternative approaches to decrease or potentially replace SO_2 within the production chain. Indeed, some winemakers and producers have been strongly oriented toward finding new emerging

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https://doi.org/10.1016/j.afres.2024.100513
Received 10 July 2024; Received in revised form 6 September 2024; Accepted 17 September 2024
Available online 18 September 2024

procedures as possible alternatives to SO_2 while maintaining wine quality and stability. Sulfites in wine are governed by European Union (EU) Regulation No. 1129/2011 which establishes rules and requirements for the production and labelling of wine within the EU (Lisanti et al., 2019). The key points define both the maximum allowable limits (expressed as the total amount of SO_2 in mg/L that depends on the type of wine (e.g., red, white, rosé) and its sugar content, labeling requirements, differences between organic and conventional wines, and guidelines for all winemaking practices, storage and aging of wines. The maximum amount of total SO_2 is set up at 150 mg/L in red wines and 200 mg/L in white and rosé wines containing a maximum of 5 g/L of reducing sugars (Commission Delegated Regulation (EU) 2019/934 replacing EU Regulation No. 606/2009) Lisanti et al., 2019. When the reducing sugars are equal to or exceed 5 g/L, the limit of total SO_2 is 200 mg/L in conventional red wines and 170 mg/L in organic wines (EU Regulation No. 203/2012) (Guerrero and Cantos-Villar, 2015; Lisanti et al., 2019; Yıldırım and Darıcı, 2020)).

In recent times, there has been a growing interest in alternatives to SO_2 (Lisanti et al., 2019; Raposo et al., 2016a; Raposo et al., 2016b) within the context of the European Union's Green Deal policy. This heightened interest aligns with an increased awareness among consumers and producers, aiming to reduce the allergenic potential of foods. Some alternative methods have already been exploited, such as low-SO_2 yeast selection, temperature control, oxygen management, and pH regulation. Also, alternative additives are studied to overcome drawbacks of the use of SO_2, such as bacteriocins, silver nanoparticles, hydroxytyrosol (abbrev. HT), short-/medium-chain fatty acids, yeast killer toxins, and antimicrobial peptides (Guerrero and Cantos-Villar, 2015; Lisanti et al., 2019).

HT has been shown to have human health benefits, including anti-inflammatory, cardioprotective, and neuroprotective effects (Martínez et al., 2018). In detail, HT was approved by the European Food Safety Authority (EFSA) (Panel on Dietetic Products, Nutrition, & Allergies, 2011)) to maintain normal blood concentrations of HDL-cholesterol. EFSA has declared the levels of hydroxytyrosol up to 215 mg/kg in vegetable oils and 175 mg/kg in margarine safe for consumption (Turck et al., 2017). Panel on Dietetic Products et al. (2017)/2373 of 14 December 2017 authorized the placing on the market of hydroxytyrosol as a novel food ingredient under Regulation (EC) No 258/97 of the European Parliament and the Council (notified under document number C (2017) 8423). The maximum level is set at 215 mg/kg in fish and vegetable oils, (except olive oils and olive pomace oils as defined in Part VIII of Annex VII of Regulation (EU) No 1308/2013 (1)) Panel on Dietetic Products et al. (2017)/ 2373 - of 14 December 2017- authorising the placing on the market of hydroxytyrosol as a novel food ingredient un-der Regulation (EC) No 258 / 97 of the European Parliament and of the Council - (notified under document number C (2017) 8423)). As reported by Soni et al. (2006), the consumption of an aqueous olive pulp extract, is considered safe at levels up to 1200 mg/kg/day.

Faced with the beneficial effects of HT for the human health, several authors studied the applicability of HT as an antioxidant and antimicrobial during winemaking procedures (Piñeiro et al., 2011; Raposo et al., 2016a; Raposo et al., 2016b; Ruiz-Moreno et al., 2015; Tedesco et al., 2022). Within the framework of a circular economy, harnessing high-value compounds from by-products is a comprehensive approach that focuses on more sustainable winemaking processes. In this context, phenolic compounds or polyphenols, naturally present in grapes and wines and extracted from natural products (International Oenological Codex Oenological tannins COEI-1-TANINS 1 Oenological tannins INS n°:181), are used as additives and their application is allowed in the winemaking process by the OIV (International Organization of Vine and Wine), in accordance with the EU legislation (Regulation EC No. 606/2009 and subsequent amendments) (Lisanti et al., 2019). Polyphenols and tannins content depend on several factors, such as environment (cultivar, soil, and weather conditions) and winemaking practices (maceration time and temperature, fermentation with skins, stems, and seeds, malolactic fermentation).

The most common polyphenols in wine are phenolic acids, flavonoids, condensed tannins, and stilbenes that influence sensory properties, organoleptic characteristics, stability, and colour. Among the various naturally occurring polyphenols, scientific research has currently focused on hydroxytyrosol (2-(3,4-dihydroxyphenyl) ethanol) as a natural additive (Martínez et al., 2018; Tedesco et al., 2022). HT is a phenolic compound naturally occurring in olive oil (Fiori et al., 2014) in the free and bound form (Fernández-Prior et al., 2022) and in wine (Boselli et al., 2006); it can be derived as a by-product from olive oil production or other plant sources (Dias et al., 2024). It is considered the second most powerful antioxidant among phenolic compounds, surpassed only by gallic acid (Martínez et al., 2018).

As already known in the literature, substances containing an ortho-diphenol (catechol), such as hydroxytyrosol, exert a strong antioxidant action. The scavenging activity of catechol was studied by Rietjens et al., 2007. Catechol was found to be a potent scavenger of $OH^{\cdot}$, $O_2^{\cdot-}$ and ONOOH. In addition, hydroxytyrosol has been shown to scavenge H_2O_2 and to possess superior antioxidant properties compared with other phenolic compounds, namely homovanillic alcohol and tyrosol. The relatively high antioxidant activity of catechol can be explained by the high electron donor effect of the second hydroxyl group.

The content of HT in the wine could be influenced by microbial activity during alcoholic fermentation since it is produced by some yeast strains from tyrosine via the Ehrlich pathway (Fernández-Prior et al., 2022). However, it has been demonstrated that the amount of HT in wine is generally quite low with concentrations in white wine between 1.72–1.92 mg/L (Boselli et al., 2006, M.I. Fernández-Mar et al., 2012) and in red wine between 3.66 and 4.20 mg/L (Fernández-Prior et al., 2022, M.I. Fernández-Mar et al., 2012). On the other hand, the applicability of HT in model wines as an antimicrobial is controversial (Medina-Martínez, María S., et al. 2016); in fact, its efficacy depends on the presence of specific microorganisms. To evaluate the antimicrobial capacity of plant extracts or their metabolites, it is necessary to consider factors such as culture media, strain types, and dosages ((Medina-Martínez, María S., et al. 2016)). An olive hydroxytyrosol-enriched extract, which was tested in model wine solution, showed similar antimicrobial activity as SO_2 for *Hanseniaspora uvarum, Candida stellata, Lactobacillus plantarum, Pediococcus damnosus* and *Acetobacter aceti*; higher for *Oenococcus oeni* and lower for *Dekkera bruxellensis* and *Botryotinia fuckeliana* (Tedesco et al., 2022). In the context of winemaking, the sensitivity of *O. oeni* to HT can be both advantageous and disadvantageous, depending on the goals of winemakers, during the malolactic fermentation (MLF) (Rapeanu et al., 2013; Rodríguez et al., 2009; Tedesco et al., 2022). Although positive results have been obtained with HT extracts in model wines, further experiments on real wines are needed to prove their effectiveness (Ruiz-Moreno et al., 2015; Tedesco et al., 2022). Ruiz-Moreno et al. (2015) suggested optimizing the ratio between SO_2 and HT combinations to maximize the inhibition of spoilage microorganisms in winemaking. Furthermore, the effects of synthetic HT and by-produced HT in a dioxide-free Syrah red wine and in a Sauvignon Blanc white wine as a replacement to SO_2 were studied (Raposo et al., 2016a; Raposo et al., 2016b). These authors (Raposo et al., 2016a; Raposo et al., 2016b; Ruiz-Moreno et al., 2015) agreed that HT alone is not sufficient to replace SO_2 in wines. However, their results (Raposo et al., 2016a; Raposo et al., 2016b; Ruiz-Moreno et al., 2015; Tedesco et al., 2022) promoted the reliability of using HT-enriched products that should be combined with other antioxidant additives during vinification, thus not excluding the combination of HT and a lower concentration of SO_2.

