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Influence of oak chips, glutathione-rich inactivated yeast, and oxygen during fermentation on the analytical profile of volatile compounds in Blaufränkisch wines

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ABSTRACT

Blaufränkisch is an emerging grape variety with promising resilience to climate change due to its distinctive viticultural and oenological characteristics. This study employed a homogeneous Blaufränkisch mash to investigate the effects of oak chips, glutathione-rich inactivated yeast (GSH-IDY), and oxygen addition during fermentation on the concentration of volatile aroma compounds. The objective was to assess changes in the analytical aroma profile of the resulting wines and to evaluate the response of both varietal and fermentation-derived compounds to these treatments. Approximately 80 volatile compounds were quantified using compound-class-specific gas chromatography-mass spectrometry (GC-MS) techniques, including SIDA-HS-SPME-GC-MS for esters and alcohols, HS-SPME-GC-SIM-MS for monoterpenes and C_{13}-norisoprenoids, and derivatisation-based SPE-GC-TQMS for polyfunctional thiols. Oak chips significantly altered the concentration of specific aroma compounds: polyfunctional thiols such as 3-sulfanylhexan-1-ol (–24 %) and 4-methyl-4-sulfanylpentan-2-one (–26 %)—associated with grapefruit and blackcurrant notes—were reduced, whereas GSH-IDY and oxygen showed only marginal effects. Oak chips also enhanced the formation of 2-furfurylmethanethiol (+26 %), a compound that contributes to roasted aroma characteristics. Monoterpenes (*e.g.*, linalool and citronellol) and C_{13}-norisoprenoids remained largely stable, with GSH-IDY exerting a slight preservative effect. There were notable changes among C_6 alcohols: oak chips decreased *trans*-2-hexen-1-ol (–40 %) and hexanol (–15 %), while GSH-IDY promoted hexanol stability. Higher alcohols and esters responded differently: oak chips increased isoamyl alcohol concentrations (+10 %), whereas GSH-IDY promoted the formation of butanol (+16 %), while oxygen increased levels of key esters such as diethyl succinate (+36 %) and ethyl octanoate (+15 %). Overall, this study highlights how targeted fermentation treatments influence the volatile profile of Blaufränkisch wines.

KEYWORDS: Blaufränkisch, polyfunctional thiols, norisoprenoids, monoterpenes, esters

INTRODUCTION

The Austrian red grape variety Blaufränkisch (*Vitis vinifera* L.), also known as Lemberger (Germany), Kékfrankos (Hungary), or Frankovka modrá (Slovakia), has gained prominence due to its adaptability to climatic changes and is cultivated extensively in countries such as Hungary (~7,200 ha; Hungarian Ministry of Agriculture, 2023), Austria (~2,500 ha; Austrian Wine Marketing Board, 2024), and Germany (~1,900 ha; German Wine Institute, 2023), among others. Presumed to have originated in Central Europe, Blaufränkisch is a cross between *Vitis vinifera* L. cv. Gouais blanc and Sbulzina (Maul *et al.,* 2016; Regner, 2000). The typical aroma compounds of this variety include selected monoterpenes, C_{13}-norisoprenoids, and polyfunctional thiols (Philipp *et al.*, 2023).

The use of oak chips and controlled oxygen addition during alcoholic fermentation is considered an effective strategy for modulating the aromatic profile of red wines. Oak chips release not only typical oak-derived aroma compounds but also small amounts of oxygen, which can promote the formation of fruit-associated esters (*e.g.*, isoamyl acetate, ethyl hexanoate) and higher alcohols (*e.g.*, isoamyl alcohol, 2-phenylethanol) (Sánchez-Palomo *et al.*, 2017). Studies have reported an enhancement of floral and fruity notes under these conditions (Sánchez-Palomo *et al.*, 2017). The influence on norisoprenoids and thiols remains poorly understood, and there is no conclusive evidence for the reduction of low-boiling-point sulfur compounds through oak addition. Nevertheless, components such as ellagitannins may bind or oxidise reactive sulfur species. Model experiments using oak extracts have demonstrated a reduction in volatile sulfur compounds (Nunes *et al.*, 2020), but robust data from actual fermentation trials are lacking.

In contrast, the effects of oxygen are more thoroughly documented: early, moderate oxygen additions—ideally from the first third of fermentation—have been shown to enhance ester production, reduce higher alcohol concentrations, and prevent the development of reductive off-flavours (Lyu *et al.*, 2021; Yan *et al.*, 2020; Schmidtke *et al.*, 2011; Day *et al.*, 2021). A slightly oxidative environment also helps preserve sensitive norisoprenoids, such as β-damascenone, although thiols may be diminished through oxidative degradation. In an Australian Shiraz study, early oxygenation completely prevented the formation of undesirable sulfur compounds, resulting in a clean, fruit-driven aroma profile (Bekker *et al.*, 2016).

The use of reduced glutathione in winemaking is currently permitted under OIV regulations, both as a pure additive (up to 20 mg/L) and in the form of glutathione-rich inactivated yeasts (GSH-IDY), which are more commonly used due to cost and handling advantages (OIV, 2015a; OIV, 2015b; Bustamante *et al.*, 2024). GSH-IDY preparations are derived from specific yeast strains inactivated after fermentation and are enriched in intracellular glutathione and other antioxidant compounds (Bahut *et al.*, 2020). Their application serves as an alternative to SO_2, particularly in protecting grape musts and wines from oxidative degradation (Giménez *et al.*, 2023). Natural reduced glutathione concentrations vary depending on the vintage, vineyard location, and processing conditions, and may range from non-detectable levels to approximately 100 mg/L in grape must (Kritzinger *et al.*, 2013). During fermentation, reduced glutathione levels fluctuate significantly (Du Toit *et al.*, 2007; Andújar-Ortiz *et al.*, 2012; Philipp *et al.*, 2017). Glutathione can react with oxidised phenolic compounds (ortho-quinones), thereby preventing browning in must (Bustamante *et al.*, 2024). Owing to its free thiol group, it can also protect a variety of aroma compounds from oxidative degradation. These include esters (*e.g.*, isoamyl acetate and ethyl hexanoate), monoterpenes (*e.g.*, linalool), C_{13}-norisoprenoids (*e.g.*, β-damascenone), and polyfunctional thiols (*e.g.*, 3-SH, 3-SHA, and 4-MSP) (Papadopoulou & Roussis, 2008; Gabrielli *et al.*, 2017). Furthermore, reduced glutathione plays a central role in maintaining redox balance, detoxification processes, and sulfur metabolism (Pastore *et al.*, 2003). However, its use under nitrogen-deficient conditions may carry certain risks: reduced glutathione can be broken down by yeast into its constituent amino acids, and H_2S may be released from cysteine by cysteine desulfhydrase in the presence of metal ions (Wegmann-Herr *et al.*, 2016).

Sulfur-containing compounds play a complex role in wine aroma, contributing both desirable and undesirable notes. Polyfunctional thiols such as 3-SH and 4-MSP are key contributors to fruity aromas due to their low odour thresholds, while other sulfur compounds like 2-FM and BM may also influence wine style, depending on concentration and context (Tominaga *et al.*, 2003; Carlin *et al.*, 2022; Zhang *et al.*, 2023).

In contrast, sulfur-containing compounds with a low boiling point (< 90 °C), such as H_2S, methanethiol (MeSH), and ethanethiol (EtSH), as well as thioacetate esters such as methylthioacetate (MeSAc) and ethylthioacetate (EtSAc), can become sensorially objectionable at elevated concentrations (*e.g.*, 80–150 µg/L for H_2S). When the yeast-assimilable nitrogen (YAN) content in the must is sufficient (~250 mg/L), yeast can utilise H_2S for the biosynthesis of sulfur-containing amino acids such as cysteine and methionine, as well as the tripeptide glutathione (Bell & Henschke, 2005; Petrovic *et al.*, 2019), which are critical for protein biosynthesis during yeast growth (Smith *et al.*, 2015). However, when the YAN level falls below 150 mg/L, surplus H_2S and its derivatives, along with potentially formed thioacetate esters, may be excreted by the yeast, as cysteine and methionine concentrations regulate the activity of the sulphite and sulphate reductase enzymes (Bell & Henschke, 2005).

Although the effects of oak chips, GSH-enriched inactivated yeast (GSH-IDY), and oxygen during fermentation have been investigated in various grape varieties (Kritzinger *et al.*, 2013; Papadopoulou & Roussis, 2008; Gabrielli *et al.*, 2017), their specific impact on the aromatic composition of Blaufränkisch wines remains insufficiently documented. GSH-IDY is primarily associated with the modulation of reductive aroma

compounds during fermentation (Wegmann-Herr *et al.*, 2016), while oak addition and oxygen exposure are expected to influence the formation of monoterpenes, norisoprenoids, and esters (Sánchez-Palomo *et al.*, 2017; Nunes *et al.*, 2020; Lyu *et al.*, 2021; Bekker *et al.*, 2016).

Given the distinctive characteristics of this cultivar and its relevance to Austrian viticulture, a more detailed investigation into the aromatic implications of these treatments is of particular scientific interest. Therefore, this study aimed to investigate the effects of targeted application of oak chips, GSH-IDY, and oxygen on the volatile compound profile of Blaufränkisch wines, with a specific focus on polyfunctional thiols, low-boiling-point sulfur-containing compounds, monoterpenes, norisoprenoids, esters, higher alcohols, C_6 alcohols, and carboxylic acids. It is hypothesised that these oenological interventions significantly influence the balance between reductive and oxidative aroma components, thereby enabling directed modulation of the analytical profile of both varietal and fermentation-derived aroma compounds.

MATERIALS AND METHODS

1. Grape processing and vinification

The Blaufränkisch grapes used in this study were provided by the department of Grapevine Breeding at the Federal College and Research Institute for Viticulture and Pomology in Klosterneuburg, Austria (Götzhof, Rehgraben 2, 2103 Langenzersdorf). The grapes were harvested on 21 September 2023. They exhibited no signs of common vine diseases such as powdery mildew *(Oïdium)*, downy mildew *(Peronospora)*, or *Botrytis*. Immediately after harvesting, the grapes were destemmed and crushed. Must parameters were determined using Fourier-transform infrared (FT-IR) spectroscopy in accordance with OIV/OENO Resolution 390/2010 and are summarised in Table 1 (OIV, 2010). The mash temperature was 14 °C. Potassium metabisulfite ($K_2S_2O_5$; Lallemand, Guntramsdorf, Austria) was added at a rate of 10 g/100 kg mash.

A total of 650 kg of Blaufränkisch mash was available. Following sulphiting, the mash was homogenised in a fermentation vessel and divided into aliquots, each transferred into 50-L fermentation containers. Three fermentation replicates were prepared per treatment, resulting in a total of 15 vessels, each containing 40 kg of mash. Immediately after aliquoting, yeast strain ES488 (Enartis, Bratislava, Slovakia) was added at a rate of 60 g/100 kg after rehydration. The experimental design and treatment setup are shown in Figure 1.

Co-inoculation with lactic acid bacteria was carried out on 22 September 2023, *i.e.*, 24 hours after yeast inoculation, using ML Prime (Lallemand, Guntramsdorf, Austria) at 10 g per 100 kg must. Although ML Prime is typically recommended for musts with pH ≥ 3.4 and total SO_2 ≤ 50 mg/L, our must (pH 3.36; total SO_2 ≈ 50 mg/L) fitted within these recommendations and has been shown to support rapid malolactic fermentation with minimal lag phase and no volatile acidity formation. The 24-hour delay aligns with the recommended co-inoculation protocol and is intended to balance nutrient competition between yeast and bacteria, ensuring robust MLF onset while preserving yeast performance. Depending on the treatment, oak chips (Incanto Natural, Enartis) were added at 80 g/100 kg, and glutathione was applied in the form of a GSH-IDY yeast product (ProBlanco, Enartis) at 30 g/100 kg.

TABLE 1. Quantified must parameters of the homogeneous Blaufränkisch mash (*n* = 3).

Basic parameters of the Blaufränkisch mash	
Density [g/cm³]	1.0866 ± 0.0004
Glucose [g/L]	101.0 ± 0.0
Fructose [g/L]	108.3 ± 0.7
°Brix	20.0 ± 0.1
Titratable acidity c. a. tartaric acid [g/L]	8.1 ± 0.0
pH [1]	3.36 ± 0.02
Malic acid [g/L]	4.1 ± 0.0
Volatile acidity [g/L]	n.d.
NOPA [mg/L]	210 ± 3
Ammonium [mg/L]	105 ± 1
YAN (calculated) [mg/L]	296 ± 2

Note that the limit of detection was 0.1 g/L, and the limit of quantification was 0.2 g/L. n.d., not detectable; NOPA, α-amino nitrogen; YAN, yeast-assimilable nitrogen.

In addition, the Blaufränkisch mash was enriched to 23.5 °Brix using sucrose. According to the manufacturer, ProBlanco contains a minimum of 1.5 % reduced glutathione, as regulated by the OIV Codex (COEI-1-LEVGLU; OIV, 2017), which corresponds to an application rate of 4.5 mg/kg of reduced glutathione.

Fermentation was conducted at 25.0 ± 1.0 °C. The cap was punched down twice daily and monitored using a handheld density meter (DMA 35, Anton Paar, Graz, Austria). No nitrogen supplementation (*e.g.*, diammonium phosphate (DAP)) was applied, as the must's YAN concentration was approximately 300 mg/L. Macro-oxygenation was carried out for selected treatments using a MicroOx system (Enartis) at the end of the second third of fermentation (between 1.3402 and 1.3888 g/cm^3), applying 15 mg/kg of oxygen (Linde, Vienna, Austria).

After seven days, on 28 September 2023, the mash was pressed. The analytical parameters of the resulting young wines are summarised in Table 2. All wines completed both alcoholic fermentation and malolactic conversion. The wines were transferred into 25-L demijohns and racked off the lees on 12 October 2023. Subsequent sulphiting was carried out with 16 g/100 L $K_2S_2O_5$ (corresponding to 80 mg/L free SO_2). Free SO_2 was adjusted twice to 50 mg/L prior to bottling and set to 60 mg/L at bottling on 15 December 2023.

The wines were bottled in olive-green 500 mL 'BD Exklusiv' bottles (Vetropack, Bülach, Switzerland) and sealed with Tin-Saran screw caps (BT-Watzke GmbH, Pinggau, Austria). The bottles were stored horizontally at 16 ± 1 °C in the *vinothèque* of the Enology department at HBLAuBA Klosterneuburg until analysis.

2. Experimental setup

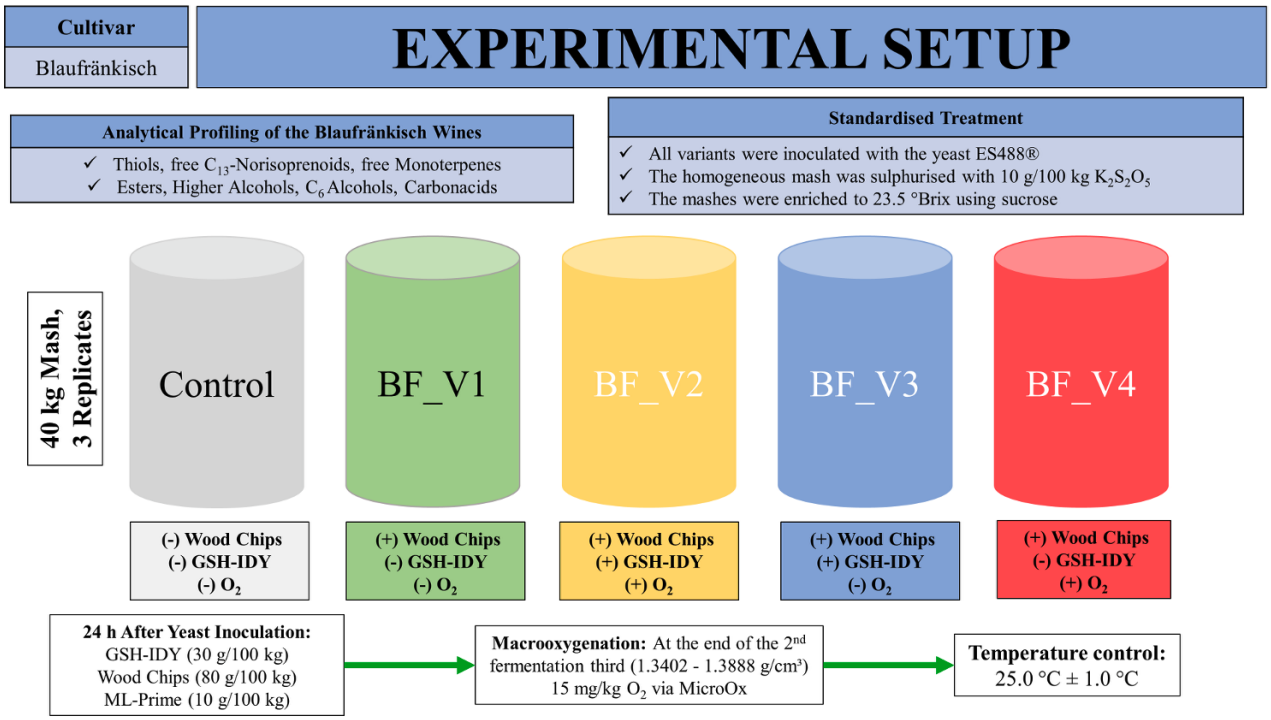


FIGURE 1. Experimental setup.

3. Analysis and instrumentation

The experimental wines were analysed between May and August 2024. Prior to sample preparation, the bottles were removed from cold storage and allowed to equilibrate to room temperature.

4. Basic must and wine parameters

Quantification of basic must and wine parameters was conducted in the department of Chemistry at the Federal College and Research Institute for Viticulture and Pomology, Klosterneuburg. Measurements were performed using FT-IR spectroscopy in accordance with OIV/OENO Resolution 390/2010 (OIV, 2010), employing a FOSS WineScan (FT 120 Reference Manual; Foss, Hamburg, Germany).

The following parameters were quantified: °Brix, α-amino nitrogen (NOPA), ammonium, density, ethanol, glucose, fructose, titratable acidity, pH, tartaric acid, malic acid, lactic acid, volatile acidity, and citric acid. YAN was calculated from the individual values for NOPA and ammonium using the following formula: YAN = NOPA + (Ammonium × 0.8225) (Bell & Henschke, 2005).

5. Aroma analysis

Quantification of esters, higher alcohols, C_6 alcohols, carboxylic acids, free monoterpenes, and C_{13}-norisoprenoids was carried out at the department of Chemistry-Research and Isotope Analysis of the Federal College and Research Institute for Viticulture and Pomology, Klosterneuburg. Analysis of polyfunctional thiols and low-boiling-point

TABLE 2. Quantified parameters of the young wines (n = 3).

Parameter	Control and experimental variants [$\bar{x} \pm \sigma$] (n = 3)				
	Control	BF_V1 (+) Oak chips (–) GSH-IDY (–) O_2	BF_V2 (+) Oak chips (+) GSH-IDY (+) O_2	BF_V3 (+) Oak Chips (+) GSH-IDY (–) O_2	BF_V4 (+) Oak chips (–) GSH-IDY (+) O_2
Density [g/cm^3]	0.9942 ± 0.0003	0.9932 ± 0.0001	0.9935 ± 0.0002	0.9935 ± 0.0001	0.9932 ± 0.0001
Alcohol [% vol.]	12.9 ± 0.2	13.1 ± 0	13.0 ± 0.1	13.1 ± 0.0	13.1 ± 0.1
Glucose [g/L]	n.d.	n.d.	n.d.	n.d.	n.d.
Fructose [g/L]	n.d.	n.d.	n.d.	n.d.	n.d.
Titratable acidity c. a. Tartaric acid [g/L]	6.2 ± 0.1	6.5 ± 0.1	6.5 ± 0.1	6.4 ± 0.0	6.6 ± 0.0
pH [1]	3.56 ± 0.01	3.51 ± 0.00	3.53 ± 0.01	3.55 ± 0.01	3.51 ± 0.01
Tartaric acid [g/L]	2.5 ± 0.1	2.8 ± 0.1	2.8 ± 0.0	2.7 ± 0.1	2.8 ± 0.1
Malic acid [g/L]	n.d.	n.d.	n.d.	n.d.	n.d.
Lactic acid [g/L]	2.3 ± 0.0	2.3 ± 0.0	2.3 ± 0.0	2.3 ± 0.0	2.2 ± 0.0
Volatile acidity [g/L]	0.3 ± 0.0	0.3 ± 0.0	0.3 ± 0.0	0.3 ± 0.0	0.3 ± 0.0
Citric acid [g/L]	n.d.	n.d.	n.d.	n.d.	n.d.

Note that the limit of detection was 0.1 g/L, and the limit of quantification was 0.2 g/L. GSH-IDY, glutathione-rich inactivated yeast; n.d., not detectable. All fermentations were completed on 28 September 2024.

sulfur-containing compounds was conducted at the department of Microbiology and Biochemistry, Hochschule Geisenheim University (HGU).

In total, five gas chromatography systems (Agilent Technologies, Santa Clara, CA, USA) were employed, each equipped with specific column technologies, detectors, and sample preparation procedures tailored to the physicochemical characteristics of the respective analytes. This multi-system approach ensured analytically differentiated detection of volatile aroma compounds.