In this study, a red wine low in SO_2 (an organic wine) and HT-fortified was prepared and the effects of HT supplementation on wine storage in the bottle were compared with a control wine not fortified with HT. This study aimed to assess the use of HT as a supplementary antioxidant additive in red winemaking, in combination with a quantity of sulfur dioxide admitted for organic red wine. The impact of HT on

specific quality parameters (colour, oenological parameters, elemental composition, phenolic profile, and sensory profile) was discussed.

2. Materials and methods

2.1. Materials

All chemical reagents, solvents, and HPLC-phase organic modifiers used for the HPLC-MS analyses were at MS grade and were purchased from Merk Life Science S.r.l. (Milano, Italy). Ultrapure water was generated *in-house* using an Arium Mini generator (Sartorius Italy S.r.l., Varedo, Monza Brianza, Italy). Polyphenol HT-1®, a food-grade hydroxytyrosol (≥98 % purity, with a relative density of 1.15 kg/m^3 (65 % (w/v) HT solution)) used in this study was produced by Nova Mentis Ltd (Dublin, Ireland) using their proprietary biotechnological process.

2.2. Sampling and winemaking

Sangiovese grapes were harvested in the last week of September 2020, while Cabernet Sauvignon grapes were harvested in the first week of October 2020; all grapes were harvested in the Tuscany region (Italy). The grapes underwent destemming and crushing, followed by passage through a temperature exchanger, and finally deposited in cement tanks at approximately 15°C. In the same evening, selected yeast strains were inoculated (LIOZIMAS Natura Due, Pietraia di Cortona (AR), Italy). Over the ensuing days, fermentation started, lasting approximately 10 days within a temperature range of 25 to 28°C. During this phase, 2 to 3 daily pump-overs were performed. In the initial days, aeration of the grape must facilitated the initiation of fermentation. Then, the fermentation and maceration were carried out in a closed tank; the pomace hat was frequently wetted. After approximately 18 days, racking was conducted. The gently pressed wine was then subjected to natural malolactic fermentation. Following malolactic fermentation, the lees were removed, and later two distinct wines Sangiovese and Cabernet Sauvignon were blended. 50 mg/L of SO_2 (Esseco, Trecate (NO), Italy) was first added to the must on the day of harvest. Before bottling, the wine was formed by 85 % Sangiovese and 15 % Cabernet Sauvignon. The wine was matured in concrete tanks for six months before bottling. At bottling, polyphenol HT-1®, was added to 750-mL wine bottles in three different quantities (30, 60, 120 mg/750mL), and these were compared with a wine control sample that received no addition of HT (control). Since the bottles in the market have a volume of 750 mL we have reported the HT added amount *per* unit bottle. The conversion into mg/L is the following: 40, 80, 160 mg/L. For each sample, three replicates were produced and twelve bottles at each time storage point were analyzed. In total, sixty samples were studied across twelve months of bottle storage. At bottling, the free and titratable acidity of the wine were 0.48 g/L acetic acid and 5.05 g/L tartaric acid, respectively, and the free and total SO_2 were 17 and 61 mg/L, respectively. The alcohol content was 13 %vol and the total dry extract was 22.7 ± 0.1 g/L. The maximum amount of HT added to the wine was 160 mg/L, corresponding to 0.16 g/L, which is negligible (0.7 %) compared to the total dry extract.

2.3. LC-MS/MS analysis of polyphenols

The LC-MS analysis of polyphenols was conducted using a UHPLC-QqQ/MS instrument (Agilent LC/TQ 6465 system) equipped with a 1260 Infinity II UHPLC with a quaternary pumps system, a 1260 Infinity II WR PDA detector, in series to an AJS ESI QqQ mass analyzer. The method was adapted from a published report (Poggesi et al., 2022). The separation of the compounds was performed using a Poroshell 120, SB-C18 2.1 mm × 100 mm × 2.7 μm (Agilent Technologies Italia, Milan, Italy) kept at 30°C. The mobile phase was B (0.1 % [*v/v*] formic acid in acetonitrile LC-MS analytical grade) and A (0.1 % [*v/v*] formic acid in degassed ultrapure water). The gradient elution 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 %B), 25–28 min (5 %B) with a flow rate 0.35 mL/min. 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, nebuliser pressure = 50 psi, sheath gas heater = 350 °C, sheath gas flow = 12 L/min, capillary voltage = −3500 V, nozzle voltage = −500 V.

MS raw data were converted into mzData format with MassHunter (Agilent) and exported. The MzMine3 (http://mzmine.github.io/) application was employed for automatic alignment and pre-processing before statistical analysis. PDA retention times were corrected to match the MS retention times. The workflow applied to obtain the dataset of phenols was adapted by Poggesi et al. 2022. The HT added to the wines was quantified using the analytical standard of HT (3-hydroxytyrosol, CAS RN: 10597-60-1, purity > 98.0 %) provided by TCI Europe. The calibration curves were built at each storage time to quantify the HT added in the wines (0.5–200 mg/L) and the quantification (Supplementary Materials, S1) was done using a PDA detector (wavelength = 280 nm). The results are expressed as regression curves built on an artificial wine (Boselli et al., 2006) from T1 to T12 (S2).

The following parameters were used for targeted MS/MS fragmentation experiments (product ions monitoring, or PRM) on specific ions: source parameters from the MS^1 analysis, acquisition mass range from m/z 25 to + 10 m/z from the selected precursor ion, scan time of 125 ms, fragmentor potential of 135 V, collision energy of 25 V, and cell accelerator of 5 V (Darnal et al., 2024).

2.4. Determination of elements in wine

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

2.5. CIELab colorimetric analysis

Colour parameters C* (chroma, relative saturation), L* (lightness), a* (red to green), b* (blue to yellow) (CIE, 1986) were recorded using a reflectance spectrophotometer Minolta CR-400 Chroma Meter (Minolta Corp., Osaka, Japan) (Darnal et al., 2023a). The instrument was calibrated over the provided white ceramic plate (S4).

2.6. Determination of basic enological parameters

Basic enological parameters were determined on a MIURA One automatic analyser (Exacta+Optech Labcenter SpA, San Prospero, Modena, Italy) for L-tartaric acid, L-malic acid, L-lactic acid, glucose and fructose, total sulfur dioxide, free sulfur dioxide, total polyphenols. Each parameter has been calibrated on the relative reference standard provided by the supplier. 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 29971-72 separated piercing head with ~ 1.5 bar N_2 flow and a GA2 × 00 O2 EC sensor ((Darnal et al., 2023b), S4). Total dry extract was determined experimentally using official method of the distillation (Dualstill, Exacta+Optech Labcenter SpA, San Prospero, Modena, Italy) and hydrostatic balance (Exacta+Optech Labcenter SpA, San Prospero, Modena, Italy) according

to Compendium of International Methods of Wine and Must Analysis (OIV-MA-AS2-03A and OIV-MA-AS2-03).

2.7. Sensory Analysis

For the sensory analysis, two steps (rapid training, and CATA (Check-All-That-Apply) combined with Projective Mapping test) each consisting of two training sessions (1 h each), and two CATA + Projective Mapping sessions were performed (Pineau et al., 2022). Cysensy–an SQL binding sensory analysis web software developed in collaboration with the Computer Science Faculty of the Free University of Bozen/Bolzano was used during the training (Poggesi et al., 2022), while the Projective Mapping was recorded using a sheet of white paper. These two sensory approaches were applied to obtain a rapid categorization and characterization of the product (namely, control, 30, 60, 120 mg HT in 750 mL wine) using an internal panel formed by twelve assessors (5 females and 7 males aged between 25-40 years old). Each sample was prepared in triplicate, a total of twelve wine samples were assessed by each panelist in two different sessions (6 bottles per session) on two successive days. Panelists were trained with reference standards (Noble et al., 1987) or natural standards, if references were not available (Darnal et al., 2024) (as shown in Table 1. The definition of each sensory descriptor is given in Table S3.