Quantification of key aroma compounds—including selected higher alcohols, short- and medium-length carboxylic acids, C_6 alcohols, and major esters—was performed by using a partial stable isotope dilution analysis-headspace solid-phase microextraction-gas chromatography-mass spectrometry (SIDA-HS-SPME-GC-MS) method as described by Philipp *et al.* (2019b), employing an Agilent 6890N GC system equipped with a ZB-Wax Plus column. Small and large ester compounds were analysed on an Agilent 7890A GC coupled to a 5975C mass selective detector (MSD) using a ZB-5MS column and a partial stable isotope dilution analysis-solid-phase microextraction-gas chromatography-single ion monitoring mass spectrometry (SIDA-SPME-GC-SIM-MS) method based on Philipp *et al.* (2019a) and Fauster *et al.* (2020).

Free monoterpenes (11 compounds) were analysed on the same system using a headspace solid-phase microextraction-gas chromatography-single ion monitoring-mass spectrometry (HS-SPME-GC-SIM-MS) method according to Philipp *et al.* (2020). C_{13}-norisoprenoids—including β-damascenone, β-ionone, 1,1,6-trimethyl-1,2-dihydronaphthalene (TDN), and vitispirane—were determined using a Trace 1300 GC system coupled to a TSQ Duo mass spectrometer with automated SPME via a TriPlus RSH autosampler, following the method described by Philipp *et al.* (2023).

Analysis of low-boiling-point sulfur-containing compounds was conducted by using an HP 6890 GC system equipped with a sulfur-selective SPB-1 column and a cooled injection system (CIS-4). Detection was carried out using headspace gas chromatography with pulsed flame photometric detection (HS-GC-PFPD) according to Jung *et al.* (2021). All systems were equipped with autosamplers and were validated for the corresponding target analytes.

Qualitative and quantitative analysis of polyfunctional thiols was performed after derivatisation with ethyl propiolate (ETP), followed by solid-phase extraction (SPE) and subsequent gas chromatography coupled to tandem mass spectrometry (GC-TQMS). The analysis was carried out using an Agilent 8890 GC system coupled to an Agilent 7010B triple quadrupole mass spectrometer. Chromatographic separation was achieved using a polar Stabilwax-DA column (30 m × 0.25 mm × 0.25 µm; Restek GmbH, Bad Homburg, Germany) with a 5 m IP-deactivated guard column (0.25 mm ID).

The sample preparation and analysis method was based on protocols developed by Herbst-Johnstone *et al.* (2013), Coetzee *et al.* (2018), and Kiene *et al.* (2021), with modifications tailored to the available instrumentation.

For SPE, 100 µL of 3-*tert*-butylanisole (2 mM in absolute ethanol, ≥ 99.9 %, VWR, Darmstadt, Germany), 10 µL of the internal standard 4-methoxy-2-methyl-2-butanethiol (11.2 µM in ethanol), and 20 µL of ETP (99 %, Merck, Darmstadt, Germany) were added to 50 mL of the sample. After mixing briefly, the pH was adjusted to 10 using sodium hydroxide (≥ 98 %, Carl Roth, Karlsruhe, Germany). The samples were centrifuged for 15 min at 4,000 rpm, and the supernatant was loaded onto a conditioned SPE cartridge (Supelclean ENVITM-18, 500 mg, 6 mL; Merck). The cartridge was conditioned with 10 mL methanol and 10 mL of a 13 % aqueous–ethanolic solution *(v/v)*.

After loading (approximately 20 min under light vacuum), the cartridge was washed with 5 mL of ultrapure water (Gen Pure System, Thermo Scientific, Waltham, USA). Then, the cartridge was dried under vacuum with a defined nitrogen flow (5.0, Nippon Gases, Düsseldorf, Germany) for approximately 25 min. Target analytes were eluted using 10 mL of dichloromethane (DCM; 99.5 %, Carl Roth), and the eluate was dried over sodium sulphate (99.5 %, Carl Roth). The extract was reduced to 0.3 mL using a Syncore Analyst R-12 evaporator (Büchi Labortechnik AG, Flawil, Switzerland), followed by further concentration under a gentle nitrogen stream to approximately 30 µL.

2 µL of the DCM extract (kept at 10 °C at a cooled tray) were injected into the GC-TQMS system using an MPS robotic pro autosampler (Gerstel, Mülheim an der Ruhr, Germany). The injector temperature was 260 °C and operated in splitless mode for 2 min, followed by a purge flow of 50 mL/min. Helium (5.0, Nippon Gases) was used as carrier gas at a constant flow of 1.2 mL/min.

The GC oven programme was as follows: initial temperature of 60 °C (1 min), ramped at 25 °C/min to 100 °C (2 min), then at 5 °C/min to 250 °C (20 min). The transfer line temperature was set to 275 °C.

Detection of ETP-derivatised thiols was performed in the electron ionisation (EI) mode at 70 eV. Data were acquired in the multiple reaction monitoring (MRM) mode. Nitrogen (5.0) served as the collision gas (1.5 mL/min), while helium was used as quench gas (4 mL/min). The ion source temperature was 230 °C, and both quadrupoles were maintained at 150 °C.

Quantification was performed using external calibration in white wine (*Vitis vinifera* L. cv. Reichensteiner, 2021 vintage; 0.1 g/L residual sugar, 6.4 g/L total acidity, and 11.2 % vol. alcohol) obtained from the department of Plant Breeding, Hochschule Geisenheim University.

Data analysis was performed using Agilent MassHunter Workstation Software (version B10.2).

6. Statistical analysis

Statistical analyses were performed using SPSS Statistics version 29.0 (IBM, Armonk, NY, USA) and XLSTAT version 2023.3.1 (Lumivero, Denver, CO, USA). The data were first log-transformed in accordance with the approach described by Leydesdorff and Bensman (2006). The data were subjected to the Shapiro–Wilk test to check whether it followed a normal distribution, and Levene's test to assess homogeneity of variances. In SPSS Statistics, one-way analysis of variance (ANOVA) was used to assess differences among the treatment variants, followed by Tukey's B test for *post hoc* comparisons between groups. A p-value < 0.05 was considered to indicate a statistically significant difference. The main effects of the three oenological factors—oak chips, GSH-IDY, and oxygen—were analysed via a three-way ANOVA (excluding interaction terms) using XLSTAT, following the approach of Williams (1986). Additionally, a principal component analysis (PCA) was carried out in XLSTAT based on the methodology of Leyer and Wesche (2007).

RESULTS AND DISCUSSION

This study aimed to analyse the effects of the experimental factors—oak chips, GSH-IDY, and oxygen—on the development of varietal and fermentation-derived aroma compounds in wines produced from the Blaufränkisch grape variety. There were four experimental variants and a control:

- Control—no treatments
- BF_V1—(+) oak chips, (–) GSH-IDY, and (–) oxygen
- BF_V2—(+) oak chips, (+) GSH-IDY, and (+) oxygen
- BF_V3—(+) oak chips, (+) GSH-IDY, and (–) oxygen
- BF_V4—(+) oak chips, (–) GSH-IDY, and (+) oxygen.

In this section, the aroma compounds are divided into monoterpenes, C_6 alcohols, polyfunctional thiols, volatile sulfur-containing compounds, higher alcohols, carboxylic acids, esters, and free C_{13}-norisoprenoids. For each group, the effects of oak chips, GSH-IDY, and oxygen on specific compounds are presented. Significant effects are indicated with asterisks (** $p < 0.01$ and *** $p < 0.001$).

1. Monoterpenes

Table 3 presents the results of quantified monoterpenes. These compounds—including geraniol, linalool, nerol, and citronellol, as well as their derivative rose oxide—are volatile isoprenoids that contribute to characteristic aroma notes such as 'rose', 'lilac', 'pine', or 'citrus'. These C_{10} compounds tend to accumulate during late ripening, particularly in Muscat and Gewürztraminer, but are also present in smaller amounts in varieties such as Riesling, Viognier, and Chenin blanc (Robinson *et al.*, 2014).

In the present study, *trans*-linalool oxide concentrations differed significantly among the variants (p = 0.015), although none of the experimental factors (oak chips, GSH-IDY, and oxygen) had a significant effect. BF_V1 had the highest concentration, whereas the remaining variants

TABLE 3. The content of free monoterpenes and their modulation on Blaufränkisch wines after the application of selected additives and treatment aids.

Free monoterpenes [µg/L]	Significance of the experimental factors			Control and experimental variants [$\bar{x} \pm \sigma$] (n = 3)				
	Oak chips 80 g/100 kg Incanto Natural®	GSH-IDY 30 g/100 kg ProBlanco®	O_2 15 mg/kg MicroOx®	Control	BF_V1 (+) Oak chips (–) GSH-IDY (–) O_2	BF_V2 (+) Oak chips (+) GSH-IDY (+) O_2	BF_V3 (+) Oak chips (+) GSH-IDY (–) O_2	BF_V4 (+) Oak chips (–) GSH-IDY (+) O_2
trans-Linalooloxide [p = **0.015**]	n.s.	n.s.	n.s.	$3.2^{a} \pm 0.3$	$4.0^{b} \pm 0.2$	$3.5^{ab} \pm 0.2$	$3.3^{a} \pm 0.1$	$3.2^{a} \pm 0.3$
cis-Linalooloxide [p = **0.002**]	**	n.s.	n.s.	$0.8^{a} \pm 0.0$	$1.0^{c} \pm 0.0$	$0.9^{bc} \pm 0.0$	$0.9^{ab} \pm 0.0$	$0.8^{ab} \pm 0.1$
Linalool [p = 0.303]	n.s.	n.s.	n.s.	$33.0^{a} \pm 3.0$	$35.0^{a} \pm 0.5$	$36.6^{a} \pm 3.6$	$36.0^{a} \pm 4.0$	$31.8^{a} \pm 2.4$
Hotrienol [p = 0.348]	n.s.	n.s.	n.s.	$0.6^{a} \pm 0.1$	$0.7^{a} \pm 0.1$	$0.6^{a} \pm 0.0$	$0.7^{a} \pm 0.1$	$0.6^{a} \pm 0.1$
trans-Limonene oxide [p = **0.015**]	**	***	n.s.	$2.3^{ab} \pm 0.1$	$2.2^{ab} \pm 0.0$	$2.4^{b} \pm 0.1$	$2.3^{ab} \pm 0.1$	$2.1^{a} \pm 0.0$
α-Terpineol [p = **0.038**]	**	n.s.	n.s.	$11.7^{a} \pm 0.6$	$13.3^{b} \pm 0.3$	$12.5^{ab} \pm 0.4$	$12.8^{ab} \pm 0.6$	$12.1^{ab} \pm 0.8$
β-Terpineol [p = 0.098]	n.s.	n.s.	n.s.	$2.3^{a} \pm 0.3$	$1.9^{a} \pm 0.3$	$2.0^{a} \pm 0.2$	$2.4^{a} \pm 0.1$	$2.3^{a} \pm 0.1$
Nerol oxide [p = 0.205]	n.s.	n.s.	n.s.	$4.5^{a} \pm 0.2$	$5.0^{a} \pm 0.2$	$4.9^{a} \pm 0.1$	$4.8^{a} \pm 0.1$	$5.7^{a} \pm 1.3$
Nerol [p = **0.002**]	n.s.	**	n.s.	$0.8^{a} \pm 0.0$	$0.7^{a} \pm 0.1$	$0.8^{a} \pm 0.1$	$1.0^{b} \pm 0.1$	$0.8^{a} \pm 0.1$
Citronellol [p = 0.071]	**	n.s.	n.s.	$3.5^{a} \pm 0.5$	$4.3^{a} \pm 0.2$	$4.1^{a} \pm 0.2$	$4.4^{a} \pm 0.2$	$3.7^{a} \pm 0.5$
Geraniol [p = 0.073]	n.s.	**	n.s.	$5.6^{a} \pm 0.4$	$5.5^{a} \pm 0.7$	$5.7^{a} \pm 0.5$	$6.2^{a} \pm 0.2$	$4.9^{a} \pm 0.4$
Σ Free monoterpenes [p = 0.195]	-	-	-	$68.4^{a} \pm 5.1$	$73.5^{a} \pm 1$	$74.1^{a} \pm 5.1$	$74.7^{a} \pm 4.6$	$68.0^{a} \pm 3.4$

The three columns to the right of the parameter column present the results of the three-way analysis of variance, indicating the main effects of oak chips, GSH-IDY, and oxygen (** $p < 0.01$; *** $p < 0.001$; n.s., not significant). The adjacent columns to the right contain the quantified concentrations ($\bar{x} \pm \sigma$) of individual compounds for the control group and the four experimental variants. Statistically significant differences ($p < 0.05$) between the variants are indicated by different superscript letters. Bold p-values indicate statistically significant differences at $p < 0.05$. GSH-IDY, glutathione-rich inactivated yeast.

showed concentrations similar to the control. *cis*-Linalool oxide also showed significant differences (p = 0.002), with oak chips exerting a moderate effect (**). The control variant exhibited the lowest concentration, while BF_V1 displayed a significantly elevated concentration, suggesting a specific influence of oak on this compound.

There were no significant differences for linalool or hotrienol among the variants (p = 0.303 and 0.348, respectively); the concentrations of both compounds remained stable under the applied experimental conditions. Similarly, the concentrations of β-terpineol and nerol oxide showed no substantial variation across the variants (p = 0.098 and 0.205, respectively). These results indicate that their concentrations are primarily varietal in origin.

trans-Limonene oxide showed significant differences among the variants (p = 0.015), with oak chips (**) and GSH-IDY (***) exerting a significant effect. BF_V2 showed the highest concentration, while BF_V4 had the lowest concentration. α-Terpineol also showed significant differences (p = 0.038), with oak chips (**) as the only significant factor. The highest concentration was detected in BF_V1, while the other variants showed only slight increases compared with the control.

For nerol (p = 0.002), the GSH-IDY (**) effect was significant. BF_V3 showed the highest concentration, whereas the other variants showed concentrations similar to the control. These findings highlight the role of glutathione in the preservation or promotion of this compound, likely through antioxidant mechanisms.

Citronellol and geraniol did not exhibit significant differences among the variants (p = 0.071 and 0.073, respectively). Although oak chips exerted a slight influence on citronellol, and GSH-IDY had a slight effect on geraniol, these effects were not statistically significant.

Monoterpenes such as linalool, α-terpineol, and citronellol are key contributors to the floral and fruity aroma of many wines. Based on the results, the experimental factors had only a limited influence on their concentrations. The observed trends for GSH-IDY are partly consistent with previous studies that have attributed a protective and stabilising role to glutathione with respect to monoterpenes (Bahut *et al.*, 2020;

Rodríguez-Bencomo *et al.*, 2014; Gabrielli *et al.*, 2017; Cojocaru & Antoce, 2019). The relatively modest effects observed in this study may be explained by the specific combination of treatments and the relatively short storage period of five months, as glutathione is known to exhibit more pronounced antioxidant activity over longer ageing periods (Bahut *et al.*, 2020).

2. C_6 alcohols

Table 4 presents the concentrations of quantified C_6 alcohols. These compounds are formed by the enzymatic reduction of C_6 aldehydes such as hexanal and hexenal, which themselves originate from the oxidation of polyunsaturated fatty acids (*e.g.*, linoleic and linolenic acid) due to damage to grape seeds and skins during processing. They are generally characterised by 'fresh-green', 'grassy', or 'herbaceous' aromas (Dudareva *et al.*, 2013).

In the present study, *trans*-2-hexen-1-ol showed significant differences among the variants (p = 0.001), with oak chips (**) exerting a significant influence, while GSH-IDY and oxygen had no significant effect. The control variant exhibited the highest concentration, whereas BF_V3 and BF_V4 showed the lowest concentrations. These findings suggest that oak chip treatment may reduce *trans*-2-hexen-1-ol concentrations.

Hexanol also presented highly significant differences among the variants ($p < 0.001$), with both oak chips (***) and GSH-IDY (***) showing strong effects. Oak chips led to lower concentrations in BF_V1 and BF_V4, while BF_V2 and BF_V3 exhibited slightly elevated concentrations, indicating a potentially stabilising role of GSH-IDY. Oxygen had no significant effect in either case.

These findings suggest that oak chips play a key role in modulating both *trans*-2-hexen-1-ol and hexanol. They partially contrast with mechanisms that have been described in the literature, which propose that glutathione reacts with *trans*-2-hexenal to form Glut-3MHal, a precursor to thiols such as 3-SH and 3-SHA (Clark & Deed, 2018). The non-significant effect of GSH-IDY observed here may be attributed to the phenolic composition specific to Blaufränkisch wines or to the comparatively short ageing period, which may have limited the kinetics of glutathione–aldehyde reactions.

Harsch *et al.* (2013) and Kritzinger *et al.* (2013) have also reported that H_2S released during fermentation can react with *trans*-2-hexenal to form polyfunctional thiols. The relatively high YAN (296 mg/L) in this study may have limited H_2S release by yeast (Kwasniewski *et al.*, 2011), thereby reducing the availability of precursors for thiol formation.

The effect of GSH-IDY on hexanol may be explained by its function as a reducing agent that inhibits oxidative degradation processes, thus preserving hexanol stability. This view is consistent with reports indicating that hexanol is formed via the lipoxygenase/hydroperoxide lyase (LOX/HPL) pathway from enzymatic oxidation of linoleic acid (Hatanaka, 1993). Glutathione may further contribute indirectly to hexanol formation by protecting polyfunctional thiols and other C_6 compounds such as hexanol from oxidation (Kritzinger *et al.*, 2013). As noted earlier, GSH also plays a central role in thiol release during early fermentation via H_2S-mediated mechanisms (Harsch *et al.*, 2013; Araujo *et al.*, 2017).

3. Polyfunctional thiols

Table 5 presents the concentrations of the quantified thiols. Polyfunctional thiols such as 3-sulfanylhexan-1-ol (3-SH), 3-sulfanylhexyl acetate (3-SHA), 4-methyl-4-sulfanylpentan-2-one (4-MSP), 2-furfurylthiol (2-FM), and benzenemethanethiol (BM) contribute to distinct aroma characteristics, including notes of blackcurrant, citrus, roasted coffee, and smoke. 3-SH and 4-MSP are present

TABLE 4. The C_6 alcohol content and its modulation in Blaufränkisch wines after the application of selected additives and treatment aids.

C_6 alcohols [mg/L]	Significance of the experimental factors			Control and experimental variants [$\bar{x} \pm \sigma$] (n = 3)				
	Oak chips 80 g/100 kg Incanto Natural®	GSH-IDY 30 g/100 kg ProBlanco®	O_2 15 mg/kg MicroOx®	Control	BF_V1 (+) Oak chips (–) GSH-IDY (–) O_2	BF_V2 (+) Oak chips (+) GSH-IDY (+) O_2	BF_V3 (+) Oak chips (+) GSH-IDY (–) O_2	BF_V4 (+) Oak chips (–) GSH-IDY (+) O_2
cis-3-Hexen-1-ol	-	-	-	n.d.	n.d.	n.d.	n.d.	n.d.
trans-2-Hexen-1-ol [p = **0.001**]	**	n.s.	n.s.	$1.6^{c} \pm 0.2$	$1.2^{bc} \pm 0.2$	$1.0^{ab} \pm 0.1$	$0.8^{a} \pm 0.1$	$0.8^{a} \pm 0.1$
Hexanol [p < **0.001**]	***	***	n.s.	$1.7^{b} \pm 0.1$	$1.4^{a} \pm 0.1$	$1.5^{a} \pm 0.1$	$1.6^{b} \pm 0.1$	$1.3^{a} \pm 0.1$
Σ C_6 alcohols [p < **0.001**]	-	-	-	$3.3^{c} \pm 0.2$	$2.6^{b} \pm 0.2$	$2.5^{ab} \pm 0.1$	$2.5^{ab} \pm 0.1$	$2.2^{a} \pm 0.1$

The three columns to the right of the parameter column present the results of the three-way analysis of variance, indicating the main effects of oak chips, GSH-IDY, and oxygen (** $p < 0.01$; *** $p < 0.001$; n.s., not significant). The adjacent columns to the right contain the quantified concentrations ($\bar{x} \pm \sigma$) of individual compounds for the control group and the four experimental variants. Statistically significant differences ($p < 0.05$) between the variants are indicated by different superscript letters. Bold p-values indicate statistically significant differences at $p < 0.05$. GSH-IDY, glutathione-rich inactivated yeast; n.d., not detectable.

TABLE 5. The content of polyfunctional thiols and their modulation in Blaufränkisch wines after the application of selected additives and treatment aids.