The wines were evaluated in an individual spot in a temperature controlled room (23°C). Panelists were instructed to to expectorate the sample wines after tasting. Water and unsalted crackers were provided for palate cleansing. Wines were served in a randomized Latin Square incomplete block design to control for possible carry-over effects (Pineau et al., 2022). The samples were labelled with random 3-digit codes, and they were served into ISO glasses (ISO 3591:1977). The panel was given a sheet of white paper (the Napping paper, size 52 × 35 cm). The assessors were asked to place the wine samples according to their perceived sensory affinity. Then, the Projective Mapping protocol was combined with a CATA method (Esmerino et al., 2017; Pineau et al., 2022). The CATA method was applied to indicate the frequency at which each descriptor was perceived by the panelists (Esmerino et al., 2017). To facilitate the task, the possible sensory attributes for the blend of Sangiovese and Cabernet Sauvignon were listed from the scientific literature (Canuti et al., 2020; Pagliarini et al., 2013; Rinaldi et al., 2020) and provided to the assessors for their evaluation in Borrigiano control wine and experimental wines. In this way, the panel checked the generated sensory attributes that could be assigned to each wine sample (S5). After testing each sample, panelists marked the position of each sample on the Napping paper with an "X" and labelled the "X" with the sample code. The X and Y coordinates of each sample on each Napping sheet were measured to the nearest centimeter from the lower left corner of the sheet. Qualitative terms from CATA were recorded for each sample and used as a frequency of each attribute. The overall preferences were expressed as a ranking list (the three most preferred samples) from each panellist and written as a frequency table. The sensory "Overall Preference" was assessed by asking the panellists to rank the samples according to their personal preferences.

Table 1
Sensory attributes used for panel training up to twelve months.

Attribute names	Group	Reference standard
Bitterness	Taste	0-1-2 g/L caffeine in water
Sourness	Taste	1 g/L tartaric acid in water 1 g/L lactic acid in water 1 g/L malic acid in water
Warmness	Taste	8 – 12 – 14 % of alcohol in water
Saltiness	Taste	0 – 0.5 – 1 g/L sodium chloride in water
Astringent	Taste	0.5 – 1- 2 g/L alum in water
Woody	Aroma & Flavour	Fresh wood 30 pieces of wood in 500 mL of red wine 2 spoons of burned coffee in water and heating up to 40°C for 20 min
Spices	Aroma & Flavour	20 pieces of cloves in 500 mL of red wine 7g black pepper in 500 mL of red wine 7 candies in 500 mL of red wine
Red fruit	Aroma & Flavour	Syrup cherry 7 pieces in 500 mL of red wine Crushed fresh or frozen strawberries in 500 mL of red wine
Vegetative	Aroma & Flavour	Half green bell pepper in 500 mL of red wine
Dried fruit	Aroma	No. 5 spoons of strawberry jam in 500 mL of red wine

2.8. Statistical analysis

All the statistical analysis and the related graphics were performed on R software version 4.2.2 using the packages "FactoMineR", "factoextra", "agricolae", and "ggplot" (Kassambara and Mundt, 2022; Mendiburu and Yaseen, 2020; Wickham, 2016; Lê et al., 2008). To explore the relationship between experimental variables and factors, Multiple Factor Analysis (MFA) was applied to the raw data (sensory and chemical datasets) as an explorative tool (Darnal et al., 2024; Poggesi et al., 2022).

The impact of HT (abbrev. HT_meas) on the chemical profile in the studied red organic wine was evaluated by calculating the correlations between HT_meas and all other variables observed in the loading plots. The variables were discussed only if validated by significance test (Pearson's product moment correlation coefficient). Correlation test (Pearson's product-moment correlation) was applied using cor.test() function on "stats" package to assess the significance between HT_meas (HT measured) and all the other variables (sensory and chemical datasets, S8) (Best and Roberts, 1975).

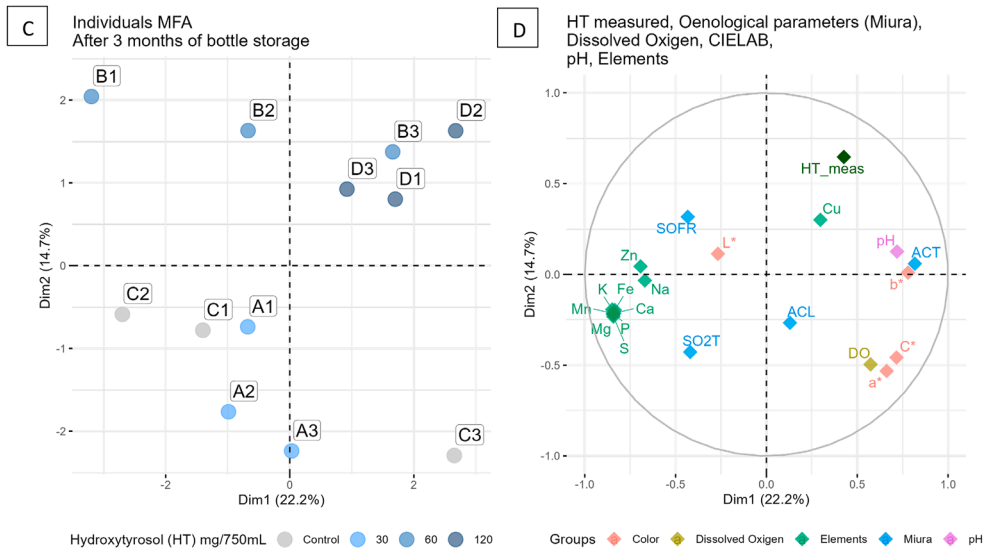
Regarding the chemical composition, the statistical impact of the temporal evolution and concentration factors and second-order interaction term (time*concentration) was assessed using 2-way ANOVA by Tukey's HSD post-hoc ($\alpha = 0.05$). Significance was represented by groups and indicated by alphabetical letters. The ANOVA was separately applied on each dataset 5 different datasets (polyphenol profile, oenological parameters, dissolved oxygen (DO mg/L), colour parameters (CIELAB), pH, and elements) (S6).

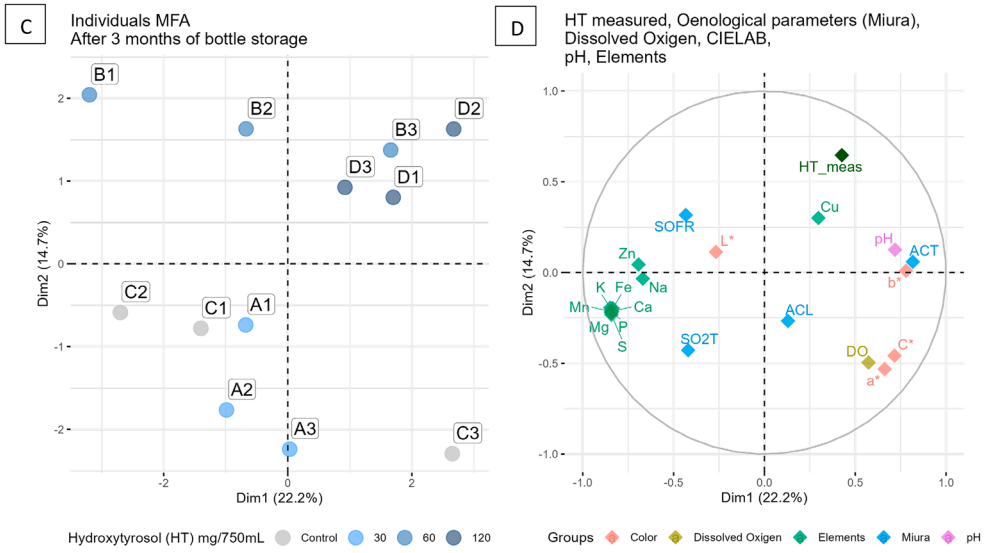
Regarding the sensory analysis, the projective mapping data were reported as two variables: x and y coordinates that expressed the participants' identification of sample locations on the paper. Additionally, the CATA results, as frequency table, were organized as groups (aroma, taste, flavour, Table 1, S3). The CATA frequency table was constructed by collecting the sum of all attributes for each sample (control, 30, 60, 120 mg/750mL) checked by the whole panel at each given time. So, each sensory attribute was represented as the frequency of a specific attribute. The panelists received the different wine samples in triplicate. Projective mapping data and CATA results were included in the MFA analysis (Heymann et al., 2014; Smith and McSweeney, 2019). The 2-way ANOVA by Tukey's HSD post-hoc ($\alpha = 0.05$) was also used to evaluate the concentration and time factors and their interactions in the CATA dataset (S7).