Polyfunctional thiols [ng/L]	Significance of the experimental factors			Control and experimental variants [$\bar{x} \pm \sigma$] (n = 3)				
	Oak chips 80 g/100 kg Incanto Natural®	GSH-IDY 30 g/100 kg ProBlanco®	O_2 15 mg/kg MicroOx®	Control	BF_V1 (+) Oak chips (–) GSH-IDY (–) O_2	BF_V2 (+) Oak chips (+) GSH-IDY (+) O_2	BF_V3 (+) Oak chips (+) GSH-IDY (–) O_2	BF_V4 (+) Oak chips (–) GSH-IDY (+) O_2
4-MSP (ODT: 1–4 ng/L) [p < **0.001**]	***	n.s.	***	$2.8^{c} \pm 0.1$	$1.9^{a} \pm 0.0$	$2.1^{ab} \pm 0.2$	$1.9^{a} \pm 0.1$	$2.4^{bc} \pm 0.4$
3-SHA	-	-	-	n.d.	n.d.	n.d.	n.d.	n.d.
3-SH (ODT: 60 ng/L) [p < **0.001**]	***	n.s.	n.s.	$187.3^{b} \pm 4.3$	$141.9^{a} \pm 3.2$	$140.7^{a} \pm 1.8$	$139.4^{a} \pm 17.3$	$144.1^{a} \pm 12.1$
2-FM (ODT: 0.4 ng/L) [p < **0.001**]	***	**	n.s.	$3.1^{a} \pm 0.2$	$4.0^{c} \pm 0.0$	$4.0^{c} \pm 0.1$	$3.6^{b} \pm 0.0$	$4.0^{c} \pm 0.0$
E3-SP [p = **0.005**]	n.s.	**	n.s.	$295.2^{ab} \pm 15.1$	$354.1^{c} \pm 17.1$	$332.6^{bc} \pm 25.2$	$278.4^{a} \pm 4.5$	$338.0^{bc} \pm 34.0$
BM (ODT: 0.3 ng/L) [p = **0.021**]	**	n.s.	n.s.	$7.4^{b} \pm 0.8$	$6.4^{ab} \pm 0.3$	$6.5^{ab} \pm 0.3$	$5.4^{a} \pm 0.7$	$6.0^{ab} \pm 0.6$

The three columns to the right of the parameter column present the results of the three-way analysis of variance, indicating the main effects of oak chips, GSH-IDY, and oxygen (** $p < 0.01$; *** $p < 0.001$; n.s., not significant). The adjacent columns to the right contain the quantified concentrations ($\bar{x} \pm \sigma$) of individual compounds for the control group and the four experimental variants. Statistically significant differences ($p < 0.05$) between the variants are indicated by different superscript letters. Bold p-values indicate statistically significant differences at $p < 0.05$. GSH-IDY, glutathione-rich inactivated yeast; n.d., not detectable; ODT, Odour Detection Threshold.

in grapes in non-volatile, bound forms, predominantly as cysteine and glutathione conjugates (*e.g.*, Cys-3SH, Glut-3SH, Cys-4MSP, Glut-4MSP), which are enzymatically cleaved by yeast during alcoholic fermentation, provided the strain expresses sufficient β-lyase activity (Tominaga *et al.*, 1998; Fedrizzi *et al.*, 2009; Swiegers *et al.*, 2007; Subileau *et al.*, 2008; Ruiz *et al.*, 2019). The release of 4-MSP from its cysteinylated precursor is catalysed by enzymes encoded by the *IRC7* gene (Cordente *et al.*, 2017), while 3-SH can be further acetylated to 3-SHA via ATF1-mediated acetyltransferase activity (Swiegers *et al.*, 2005).

There were significant differences in the 4-MSP concentrations among the variants ($p < 0.001$). Three-way ANOVA indicated that oak chips (***) and oxygen (***) had a highly significant effect, while GSH-IDY showed no significant impact. The control variant had the highest 4-MSP concentration, whereas BF_V1 and BF_V3 (both without oxygen) exhibited the lowest concentrations. This suggests that the combined application of oak chips and oxygen may moderately increase 4-MSP. The intermediate concentrations in BF_V2 and BF_V4 further confirm the joint influence of these two factors.

The 3-SH concentrations also varied significantly among the variants ($p < 0.001$). Oak chips had a highly significant impact (***), while GSH-IDY and oxygen were not significant. The control variant again showed the highest concentration, followed by BF_V4. All other variants (BF_V1, BF_V2, and BF_V3) demonstrated a significant reduction in 3-SH, underlining the dominant effect of oak chips, with only marginal contributions from GSH-IDY or oxygen.

The E3-SP concentrations differed significantly among the variants ($p = 0.005$), with GSH-IDY showing a significant effect (**), while oak chips and oxygen had no significant impact. BF_V1 showed the highest concentration, while control and BF_V3 exhibited the lowest concentrations. BF_V2 and BF_V4 showed intermediate concentrations, indicating that GSH-IDY may exert a stabilising or enhancing effect on E3-SP, with little contribution from the other two factors.

There were also significant differences in the 2-FM concentrations ($p < 0.001$). Oak chips had a highly significant effect (***), and GSH-IDY showed a significant effect (**), but oxygen had no significant influence. The control variant displayed the lowest concentration, while BF_V1 (oak chips only) exhibited a significantly elevated concentration, highlighting the dominant role of oak. BF_V2 and BF_V4 reached similarly high concentrations, whereas BF_V3 showed a slightly reduced concentration, suggesting that the effect of GSH-IDY may depend on the presence of oxygen. Overall, oak chips remained the strongest influencing factor, with GSH-IDY contributing moderately and oxygen showing no relevant effect.

There were significant differences for BM among the variants ($p = 0.021$). Specifically, oak chips had a significant effect (**), while GSH-IDY and oxygen showed no significant impact. The control variant showed the highest BM concentration. BF_V1 and BF_V2 (both with oak chips) showed moderate reductions, while BF_V3 and BF_V4 had the lowest concentrations, suggesting a modest reductive effect of oak chips, without direct influence from GSH-IDY or oxygen.

In this study, 4-MSP, associated with blackcurrant and exotic fruit aromas, seemed to be particularly influenced by the addition of oak chips. This may be explained by interactions between ellagitannins from oak and thiol precursors, as described by Petit *et al.* (2015). However, the present findings contrast with those of Papadopoulou and Roussis (2008) and Bahut *et al.* (2020), who reported an antioxidant effect of GSH-IDY on polyfunctional thiols such as 4-MSP. Such an effect was not confirmed in this study, potentially due to the specific conditions associated with the Blaufränkisch variety. Ultimately, the concentrations of 4-MSP ranged between 1.8 and 2.9 ng/L across all variants, including 2.8 ng/L in the control, potentially exceeding the odour detection threshold and making this compound perceivable in the wines (Muhl *et al.*, 2022; Carlin *et al.*, 2022).

3-SH imparts citrus and tropical fruit aromas (Tominaga *et al.*, 1996). The results indicate that oak chip treatment leads to a marked reduction of 3-SH in Blaufränkisch wines. This may again be related to ellagitannin interactions, as suggested by Petit *et al.* (2015). These findings contradict reports by Papadopoulou and Roussis (2008) and Bahut *et al.* (2020), who observed a protective effect of glutathione on 3-SH. Notably, the control variant—produced without oak—showed the highest 3-SH concentration. Given that glutathione naturally reacts with *trans*-2-hexenal to form Glut-3MHal (Clark & Deed, 2018), the significant reduction in *trans*-2-hexenal by oak observed in this study may explain the lower 3-SH concentrations in the oak chip-treated variants. Across all variants, 3-SH concentrations ranged from 139.4 to 187.3 ng/L, clearly exceeding the reported odour detection threshold of 60 ng/L (Tominaga *et al.*, 1996), and are therefore likely to contribute perceptibly to the wine's aroma profile.

The ester 3-SHA, formed through acetylation of 3-SH by yeast enzymes, was not detected in either the control or experimental variants. This is likely due to the use of a single yeast strain that potentially does not express the *ATF1* gene (Swiegers & Pretorius, 2007). Alternatively, matrix effects specific to red wine or post-fermentation losses during bottle storage may have inhibited its formation (Herbst-Johnstone *et al.*, 2011).

2-FM is associated with roasted coffee aromas and is formed by the addition of H_2S to furfural, a compound extracted from oak (Blanchard *et al.*, 2001). This reaction highlights the pivotal role of oak chips in 2-FM formation and supports the findings of this study. In the present work, 2-FM concentrations ranged from 3.1 to 4.0 ng/L, clearly exceeding the reported odour detection threshold of 0.4 ng/L, indicating a likely perceptible contribution to the wine's aroma profile.

BM is associated with smoky, mineral notes (*e.g.*, flint) and is considered a stylistic element in varieties such as Chardonnay and Sauvignon blanc, rather than a fault (Tominaga *et al.*, 2003). Although its formation is not fully understood, it is hypothesised to result from the addition of H_2S or other reductive compounds to benzaldehyde (Tominaga *et al.*, 2003). This mechanism was recently supported by Cordente *et al.* (2024), who observed increased BM formation in fermentations spiked with benzaldehyde—however, only when high-H_2S-producing yeast strains were used. The observed reduction in BM due to oak may be attributed to interactions with ellagitannins (Petit *et al.*, 2015). In the present study, BM concentrations ranged from 5.4 to 7.4 ng/L, well above the reported odour detection threshold of 0.3 ng/L, suggesting that it likely contributed perceptibly to the wines' aromatic profile.

4. Volatile sulfur-containing components

Table 6 presents the quantified concentrations of low-boiling-point sulfur-containing compounds. Volatile sulfur compounds such as H_2S, MeSH, and ethanethiol are known to contribute to off-flavours reminiscent of rotten eggs, cheese, leeks, onions, and garlic at elevated concentrations. However, at sub-threshold levels, they may enhance wine complexity (Siebert *et al.*, 2009).

H_2S concentrations differed significantly among the variants ($p = 0.011$), with oak chips (**) and GSH-IDY (***) exerting significant to highly significant effects, while oxygen had no influence. The control variant exhibited a moderate H_2S concentration. There were slight reductions in BF_V1 and BF_V4 (oak chips + oxygen), whereas BF_V2 and BF_V3 showed slightly elevated levels.

The MeSH concentrations did not differ significantly between the variants ($p = 0.083$). GSH-IDY exhibited a significant effect (**), whereas oak chips and oxygen had no significant impact. The concentrations remained relatively similar across all treatments, suggesting minimal modulation by the experimental factors.

The dimethyl sulphide (DMS) concentrations also showed no significant differences among the variants ($p = 0.090$), although oxygen exerted a highly significant effect (***), while oak chips and GSH-IDY showed no influence. The concentrations indicated no clear trends among the variants.

The carbon disulfide (CS_2) concentrations varied significantly among the variants ($p = 0.006$), with oak chips (***) and GSH-IDY (***) showing highly significant effects, while oxygen showed no influence.

These findings suggest that GSH-IDY, particularly when combined with oak chips, may promote H_2S formation. Despite these moderate concentrations—likely mitigated by the sufficiently high YAN concentration of 296 mg/L in the must (Bell & Henschke, 2005)—H_2S remains of sensory concern due to its low perception threshold and potential to cause off-aromas (Siebert *et al.*, 2009).

MeSH, associated with cheese and cooked vegetable notes, was detected at concentrations below its odour threshold, and is therefore unlikely to have contributed perceptibly to the aroma profile of the wines (Solomon *et al.*, 2010).

At low concentrations, DMS may contribute to positive aromas such as blackcurrant, red fruit, and truffle (Escudero *et al.*, 2007; Vidal & Aagaard, 2008). However,

TABLE 6. The content of volatile sulfur-containing components and their modulation in Blaufränkisch wines after the application of selected additives and treatment aids.

Volatile sulfur-containing components [µg/L]	Significance of the experimental factors			Control and experimental variants [$\bar{x} \pm \sigma$] (n = 3)				
	Oak chips 80 g/100 kg Incanto Natural®	GSH-IDY 30 g/100 kg ProBlanco®	O_2 15 mg/kg MicroOx®	Control	BF_V1 (+) Oak chips (–) GSH-IDY (–) O_2	BF_V2 (+) Oak chips (+) GSH-IDY (+) O_2	BF_V3 (+) Oak chips (+) GSH-IDY (–) O_2	BF_V4 (+) Oak chips (–) GSH-IDY (+) O_2
H_2S (ODT: 1.1–1.6 µg/L) [p = **0.011**]	**	***	n.s.	$1.9^{ab} \pm 0.2$	$1.6^{a} \pm 0.1$	$2.0^{b} \pm 0.2$	$2.0^{b} \pm 0.3$	$1.5^{a} \pm 0.1$
MeSH (ODT: 1.8–3.1 µg/L) [p = 0.083]	n.s.	**	n.s.	$2.1^{a} \pm 0.2$	$2.0^{a} \pm 0.1$	$2.2^{a} \pm 0.2$	$2.1^{a} \pm 0.1$	$2.0^{a} \pm 0.1$
EtSH	-	-	-	n.d.	n.d.	n.d.	n.d.	n.d.
DMS (ODT: 27–60 µg/L) [p = 0.090]	n.s.	n.s.	***	$9.5^{a} \pm 0.5$	$7.8^{a} \pm 0.3$	$9.0^{a} \pm 0.6$	$8.5^{a} \pm 0.9$	$7.5^{a} \pm 0.2$
CS_2 [p = **0.006**]	***	***	n.s.	$0.2^{c} \pm 0.2$	$0.1^{ab} \pm 0.1$	$0.4^{bc} \pm 0.2$	$0.2^{abc} \pm 0.1$	$0.4^{a} \pm 0.1$
MeSAc	-	-	-	n.d.	n.d.	n.d.	n.d.	n.d.
DMDS	-	-	-	n.d.	n.d.	n.d.	n.d.	n.d.
EtSAc	-	-	-	n.d.	n.d.	n.d.	n.d.	n.d.
DEDS	-	-	-	n.d.	n.d.	n.d.	n.d.	n.d.
DMTS	-	-	-	n.d.	n.d.	n.d.	n.d.	n.d.

The three columns to the right of the parameter column present the results of the three-way analysis of variance, indicating the main effects of oak chips, GSH-IDY, and oxygen (** $p < 0.01$; *** $p < 0.001$; n.s., not significant). The adjacent columns to the right contain the quantified concentrations ($\bar{x} \pm \sigma$) of individual compounds for the control group and the four experimental variants. Statistically significant differences ($p < 0.05$) between the variants are indicated by different superscript letters. Bold p-values indicate statistically significant differences at $p < 0.05$. GSH-IDY, glutathione-rich inactivated yeast; n.d., not detectable; ODT, Odour Detection Threshold.

it may become problematic at higher levels due to vegetal or unpleasant notes (Mestres *et al.*, 2000). In this study, DMS concentrations ranged between 7.5 and 9.5 µg/L, remaining well below the reported perception threshold of 27–60 µg/L. This suggests that any sensory impact would likely be subtle and potentially positive.

Recent research indicates that moderate oxygen addition during fermentation acts primarily as a preventive measure to reduce the formation of malodorous sulfur compounds such as H_2S and mercaptans (Nelson *et al.*, 2025). In the case of Blaufränkisch, where nitrogen availability was high, H_2S production was minimal from the outset, resulting in only trace levels of the downstream compound carbon disulfide (CS_2). Consequently, the single oxygen addition of 15 mg/L had no measurable effect on the already low H_2S and CS_2 concentrations. Moreover, CS_2 is relatively unreactive and is not degraded by moderate amounts of oxygen (Zeng *et al.*, 2019). These findings are consistent with other studies, which report that while oxygen additions can reduce reductive off-flavours overall, absolute H_2S and CS_2 levels remain unchanged in the low µg/L range (Lyu *et al.*, 2021).

5. Higher alcohols

Table 7 presents the quantified concentrations of higher alcohols, which are formed during yeast metabolism and contribute significantly to the sensory profile of wine (Valero *et al.*, 2002; Garde-Cerdán *et al.*, 2021). They are associated with aroma descriptors such as rose, lilac, ripe fruit, herbs, and whisky (Francis & Newton, 2005; Perestrelo *et al.*, 2020). While concentrations below 300 mg/L are generally considered to enhance aromatic complexity, levels exceeding 400 mg/L may have a detrimental effect on wine quality by contributing to harsh or solvent-like notes (Rapp & Versini, 1995; Ribéreau-Gayon *et al.*, 2006; Garde-Cerdán *et al.*, 2021).

The propanol and isobutanol concentrations did not differ significantly among the variants (p = 0.259 and 0.459, respectively). None of the three experimental factors exerted a significant influence. Isoamyl alcohol (p = 0.022) was significantly influenced by oak chips, with all oak-treated variants exhibiting higher concentrations than the control. Hence, oak chips may promote isoamyl alcohol formation during fermentation. Butanol also differed significantly among the variants (p = 0.005), with GSH-IDY emerging as the main influencing factor, while oak chips and oxygen

TABLE 7. The content of higher alcohols and carboxylic acids and their modulation in Blaufränkisch wines after the application of selected additives and treatment aids.

Higher alcohols [mg/L] and carboxylic acids [mg/L]	Significance of the experimental factors			Control and experimental variants [$\bar{x} \pm \sigma$] (n = 3)				
	Oak chips 80 g/100 kg Incanto Natural®	GSH-IDY 30 g/100 kg ProBlanco®	O_2 15 mg/kg MicroOx®	Control	BF_V1 (+) Oak chips (–) GSH-IDY (–) O_2	BF_V2 (+) Oak chips (+) GSH-IDY (+) O_2	BF_V3 (+) Oak chips (+) GSH-IDY (–) O_2	BF_V4 (+) Oak chips (–) GSH-IDY (+) O_2
Propanol [p = 0.259]	n.s.	n.s.	n.s.	$53.3^{a} \pm 2.4$	$50.3^{a} \pm 3.9$	$48.7^{a} \pm 0.7$	$51.3^{a} \pm 2.5$	$49.7^{a} \pm 1.6$
Isobutanol [p = 0.459]	n.s.	n.s.	n.s.	$12.7^{a} \pm 2.1$	$10.7^{a} \pm 0.8$	$11.7^{a} \pm 1.1$	$12.2^{a} \pm 1.1$	$11.4^{a} \pm 1.0$
Isoamyl alcohol [p = **0.022**]	***	n.s.	n.s.	$237.4^{a} \pm 2.2$	$261.4^{b} \pm 12.8$	$263.4^{b} \pm 5.3$	$263.4^{b} \pm 11.3$	$259.2^{b} \pm 10.3$
Butanol [p = **0.005**]	n.s.	***	n.s.	$1.2^{a} \pm 0.1$	$1.3^{a} \pm 0.1$	$1.3^{ab} \pm 0.0$	$1.5^{b} \pm 0.1$	$1.2^{a} \pm 0.1$
Σ Higher alcohols [p = 0.122]	-	-	-	$304.6^{a} \pm 4.3$	$323.7^{a} \pm 15.7$	$325.1^{a} \pm 5.6$	$328.4^{a} \pm 12.1$	$321.6^{a} \pm 11.6$
Propionic acid [p = 0.116]	**	n.s.	n.s.	$0.6^{a} \pm 0.1$	$0.5^{a} \pm 0.1$	$0.5^{a} \pm 0.1$	$0.4^{a} \pm 0.1$	$0.5^{a} \pm 0.1$
Isobutyric acid [p = **0.001**]	**	n.s.	n.s.	$10.3^{b} \pm 2.5$	$6.6^{ab} \pm 1.3$	$5.4^{a} \pm 0.7$	$4.1^{a} \pm 0.7$	$4.1^{a} \pm 0.8$
Butyric acid [p = **0.013**]	n.s.	n.s.	n.s.	$1.8^{a} \pm 0.2$	$1.9^{a} \pm 0.1$	$2.1^{ab} \pm 0.2$	$2.1^{ab} \pm 0.1$	$2.3^{b} \pm 0.2$
Isovaleric acid	-	-	-	n.d.	n.d.	n.d.	n.d.	n.d.
Hexanoic acid [p < **0.001**]	n.s.	n.s.	n.s.	$0.7^{a} \pm 0.0$	$0.7^{a} \pm 0.0$	$0.8^{ab} \pm 0.0$	$0.9^{b} \pm 0.0$	$0.9^{b} \pm 0.1$
Octanoic acid [p < **0.001**]	***	n.s.	n.s.	$0.5^{a} \pm 0.0$	$0.6^{b} \pm 0.1$	$0.7^{b} \pm 0.0$	$0.7^{b} \pm 0.0$	$0.8^{c} \pm 0.0$
Decanoic acid [p < **0.001**]	n.s.	n.s.	**	$0.2^{a} \pm 0.0$	$0.2^{a} \pm 0.0$	$0.2^{b} \pm 0.0$	$0.2^{b} \pm 0.0$	$0.3^{c} \pm 0.0$
Σ Carbon acids [p = **0.005**]	-	-	-	$14.1^{b} \pm 2.6$	$10.5^{a} \pm 1.5$	$9.7^{a} \pm 1.0$	$8.4^{a} \pm 0.9$	$8.9^{a} \pm 0.6$

The three columns to the right of the parameter column present the results of the three-way analysis of variance, indicating the main effects of oak chips, GSH-IDY, and oxygen (** $p < 0.01$; *** $p < 0.001$; n.s., not significant). The adjacent columns to the right contain the quantified concentrations ($\bar{x} \pm \sigma$) of individual compounds for the control group and the four experimental variants. Statistically significant differences ($p < 0.05$) between the variants are indicated by different superscript letters. Bold p-values indicate statistically significant differences at $p < 0.05$. GSH-IDY, glutathione-rich inactivated yeast; n.d., not detectable.

showed no significant effects. BF_V3 showed the highest concentration, while the control variant and BF_V4 showed the lowest concentrations.