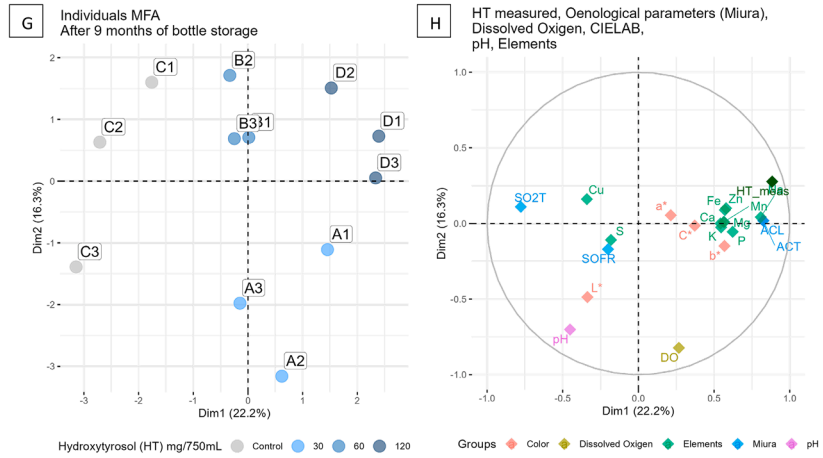
3. Results

3.1. Evolution of the quality parameters at each bottle storage time

MFA was applied as an explorative tool to analyze 5 different datasets per bottle storage time, and to evaluate possible trends between variables and samples. In details, oenological parameters, dissolved oxygen (DO mg/L), color parameters (CIELAB), pH, elemental composition, and polyphenol profile datasets were subjected to MFA. HT_meas was projected to MFA as supplementary variables (deep green), so that their effect did not affect the other variables (Fig. 1 C, D, E, F, G, H). The correlation between HT_meas and variables was discussed only if validated by a significance test (Pearson's product moment correlation







(caption on next page)

Fig. 1. MFA for the chemical dataset. (C, D) show the individual and variable plots after 3 months of bottle storage for the first 2 components and the different datasets used in the computation, (E, F) show the individual and variable plots after 6 months of bottle storage for the first 2 components and the different datasets used in the computation, (G, H) show the individual and variable plots after 9 months of bottle storage for the first 2 components and the different datasets used in the computation. The MFA plots showing the individual and variable plots after 1 month (A, B) and after 12 months (I, J) are reported in S9. A= wine samples added with 30 mg/750mL; B= 60mg/750mL; C= Control (no addition); D= 120mg/750mL. A1-A2-A3, B1-B2-B3, C1-C2-C3, D1-D2-D3 = replicates (different bottles). ACT = tartaric acid (g/L), ACL = acetic acid (g/L), SOFR = free sulfur dioxide, (mg/L), SO_2T = total sulfur dioxide (mg/L), L* = lightness, a* = redness, b* = yellowness, C* = Chroma, pH = pH, DO = dissolved oxygen (mg/L), HT_meas = HT quantified (mg/L) using PDA detector (wavelength = 280 nm), (30 (steelblue1), 60 (steelblue3), 120 (steelblue4) mg/750mL) and control (grey). Na, Mn, Fe, Cu, Zn (μg/L), Mg, Si, P, S, K, Ca (mg/L) = elements quantified using a calibration curve.

coefficient, S8). The MFAs were only reported if a sample grouping according to the HT addition was detectable (T3, T6, and T9). The other MFAs (T1 and 12) were saved in S9 as Figures A, B, and Figures I, J, respectively.

After three months of storage (T3), the highest and medium HT contents (120 and 60 mg/750mL) were grouped showing positive values on Dim.2. On the contrary, the wine with the lowest HT contents (30 mg/750mL) and controls were grouped on Dim.2 showing negative scores on the MFA (Fig. 1 C, D). Furthermore, at T3, control and 30 mg/750mL were higher in C* and a* (redness), contrary to what was found for 120 mg/750 mL and 60 mg/750mL samples, thus suggesting that these wines were redder and the intensity of colour was higher than the other samples.

After six months of storage (T6) (Fig. 1 E, F), the samples enriched with 60 mg/750 mL were poorly separated from the others and these samples showed negative values on the Dim.1. At T6, the separation between groups of samples was mostly attributed to colour parameters and DO. At T6, ACL was lower in 120 mg/750 mL samples than in the other samples (S6). The HT_meas variable was negatively and significantly correlated with the content of ACL ($R^2 = 0.81$; p-value = 0.0009) (Fig. 2 and S8), thus suggesting that the HT might preserve wines from oxidation. Indeed, Fig. 2 shows that the value of ACL (g/L) decreased as HT (mg/L) added increased.

The samples were well grouped according to the treatment of HT addition after nine months (T9) (Fig. 1 G, H). The separation between samples was mainly attributed to enological parameters, in detail SO_2T and SOFR showed negative values on the Dim1 and a strong opposite trend related to ACL and ACT. The latter showed positive values on Dim1. The DO was lower in 60 mg/750mL samples contrary to what was found at T1, thus suggesting that this sample might be less oxidized (S9). Additionally, ACL was higher in 120 mg/750mL samples, contrary to what found at T6 (S6), thus suggesting that HT could help in preserving the quality of wines until six months of bottle storage. Indeed, significant positive correlations between HT_meas and ACT, and ACL were found at T9. A significant inverse correlation was found between HT_meas and SO_2T.

The elemental composition of wine (Na, Mn, Fe, Zn, Mg, Si, P, K, Ca, and S) showed inconsistent results: their concentration did not correlate with HT concentration. On the contrary, Cu concentration showed a low level of positive correlation with HT ($R^2 = 0.41$, p-value = 0.009), with higher [Cu] in wines with 120mg/750mL and lower levels in control wines up to T3 (S12). Moreover, while Na, Si, P, S, Cu, and S concentrations significantly decreased, Mg, Mn, Fe, Ca, K, and Zn concentrations remained constant over time of storage (S12).

3.2. *Evolution of the HT across twelve months of bottle storage*

The Tukey's HSD post-hoc (α = 0.05) conducted for 2-way ANOVA (time*concentration) on HT (HT_meas) of the stored samples, expressed in terms of percentage (%) with respect of the initial HT content, highlighted significant interactions between the content of quantified HT and aging process (Table 2). The whole result is reported in S6. The significant results of Tukey's HSD post-hoc, expressed as letters, (Table 2) evidenced the statistically significant impact in terms of interaction effect on HT (%) in the wine samples (HT_meas) between storage time after twelve months and the HT added at bottling (30, 60,

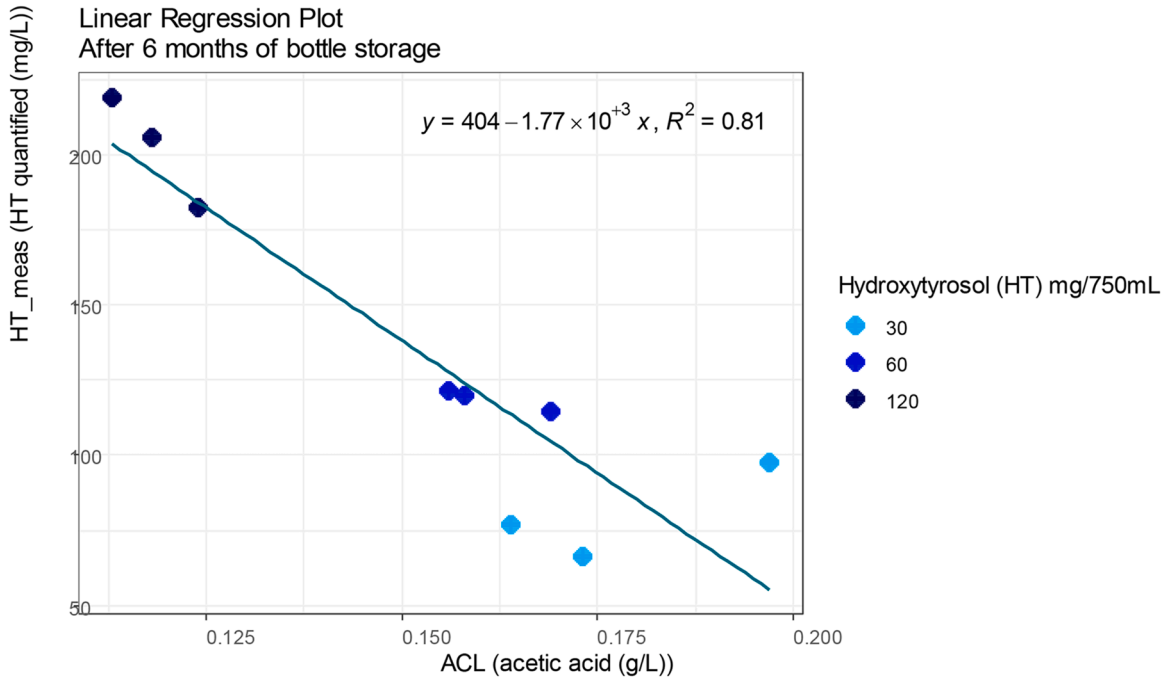


Fig. 2. Correlation between HT_meas = HT quantified (mg/L) using PDA detector (wavelength = 280 nm) and ACL = acetic acid (g/L) at T6. The other correlation plots were saved in S11. The control samples have been excluded from the regression curve since the focus was the relationship between HT_meas and ACL. The regression model is shown including the equation and coefficient of determination (R^2).

Table 2
Ratio of HT measured in wine compared to HT added in the bottle (in percentage). Significant differences are reported as letters according to 2-way ANOVA using the Tukey's HSD post-hoc (α = 0.05). The whole results are reported in S6.

	T1	T3	T6	T9	T12
Control	100 % a	63 % ab	70 % ab	64 % ab	56 % b
30mg/750mL	100 % a	99 % a	87 % ab	70 % ab	65 % ab
60mg/750mL	100 % a	98 % ab	87 % ab	69 % ab	69 % ab
120mg/750mL	100 % a	102 % a	83 % ab	73 % ab	66 % ab

120 mg/750mL).

Noteworthy, only the control sample, which represents the natural amount present in the wines under investigation, showed a significant decrease between T1 and T12. The initial values of the HT in the samples enriched at different levels of HT slowly decreased across twelve months, but no statistical significance were found. It was interesting to observe that endogenous HT decreased over the twelve months, unlike exogenous HT which remained stable.

However, an in-depth study was conducted on the major oxidation product of HT. HT has been reported to autoxidize to *o*-quinone that in water environment undergoes coupling with a second *o*-quinone leading to the formation of dimers (Zafra-Gómez et al., 2011). A tentative identification by MS/MS experiments was conducted on the peak at *m/z* 303 (rt = 12.3 min), which was tentatively identified as a dimer of quinone-quinone (Di Maio et al., 2011). A statistical negative correlation between this compound and HT_meas variable was found at T1 (*p*-value = 0.047, R^2 = 0.42, S8) and a negative correlation at T3 (*p*-value = 0.005, R^2 = 0.22, S8). Its identification was tentatively confirmed in negative ionization mode, producing ions at *m/z* 284, 272, 151, 122 and 121 and it was compared with the literature (Di Maio et al., 2011).

3.3. Evolution of the sensory attributes at each bottle storage time

MFA is widely used to generate a map that is a visual representation of how wines are positioned, based on specific data attributes, by the panel (Barton et al., 2020; Heymann et al., 2014; Llobell et al., 2020; Smith and McSweeney, 2019). The panel used for our study consisted of twelve panelists at each time session. The projective mapping data, reported as x and y coordinates, and CATA results, reported as a frequency of each attribute checked by the panel at each storage time, were submitted to MFA. MFA (Fig. 3 C, D, G, H) reported the correlation between samples and descriptors at each time of storage. MFA was applied as an explorative method to assess associations between sensory data (aroma, flavour, taste (Table 1)) and samples. The correlation between HT_meas and variables, and overall preference and variables were discussed only if validated by significance test (Pearson's product moment correlation coefficient, S8). The MFAs were only reported if a sample grouping according to the HT addition was detectable (T3 and T9), contrary, the other MFAs (T1, T6, T12) were saved in S10, namely Figure A, B, E, F, I, J.

At T3 (Fig. 3 C, D), the panel tried to group the wine samples according to HT levels. This grouping provided by the panel could be linked to specific attributes. In detail, "red fruits", "spicy" sensory features showed positive values on Dim1 dimension, on the opposite side, there were "woody" and "vegetative" attributes. Additionally, "warmness", "saltiness", and "sourness" were positively associated with Dim.1, while "bitterness" and "astringency" were negatively correlated with Dim1. The HT_meas was negatively correlated with the "red fruits" aroma and statistically significant (*p*-value = 0.0022, R^2 = 0.62, S8).

On the contrary, at T9 (Fig. 3 G, H), samples enriched with 120 mg/750mL were tentatively clustered. In detail, "red fruits" sensory features showed positive values on Dim1, on the opposite side, there was "vegetative" attributes that was negatively associated with the Dim1. These results agree with those found at T3, suggesting that the panel tended to separate the sample based on the attributes "red fruit" and "vegetative". At T9, HT_meas was sightly significantly and positively correlated with "warmness" (*p*-value = 0.049, S8) and again negatively with "red fruit" flavour (*p*-value = 0.034, S8). So, the panel detected differences in the sensory characteristics of these samples. In contrast, the other samples were not recognized as different and were juxtaposed with each other by the panelist.

The overall preference was negatively correlated with "vegetative" aroma and statistically significant only at T9 (*p*-value = 0.037, S8). "Sourness" and "vegetative" flavour (*p*-value = 0.013, *p*-value = 0.034, respectively, S8)

Furthermore, the significance (2-way ANOVA, *p*-value < 0.05) was calculated across time, HT concentrations, and interactions (time*-concentration). Interaction results (time*concentration) were expressed as LS mean and the corresponding significant differences (represented by letters). The complete results have been saved in S7. In details, "red fruit" (*p*-value = 0.027, S7), "spicy" (*p*-value = 0.023, S7) aroma, and astringency (*p*-value = 0.0041, S7) resulted to be statistically significant for the interaction factor (time*concentration). In addition, the 120mg/750mL sample was tentatively grouped by the panel with respect to the other samples at T9. Therefore, the time evolution of these attributes that were statistically significant in this sample enriched with the highest content of HT for the interaction factor (time*concentration, 2-way ANOVA, *p*-value < 0.05) was displayed in (Fig. 4).

Fig. 4 shows the time evolution in the perception of significant sensory attributes in the 120mg/750mL sample for the interaction factor (time*concentration). The "red fruit" aroma increased after three months of storage, and then it seemed to be more stable from T6 to T12. The maximum level in terms of perception of astringency was perceived by the panel at T12. On the contrary, the "spicy" aroma remained stable across twelve months without any statistical differences. Thus, the different perceptions of the attributes "red fruits" and astringency at five bottle storage points could be responsible for the grouping of 120 mg/750 mL at T9.