These stable concentrations of propanol and isobutanol are consistent with the findings of Gabrielli *et al.* (2017), who observed no clear effect of glutathione on these alcohols. At moderate levels, both compounds contribute to subtle lilac and herbal notes and enhance the aromatic complexity of wine without reaching concentrations that negatively affect sensory perception (Ribéreau-Gayon *et al.*, 2006).

Isoamyl alcohol contributes to the aromas of ripe fruit and herbs (Francis & Newton, 2005) and may have enhanced the aromatic complexity of the Blaufränkisch wines evaluated in this study. However, these findings contrast with those of Gabrielli *et al.* (2017), who reported a positive effect of glutathione on the isoamyl alcohol concentration.

The findings regarding butanol underscore the role of glutathione in maintaining redox balance and stabilising volatile compounds, as described in the literature (Gabrielli *et al.*, 2017). Butanol was present at levels unlikely to produce sensory faults but may contribute to subtle, alcohol-related aroma nuances (Francis & Newton, 2005).

The total higher alcohol concentration ranged from 304 mg/L (control) to 328 mg/L (BF_V3), which falls within the typical range reported in the literature (Francis & Newton, 2005; Gabrielli *et al.*, 2017). Overall, these results suggest that higher alcohols in Blaufränkisch wines may contribute positively to aromatic complexity and mouthfeel. The findings further indicate that oak chips play a significant role in modulating isoamyl alcohol, while GSH-IDY appears to enhance butanol formation.

6. Carboxylic acids

Table 7 also presents the concentrations of quantified carboxylic acids. These compounds are associated with aroma descriptors such as fruity, fatty, cheesy, sweaty, buttery,

and rancid (Francis & Newton, 2005), and contribute to the sensory profile of wine depending on their concentration and composition. Carboxylic acids, particularly those responsible for buttery or rancid notes, can be modulated by different vinification treatments (Carpena *et al.*, 2020; Garde-Cerdán *et al.*, 2021).

Propionic acid remained largely stable under the tested conditions, with only oak chips exerting a moderate influence, while GSH-IDY and oxygen had no significant effects. In contrast, isobutyric acid was significantly reduced by oak chip treatment. Neither GSH-IDY nor oxygen had a notable effect.

There were significant differences in butyric acid among the variants, but there were no clear effects of the three experimental factors. Some variants exhibited slightly higher concentrations than the control, but the differences did not reach statistical significance. Hexanoic acid showed a similar pattern: the concentrations varied among the variants, but oak chips, GSH-IDY, and oxygen did not show a significant influence. BF_V3 and BF_V4 had the highest concentrations, while the control and BF_V1 showed the lowest concentrations.

Octanoic acid emerged as a clear exception, with oak chips having a highly significant impact. The control variant showed the lowest concentration, while BF_V4 had the highest, suggesting that oak chips may promote octanoic acid formation. There was a similar trend for decanoic acid, for which oxygen exerted a significant influence. However, the differences between the variants were minor and may have been affected by analytical variability.

These results align with Gabrielli *et al.* (2017), who noted that treatments such as GSH-IDY addition can differentially affect the concentrations of specific fatty acids, including hexanoic, octanoic, and decanoic acid.

These findings highlight the partially significant role of oak chips in modulating fatty acid concentrations, while GSH-IDY and oxygen had less consistent effects. Previous studies, such as the one by Gabrielli *et al.* (2017), have reported that glutathione increases the concentrations of certain fatty acids, including hexanoic and octanoic acid. The discrepancies may be explained by different experimental conditions, such as nitrogen availability and/or storage time. The present results suggest that further research is needed to better understand the specific interactions between oak chips, GSH-IDY, and oxygen and their influence on wine aroma development.

7. Esters

Table 8 presents the quantified concentrations of esters. These volatile aroma compounds are synthesised during alcoholic fermentation through the reaction of alcohols with carboxylic acids and play a central role in the aromatic expression of wine by contributing fruity and floral notes. Ethyl and acetate esters, in particular, are considered key contributors to sensory quality due to their typically high concentrations, which often exceed olfactory thresholds (Carpena *et al.*, 2020; Garde-Cerdán *et al.*, 2021).

The ester concentrations in the analysed Blaufränkisch wines were partially influenced by oak chips, GSH-IDY, and oxygen. However, compounds such as ethyl benzoate, ethyl lactate, ethyl propanoate, ethyl decanoate, and ethyl palmitate showed no significant differences among the variants, suggesting that their formation is less susceptible to oxidative or fermentative modulation. This observation is consistent with Gabrielli *et al.* (2017), who reported similar stability for ethyl esters of long-chain fatty acids under varying oenological conditions.

In contrast, esters such as ethyl phenylacetate, diethyl succinate, and ethyl butanoate exhibited significant variations, particularly due to oak chips, GSH-IDY, and oxygen. Oak chips appeared to promote the formation of ethyl phenylacetate and diethyl succinate, possibly due to indirect effects on yeast metabolism, such as modulating the fermentation environment (*e.g.*, oxygen availability or adsorption of inhibitory compounds), which may favour enhanced ester biosynthesis during alcoholic fermentation (Sánchez-Palomo *et al.*, 2017). Diethyl succinate was also enhanced by oxygen. GSH-IDY showed a predominantly stabilising effect and significantly influenced compounds such as ethyl butanoate and ethyl octanoate, consistent with its known antioxidant properties (Papadopoulou & Roussis, 2008; Roussis *et al.*, 2009). Ethyl octanoate, known to contribute significantly to the fruity aroma profile of wine, increased in response to both oak chips and GSH-IDY (Francis & Newton, 2005; Swiegers *et al.*, 2005).

Hexyl acetate showed significant variations across the variants but no consistent correlation with the three experimental factors. In contrast, isobutyl acetate concentrations, which also varied significantly, were reduced by the addition of oak chips. There were similar trends for methyl hexanoate and methyl laurate, which presented significant differences, although the practical impact was minor. These variations may be attributable to analytical fluctuations or unaccounted factors, as has been reported in studies involving comparable grape varieties (Andújar-Ortiz *et al.*, 2014).

Interestingly, isoamyl acetate, a key contributor to fruity aromas, remained stable under the applied experimental conditions, despite its known sensitivity to fermentation parameters (Valero *et al.*, 2002). In contrast, esters such as ethyl hexanoate and ethyl laurate showed significant changes, particularly following the addition of oak chips and GSH-IDY, suggesting that the formation of these esters may be particularly influenced by specific oenological factors. (Papadopoulou & Roussis, 2008).

With regard to esters, the results of this study highlight the central role of GSH-IDY in their stabilisation and enhancement, while oak chips promoted the formation of compounds such as diethyl succinate. These findings are consistent with previous studies highlighting the antioxidant and aroma-stabilising properties of glutathione (Roussis *et al.*, 2009; Bahut *et al.*, 2020) and underline the potential to improve wine sensory quality through targeted oenological strategies.

TABLE 8. The ester content and its modulation in Blaufränkisch wines after the application of selected additives and treatment aids. (part 1/2)

Esters [µg/L]	Significance of the experimental factors			Control and experimental variants [$\bar{x} \pm \sigma$] (n = 3)				
	Oak chips 80 g/100 kg Incanto Natural®	GSH-IDY 30 g/100 kg ProBlanco®	O_2 15 mg/kg MicroOx®	Control	BF_V1 (+) Oak chips (–) GSH-IDY (–) O_2	BF_V2 (+) Oak chips (+) GSH-IDY (+) O_2	BF_V3 (+) Oak chips (+) GSH-IDY (–) O_2	BF_V4 (+) Oak chips (–) GSH-IDY (+) O_2
Ethyl benzoate [p = 0.074]	n.s.	n.s.	n.s.	$0.3^{a} \pm 0.0$	$0.3^{a} \pm 0.0$	$0.4^{a} \pm 0.0$	$0.4^{a} \pm 0.0$	$0.4^{a} \pm 0.0$
Ethyl phenylacetate [p = **0.012**]	**	n.s.	n.s.	$4.2^{a} \pm 0.2$	$4.9^{ab} \pm 0.4$	$5.3^{b} \pm 0.0$	$5.5^{b} \pm 0.8$	$5.7^{b} \pm 0.3$
Σ Aromatic esters [p = **0.011**]	-	-	-	$4.6^{a} \pm 0.2$	$5.3^{ab} \pm 0.4$	$5.7^{b} \pm 0.1$	$5.9^{b} \pm 0.9$	$6.1^{b} \pm 0.3$
Ethyl lactate [mg/L; p = 0.472]	n.s.	n.s.	n.s.	$162.9^{a} \pm 24.5$	$178.9^{a} \pm 28.7$	$156.2^{a} \pm 7.2$	$153.3^{a} \pm 2.6$	$160.6^{a} \pm 2.9$
Ethyl acetate [mg/L; p = **0.007**]	***	**	n.s.	$39.8^{b} \pm 0.7$	$34.5^{a} \pm 2.4$	$38.4^{b} \pm 0.9$	$39.0^{b} \pm 1.3$	$37.5^{b} \pm 0.3$
Ethyl propanoate [mg/L; p = 0.075]	n.s.	n.s.	n.s.	$0.1^{a} \pm 0.0$	$0.1^{a} \pm 0.0$	$0.1^{a} \pm 0.0$	$0.1^{a} \pm 0.0$	$0.1^{a} \pm 0.0$
Diethyl succinate [mg/L; p < **0.001**]	**	n.s.	***	$1.1^{a} \pm 0.0$	$1.2^{b} \pm 0.0$	$1.4^{c} \pm 0.0$	$1.4^{c} \pm 0.1$	$1.6^{d} \pm 0.1$
Σ Major ethyl esters [mg/L; p < **0.001**]	-	-	-	$204.0^{a} \pm 24.5$	$214.7^{a} \pm 29.5$	$196.2^{b} \pm 7.0$	$193.8^{b} \pm 3.3$	$199.9^{c} \pm 2.7$
Pentyl acetate [p = 0.340]	n.s.	n.s.	n.s.	$0.2^{a} \pm 0.0$	$0.1^{a} \pm 0.0$	$0.1^{a} \pm 0.0$	$0.1^{a} \pm 0.0$	$0.2^{a} \pm 0.0$
Isobutyl acetate [p < **0.001**]	***	n.s.	n.s.	$44.7^{c} \pm 1.2$	$37.4^{a} \pm 1.9$	$40.9^{b} \pm 1.4$	$42.9^{bc} \pm 0.7$	$42.2^{bc} \pm 0.7$
Isoamyl acetate [p = 0.062]	n.s.	n.s.	n.s.	$412.3^{a} \pm 20.1$	$424.1^{a} \pm 40.4$	$456.5^{a} \pm 20.8$	$468.6^{a} \pm 8.5$	$450.5^{a} \pm 3.8$
Hexyl acetate [p < **0.001**]	n.s.	n.s.	n.s.	$2.6^{bc} \pm 0.2$	$2.0^{a} \pm 0.1$	$2.4^{b} \pm 0.1$	$2.8^{c} \pm 0.2$	$2.5^{bc} \pm 0.1$
Σ Acetate esters of higher alcohols [p = 0.070]	-	-	-	$459.7^{a} \pm 21.2$	$463.6^{a} \pm 41.9$	$499.9^{a} \pm 21.8$	$514.5^{a} \pm 8.8$	$495.5^{a} \pm 3.2$
Ethyl butanoate [p < **0.001**]	n.s.	**	n.s.	$153.0^{b} \pm 3.0$	$138.5^{a} \pm 4.9$	$152.6^{b} \pm 1.0$	$157.2^{b} \pm 4.4$	$155.0^{b} \pm 0.4$
Ethyl hexanoate [p < **0.001**]	**	n.s.	n.s.	$203.3^{a} \pm 6.3$	$212.0^{b} \pm 0.6$	$223.1^{c} \pm 5.8$	$225.3^{c} \pm 0.6$	$227.8^{c} \pm 2.0$
Ethyl octanoate [p < **0.001**]	***	**	**	$160.2^{a} \pm 3.9$	$179.7^{bc} \pm 9.9$	$180.3^{bc} \pm 3.3$	$175.3^{b} \pm 2.7$	$189.3^{c} \pm 1.2$
Ethyl decanoate [p = 0.178]	n.s.	n.s.	n.s.	$1.7^{a} \pm 0.1$	$1.7^{a} \pm 0.2$	$1.7^{a} \pm 0.0$	$1.7^{a} \pm 0.1$	$1.9^{a} \pm 0.0$
Ethyl laurate [p < **0.001**]	***	**	n.s.	$17.5^{c} \pm 1.4$	$11.1^{a} \pm 1.3$	$12.9^{ab} \pm 0.7$	$14.6^{bc} \pm 1.6$	$12.6^{ab} \pm 0.7$
Ethyl myristate [p < **0.001**]	**	**	n.s.	$2.1^{b} \pm 0.2$	$1.3^{a} \pm 0.1$	$1.8^{b} \pm 0.2$	$2.1^{b} \pm 0.2$	$1.9^{b} \pm 0.2$
Ethyl palmitate [p = 0.070]	n.s.	n.s.	n.s.	$12.3^{a} \pm 1.1$	$9.5^{a} \pm 1.6$	$11.7^{a} \pm 2.1$	$14.1^{a} \pm 1.6$	$12.1^{a} \pm 1.8$
Σ Ethyl esters of carboxylic acids with an even number of carbon atoms [p < **0.001**]	-	-	-	$550.1^{a} \pm 11.0$	$553.9^{a} \pm 16$	$584.1^{b} \pm 8.1$	$590.3^{b} \pm 7.9$	$600.5^{b} \pm 4.9$