During the prolonged storage (Fig. 5), an evolution in the trend related to the overall preference expressed by the panel was observed. In detail, at T1 and T3 the control samples were preferred by the panel, while the 120mg/750 mL was preferred at T6. At T9, the preference was for 60 mg/750 mL and at T12 for control and 30 mg/750 mL.

4. Discussion

The objective of this study was to assess whether the addition of a biotechnologically produced HT could influence the chemical and sensory profile of an organic wine to which a low sulphur dioxide content had been added.

Measured HT **(**HT_meas**)**: Our study evaluated the interaction factor of the variable HT_meas with time evolution and the different concentrations added. The initial values of endogenous HT, which reflected the natural presence of HT in these wines, changed quite rapidly over twelve months. On the contrary, the content of exogenous HT, added at three different concentrations, did not change. The evolution of natural polyphenols during aging in the bottles has been already demonstrated and it depends on the variety under investigation (Monagas et al., 2005). Furthermore, Di Maio et al., 2011 (Di Maio et al., 2011) reported that quinones, dimers, and acids, which are reported as oxidation products of HT, were identified in virgin olive oil during the initial stage of autoxidation process. On the other hand, fresh olive oil did not contain these oxidized chemicals, which could be created by the endogenous activity of polyphenol oxidases during malaxation (Di Maio et al., 2011). Our findings demonstrated that the amount of added HT in wine was stable across twelve months, while the endogenous HT might evolve into other polyphenolic forms such as dimers (Di Maio et al., 2011). Significant correlations were found between HT_meas and the molecular species with M^- = *m/z* 303 (V135_303, S8) up to T3 (*p*-value = 0.005, R^2 = 0.22, S8), while no significant correlations were found at all other time points.

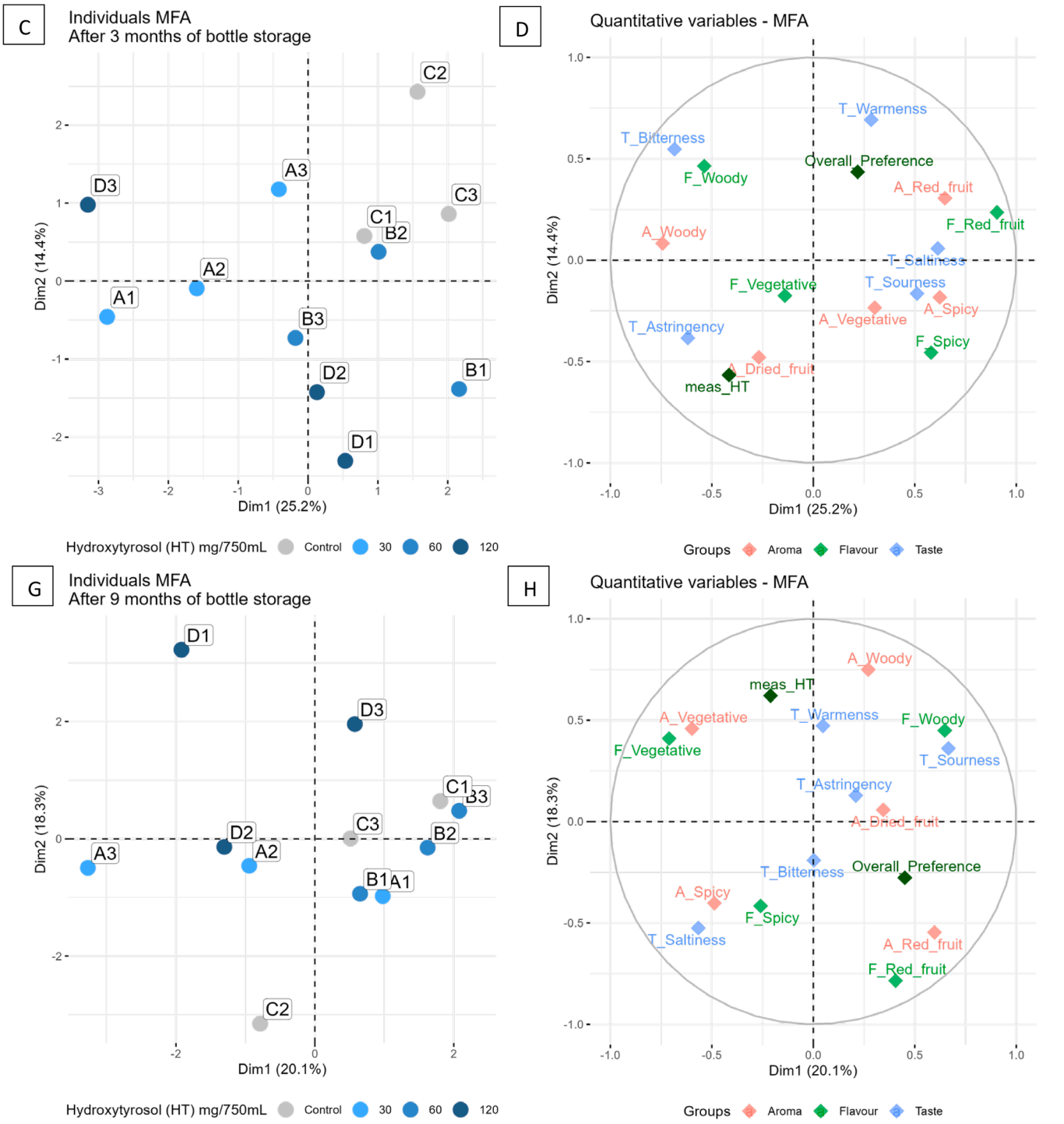


Fig. 3. MFA for the sensory dataset. (C, D) show the individual and variable plots after 3 months of bottle storage for the first 2 components and the different datasets used in the computation, (G, H) show the individual and variable plots after 9 months of bottle storage for the first 2 components and the different datasets used in the computation. The MFA plots showing the individual and variable plots after 1 month (A, B), after 6 months (E, F), and after 12 months (I, J) are reported in S10. A= wine samples added with 30 mg/750mL; B= 60mg/750mL; C= Control (no addition); D= 120mg/750mL. A1-A2-A3, B1-B2-B3, C1-C2-C3, D1-D2-D3 = replicates (different bottles). A = Aroma, T = Taste, F = flavour, HT_meas = HT quantified (mg/L) using PDA detector (wavelength = 280 nm), (30 (steelblue1), 60 (steelblue3), 120 (steelblue4) mg/750mL) and control (grey), Overall_Preference = expressed by the panel composed by twelve panelists.

HT might generate as oxidation product the dimer up to T3, and then start reacting with other molecules after three months. For example, 3, 4-dihydroxyphenylacetic acid, which was reported (Di Maio et al., 2011) as an autoxidation product of HT, was not found in our samples. Studies on wine matrices are lacking in the scientific literature, so the present results provide the first evidence that the HT oxidation products might be found also in wine. In wine, HT is produced by yeast strains from tyrosine during alcoholic fermentation (Álvarez-Fernández et al., 2018); however, no further information regarding the oxidation products of HT in wines during bottle storage was found in the literature. To date, the average contents of HT are as follows: 629.1, 5.2 and 2.1 μg/g for olives, olive oil and wine, respectively (Gallardo-Fernández et al., 2022). Our findings agreed with the literature since the natural amount of HT in the control wines ranged from 1.09 to 6.10 μg/mL (1.22 to 6.78 μg/g,

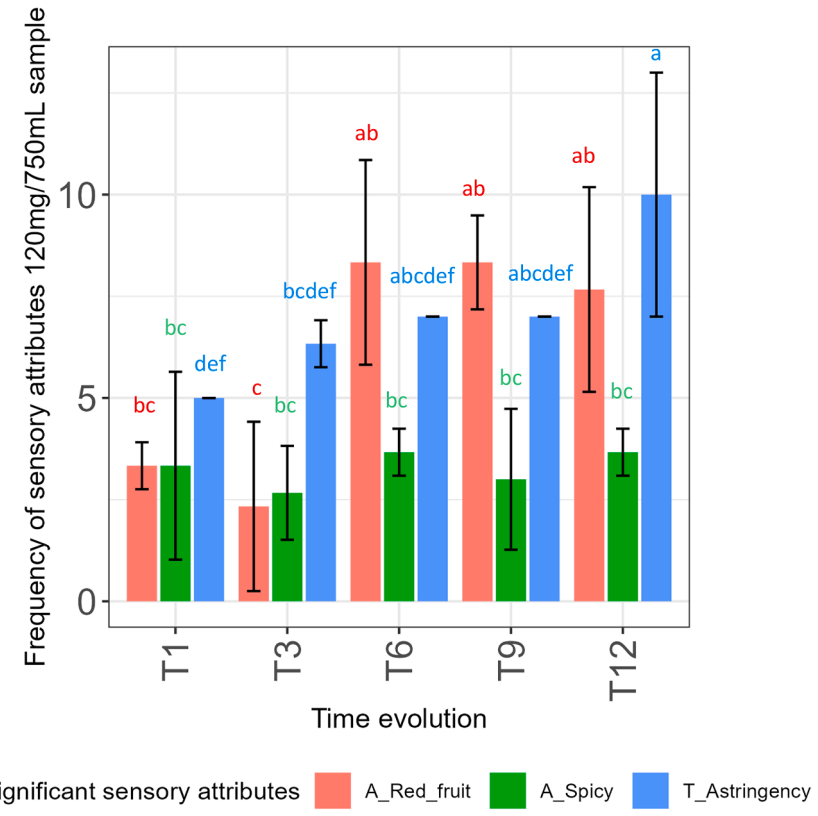


Fig. 4. Results from 2-way ANOVA by Tukey's HSD post-hoc (α = 0.05). A = Aroma, T = Taste, F = flavour. Only 120mg/750mL sample and the time evolution of sensory attributes statistically significant for the interaction factor (time*concentration) are displayed. The whole results are reported in S7. Three replicates for each sample were analyzed and the standard deviations are represented by error bars.

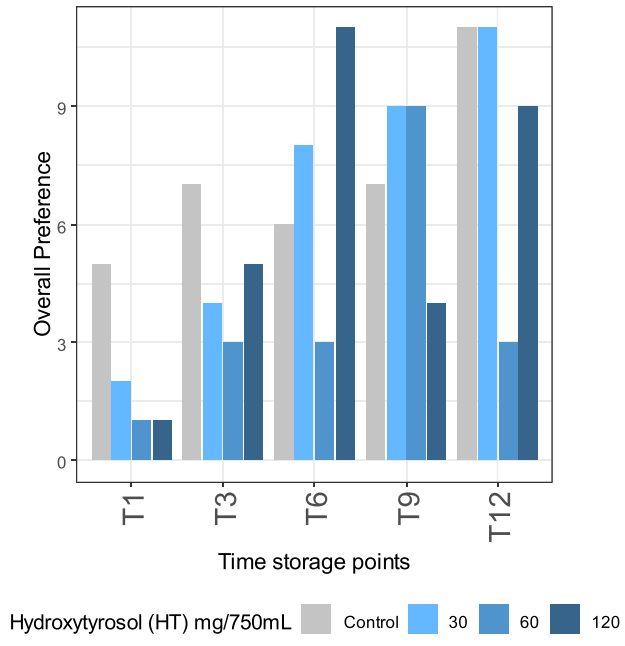


Fig. 5. The overall preference expressed by the assessors on 12 wine samples across 5 time points. The bars represent the number of times a specific sample was preferred (first preference) by the panel to construct a ranking at each time point of storage (T1, T3, T6, T9, T12).

respectively) across twelve months (S1).

Total and free SO_2 (SO_2T and SOFR). Monitoring both total and free SO_2 levels in wine plays an important role in evaluating wine storage and processing to prevent potential chemical and microbiological threats. The addition of HT to this organic wine did not influence the free and the total SO_2 content between treatments, as already reported (Raposo et al., 2016a; Raposo et al., 2016b). Conversely, in terms of time evolution, free SO_2 showed a significant increase during the first six months of bottle storage (52 %). Following this initial period, its content decreased by 28% for the subsequent six months independently of the treatment.

Dissolved oxygen (DO): Dimkou et al. (2013) showed that DO started to decrease immediately after bottling; the same authors reported that however higher DO treatments showed an increment in the first week of storage, followed by a decrement in the second week of storage. This might occur because oxygen could initially move from the wine into the headspace, driven by the variance in oxygen partial pressure between the liquid and gas phases (Dimkou et al., 2013). The higher the initial DO the higher is this movement during wine bottle storage conditions (Dimkou et al., 2013). However, our results showed that the DO content was not affected by the time evolution and by the content of HT added, indeed, no statistical differences in DO between wines enriched with HT and control samples were found (S6). Probably the cork stoppers used for the wine were of high quality.

Color parameters: The color of red wine is a crucial quality parameter, contributing a substantial influence on sensory assessment. Typically, it constitutes the initial attribute perceived, thus assuming an essential role in consumers' decision-making. No statistical differences in color parameters between wines enriched with HT and control samples were found, thus suggesting that the color parameters were not influenced by the addition of HT (S6). Only the time evolution affected the color parameters in the organic red wine studied as already reported (Raposo et al., 2016a; Raposo et al., 2016b). Regarding the bottle storage, lightness (L*) showed a decrease of 11 % from T1 to T12, while a*, b*, and C* increased by 21 %, 26 %, and 10 % from T1 to T12,

respectively, therefore the wines were more red and more yellow. Instead, Raposo et al., 2016a reported that the addition of HT, in combination with SO_2, could contribute to the preservation of polyphenols, thereby slowing down the color evolution in wines.

Acetic acid (ACL). ACL is associated with oxidation problems in wine, as both result from overexposure to oxygen and/or lack of sulfur dioxide management. Acetic acid bacteria need oxygen to grow and proliferate (Robles et al., 2019). Like many other microorganisms related to wine production, acetic acid bacteria can be managed with proper sulfur dioxide treatment. In the case of the red wine used in this experiment, the natural amount of HT (present in the control wine) ranged from 1.09 to 6.10 μg/mL. When the HT was added to wine at T6, a negative correlation between HT_meas and ACL was found (p-value = 0.001, $R^2 = 0.81$, S8), thus suggesting that HT could slow wine oxidation problems for up to six months of bottle storage. However, the ACL content increased 5-fold from the initial value from T1 to T12. Remarkably, the ACL content started to increase after six months of bottle storage.

Elemental composition: The elemental composition of wines is known to vary according to vinification and aging processes (Castiñeira Gómez et al., 2004). In the present study, Na, Mg, Si, P, S, K, Ca, Mn, Fe, and Zn, (S6) concentration did not show any statistically significant difference between treatments, i.e. addition of HT, as previously reported (Raposo et al., 2016a; Raposo et al., 2016b). Interestingly though, Na, Si, P, S and Cu content decreased over time of storage probably due to precipitation (Suhaj and Koreovská, 2005). In addition, our findings showed a positive correlation between Cu and HT_meas. These results suggest that the addition of HT enhances the stability of Cu in solution, i.e. wine, especially in the first months of storage. Consequently, the decrease, i.e. degradation, of HT during storage induces a precipitation of Cu. Considering the molar ratio of the Cu-HT complex and that the molecular Cu-HT ratio in the complex is suggested to be ½ or ¼ (Perta et al., 2023), it appears that there is an excess of HT. Hence, at the beginning of the storage period it is reasonable to think that all Cu in wine is complexed by HT. These findings are in agreement with literature showing that HT can chelate and increase the stability of metal ions, such as Fe and Cu, in olive oil and or oil-in-water emulsions (Zafra-Gómez et al., 2011). To our knowledge, literature on wine matrices is still missing, thus the present results provide the first pieces of evidence that Cu complexation by HT occurs also in wine. It was noteworthy to mention that a decrease in copper concentration from T1 to T12 was observed in the current study.

According to the OIV standards (Gajek et al., 2021) for Cu, as shown in Table S4, all analysed wine samples were within acceptable limits. Cu levels ranged from 1.74 to 83.92 μg/L, consistent with findings from another study (Gajek et al., 2021). In the past, Cu in wine primarily originated from the Bordeaux mixture, a fungicide containing copper sulfate ($CuSO_4$) and calcium hydroxide ($Ca(OH)_2$), used in vineyards to combat fungal diseases. However, due to potential soil accumulation, the European Community has banned its use (Gajek et al., 2021). Moreover, Cu is added to wine to counteract odorous organic sulphur compounds formed during fermentation and aging. Excessive Cu concentrations exceeding 1 mg/L can impart a metallic bitter taste and cause turbidity (Gajek et al., 2021). Additionally, elevated levels of Cu can pose significant health risks (Brewer, 2010). Hence, Cu levels are typically maintained below 0.5 mg/L during wine production (Gajek et al., 2021).

Sensory profile: In general, except for specific cases, the addition of HT did not significantly change the sensory profile of the wines, although trends can be identified (S7). The samples fortified with the highest content of HT were tentatively grouped at T9. It is worth mentioning that the attribute "red fruits" was negatively correlated with HT_meas at both T3 and T9. Furthermore, “red fruit”, “spicy” aroma, and astringency were significant for the interaction factor (time*-concentration) (Fig. 4). At T3 and T9, the 120mg/750mL were higher in astringency and higher in “red fruit” aroma.

All these findings agreed with the results found by Sáenz-Navajas et al. (2014). The increase of “red fruits” aroma was attributed to the formation of branched ethyl esters during wine aging (Sáenz-Navajas et al., 2014). The increase in the perception of “astringency” by the panel could be linked to an oxidative process in wine after twelve months of bottle storage. Indeed, the ACL and yellowness (b*) increased significantly (5-fold and 0.26-fold from the initial value, respectively) from T1 to T12. Additionally, ACL was higher in 120mg/750mL sample than the control samples at T9. Increased oxidation can also affect the perception of tannins, making them more noticeable and accentuating the sensation of astringency (Ferrer-Gallego et al., 2016). Therefore, the aging of wine in bottles rather than the addition of HT was more closely associated with the change in the perception of sensory attributes (Sáenz-Navajas et al., 2014).

In general, the panel grouped the wine samples according to the presence or absence of red or non-red fruit sensory attributes (e.g., "vegetative" attributes) over twelve months rather than the presence of HT. However, our results were consistent with the literature in that the panel was able to identify and characterize the wines by “red fruit” and “spicy” attributes, usually separating them from samples characterized by “woody” and “vegetative” attributes (Smith and McSweeney, 2019).

5. Conclusions

In conclusion, our study shows that the enrichment of organic wine with HT exerted an additional antioxidant effect during the first six months of storage, based on the markers that were analyzed, such as acetic acid (ACL). In detail, ACL was found to be inversely correlated to the HT addition at T6, so, the enrichment of wine with HT has preserved the wine from oxidation until six months of bottle storage. Storage time had a significant impact on color parameters, with a decrease of 11% in lightness (L*) and increases of 21% in a* (red/blue), 26% in b* (yellow/blue), and 10% in C* (chroma). At T6, wines spiked with 120 mg/750 mL of HT were preferred by the panel. After 9 months of bottle storage, wines spiked with 120 mg/750 mL of HT were precepted as more complex (more astringent and lower in red fruit aromas), however, the sensory overall preference changed. Indeed, wines spiked with 120 mg/750 mL of HT showed the lowest sensory overall preference at T9, whereas wine samples spiked with 30 mg/750 mL of HT were appreciated at T9 and T12.

The interest of researchers, oenologists, markets, and consumers in producing wines with less sulfur dioxide or even without it is increasing. Indeed, reducing the amount of sulfur dioxide can result in better wines that can be consumed even by people who are allergic or intolerant to sulfur dioxide. The utilization of compounds, such as HT, that can improve wine quality and are recognized as beneficial to human health is an area of growing interest and evaluation by the scientific community.

The well-known health properties of HT, combined with its ability to act synergistically with sulfur dioxide in terms of antioxidant action, make it a promising candidate for enhancing food preservation, winemaking, and other industrial processes where sulfur dioxide is traditionally used. However, several areas of concern need to be addressed in future research to ensure HTs widespread and safe application. The interaction of HT with other food ingredients, preservatives, and additives must be explored. Public awareness campaigns and education about the benefits and safety of HT can help in gaining consumer trust and acceptance. Moreover, regulatory frameworks for the use of HT as a supplement to sulfur dioxide are not yet well-established. It is important to work with regulatory bodies to develop clear guidelines and obtain approvals to extend for its use to other food sectors.

Declaration

Ethical statement

The panellists were recruited on a voluntary basis and they were asked to sign an informed consent form, in which they were informed about the presence of sulphite in the sample wines to avoid any related issues. An affirmative reply was required to enter the survey. The maximum quantity of wine per glass per session was 30 mL per glass per session and the wines contained approximately 13 % v/v ethanol (3.9 g of ethanol per 30 mL sample). The study was conducted following the Declaration of Helsinki (World Medical Association, 2013). There was no cost to participate, refusal to participate did not imply any penalties or loss of benefits, and participants were permitted to withdraw at any time without giving any reason. Participants were disclosed that an organic Tuscany wine (Sangiovese and Cabernet Sauvignon blend wine) from the commerce, containing around 13 % v/v ethyl alcohol, was enriched by food-grade HT product (naturally produced in wine grapes in a concentration around 1.98 – 3.89 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). The participants were instructed to spit the wine after the analysis, and the samples were commercial products available on the market and enriched with food-grade HT (already present in wine and other food products), approval from an ethics committee was not required.

Ethical statement – Studies in humans

The study was evaluated low-risk by the researchers involved, and therefore ethical committee approval was not sought for the following reasons: participants were instructed to expectorate the wine samples after analysis, the samples and additives used were commercially available products, and hydroxytyrosol (HT) was certified as food-grade. The European Food Safety Authority (EFSA) has deemed hydroxytyrosol levels of up to 215 mg/kg in vegetable oils and 175 mg/kg in margarine safe for consumption. The concentration of HT used in this study was well below these established safety limits (Panel on Dietetic Products et al., 2017). All participants provided informed consent and were informed that they could withdraw from the study at any time without consequences. Participation was voluntary, and refusal did not result in any penalties or loss of benefits. The study was conducted in accordance with the Declaration of Helsinki (World Medical Association, 2013). Participants were informed that the wine used for the study was an organic Tuscan blend of Sangiovese and Cabernet Sauvignon, commercially available and containing approximately 13% v/v ethyl alcohol, enriched with naturally occurring, food-grade HT at a concentration of 1.98–3.89 mg/L.

CRediT authorship contribution statement

Adriana Teresa Ceci: Conceptualization, Methodology, Software, Validation, Formal analysis, Investigation, Data curation, Writing – review & editing, Writing – original draft, Visualization. **Aakriti Darnal:** Methodology, Validation, Formal analysis, Investigation, Writing – review & editing. **Simone Poggesi:** Methodology, Validation, Formal analysis, Investigation, Writing – review & editing. **Prudence Fleur Tchouakeu Betnga:** Methodology, Validation, Formal analysis, Investigation, Writing – review & editing. **Edoardo Longo:** Methodology, Validation, Formal analysis, Investigation, Writing – review & editing. **Renzo Nicolodi:** Resources, Writing – review & editing, Supervision. **Reeta Davis:** Resources, Writing – review & editing, Supervision. **Meg Walsh:** Resources, Writing – review & editing, Supervision. **Kevin E. O'Connor:** Resources, Writing – review & editing, Supervision. **Enrico Angelo Altieri:** Resources, Writing – review & editing, Supervision. **Fabio Trevisan:** Writing – review & editing. **Tanja Mimmo:** Resources, Writing – review & editing, Funding acquisition. **Emanuele Boselli:** Resources, Writing – review & editing, Supervision, Funding acquisition.

Declaration of competing interest

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

Data availability

Data will be made available on request.

Acknowledgements

Salvatore Ferragamo and Stefano Chioccioli are acknowledged for their technical assistance.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.afres.2024.100513.

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