The three columns to the right of the parameter column present the results of the three-way analysis of variance, indicating the main effects of oak chips, GSH-IDY, and oxygen (** $p < 0.01$; *** $p < 0.001$; n.s., not significant). The adjacent columns to the right contain the quantified concentrations ($\bar{x} \pm \sigma$) of individual compounds for the control group and the four experimental variants. Statistically significant differences ($p < 0.05$) between the variants are indicated by different superscript letters. Bold p-values indicate statistically significant differences at $p < 0.05$. GSH-IDY, glutathione-rich inactivated yeast; n.d., not detectable.

TABLE 8. The ester content and its modulation in Blaufränkisch wines after the application of selected additives and treatment aids. (part 2/2)

Isobutyl propionate [p = 0.063]	**	**	n.s.	$29.8^{a} \pm 2.7$	$25.9^{a} \pm 2.2$	$28.1^{a} \pm 0.9$	$29.4^{a} \pm 1.6$	$25.9^{a} \pm 1.2$
Propyl propionate [p = 0.695]	n.s.	n.s.	n.s.	$1.6^{a} \pm 0.1$	$1.6^{a} \pm 0.0$	$1.6^{a} \pm 0.0$	$1.6^{a} \pm 0.1$	$1.6^{a} \pm 0.0$
Pentyl butanoate [p = 0.902]	n.s.	n.s.	n.s.	$0.1^{a} \pm 0.0$	$0.1^{a} \pm 0.0$	$0.1^{a} \pm 0.0$	$0.1^{a} \pm 0.0$	$0.1^{a} \pm 0.0$
Isobutyl hexanoate [p = **0.013**]	n.s.	n.s.	***	$0.4^{a} \pm 0.0$	$0.5^{ab} \pm 0.0$	$0.5^{b} \pm 0.0$	$0.5^{ab} \pm 0.0$	$0.5^{b} \pm 0.0$
Butyl butanoate [p = 0.471]	n.s.	n.s.	n.s.	$14.8^{a} \pm 0.3$	$15.1^{a} \pm 0.1$	$15.1^{a} \pm 0.1$	$14.9^{a} \pm 0.2$	$15.0^{a} \pm 0.0$
Propyl octanoate [p = 0.903]	n.s.	n.s.	n.s.	$0.1^{a} \pm 0.0$	$0.1^{a} \pm 0.0$	$0.1^{a} \pm 0.0$	$0.1^{a} \pm 0.0$	$0.1^{a} \pm 0.0$
Isobutyl octanoate	-	-	-	n.d.	n.d.	n.d.	n.d.	n.d.
Σ Esters of higher alcohols and medium-length carboxylic acids [p = 0.073]	-	-	-	$46.7^{a} \pm 2.4$	$43.1^{a} \pm 2.3$	$45.4^{a} \pm 1$	$46.5^{a} \pm 1.6$	$43.1^{a} \pm 1.3$
Ethyl valerate [p = **0.017**]	n.s.	***	n.s.	$1.7^{a} \pm 0.2$	$1.7^{a} \pm 0.1$	$1.8^{ab} \pm 0.0$	$2.1^{b} \pm 0.1$	$1.7^{a} \pm 0.1$
Ethyl heptanoate [p = **0.004**]	***	***	n.s.	$1.1^{b} \pm 0.0$	$1.0^{a} \pm 0.0$	$1.1^{b} \pm 0.1$	$1.1^{b} \pm 0.1$	$0.9^{a} \pm 0.0$
Σ Ethyl esters of carboxylic acids with an odd number of carbon atoms [p = **0.006**]	-	-	-	$2.8^{ab} \pm 0.2$	$2.7^{a} \pm 0.1$	$2.9^{ab} \pm 0.1$	$3.1^{b} \pm 0.2$	$2.6^{a} \pm 0.1$
Butyl isobutyrate [p = 0.258]	n.s.	n.s.	n.s.	$0.1^{a} \pm 0.0$	$0.1^{a} \pm 0.0$	$0.1^{a} \pm 0.0$	$0.1^{a} \pm 0.0$	$0.1^{a} \pm 0.0$
Propyl isoamylate [p = 0.690]	-	-	-	n.d.	n.d.	n.d.	n.d.	n.d.
Isoamyl isovalerate [p = 0.095]	n.s.	n.s.	n.s.	$0.1^{a} \pm 0.0$	$0.1^{a} \pm 0.0$	$0.1^{a} \pm 0.0$	$0.1^{a} \pm 0.0$	$0.1^{a} \pm 0.0$
Σ Esters of branched carboxylic acids [p = 0.327]	-	-	-	$0.2^{a} \pm 0.0$	$0.2^{a} \pm 0.0$	$0.2^{a} \pm 0.0$	$0.2^{a} \pm 0.0$	$0.2^{a} \pm 0.0$
Isoamyl butyrate [p = 0.934]	n.s.	n.s.	n.s.	$0.1^{a} \pm 0.0$	$0.1^{a} \pm 0.0$	$0.1^{a} \pm 0.0$	$0.1^{a} \pm 0.0$	$0.1^{a} \pm 0.0$
Isoamyl hexanoate [p = 0.832]	n.s.	n.s.	n.s.	$0.4^{a} \pm 0.0$	$0.4^{a} \pm 0.0$	$0.4^{a} \pm 0.0$	$0.4^{a} \pm 0.0$	$0.4^{a} \pm 0.0$
Σ Isoamyl ester of medium-length carboxylic acids [p = **0.009**]	-	-	-	$0.4^{a} \pm 0.0$	$0.5^{b} \pm 0.0$	$0.5^{b} \pm 0.0$	$0.5^{b} \pm 0.0$	$0.5^{b} \pm 0.0$
Methyl hexanoate [p = **0.002**]	***	***	n.s.	$0.6^{b} \pm 0.0$	$0.4^{a} \pm 0.0$	$0.5^{ab} \pm 0.0$	$0.5^{b} \pm 0.1$	$0.4^{a} \pm 0.0$
Methyl octanoate [p = **0.026**]	***	n.s.	n.s.	$0.7^{b} \pm 0.0$	$0.6^{a} \pm 0.0$	$0.7^{ab} \pm 0.0$	$0.7^{ab} \pm 0.0$	$0.7^{ab} \pm 0.0$
Methyl decanoate [p = 0.789]	n.s.	n.s.	n.s.	$0.3^{a} \pm 0.0$	$0.3^{a} \pm 0.0$	$0.3^{a} \pm 0.0$	$0.3^{a} \pm 0.0$	$0.3^{a} \pm 0.0$
Methyl laurate [p < **0.001**]	**	**	n.s.	$0.2^{b} \pm 0.0$	$0.1^{a} \pm 0.0$	$0.1^{a} \pm 0.0$	$0.2^{b} \pm 0.0$	$0.1^{a} \pm 0.0$
Methyl myristate [p < **0.001**]	**	***	**	$0.2^{b} \pm 0.0$	$0.1^{a} \pm 0.0$	$0.1^{a} \pm 0.0$	$0.2^{b} \pm 0.0$	$0.1^{a} \pm 0.0$
Σ Methyl esters [p < **0.001**]	-	-	-	$2.0^{b} \pm 0.1$	$1.6^{a} \pm 0.1$	$1.7^{a} \pm 0.0$	$1.9^{b} \pm 0.1$	$1.6^{a} \pm 0.1$

The three columns to the right of the parameter column present the results of the three-way analysis of variance, indicating the main effects of oak chips, GSH-IDY, and oxygen (** $p < 0.01$; *** $p < 0.001$; n.s., not significant). The adjacent columns to the right contain the quantified concentrations ($\bar{x} \pm \sigma$) of individual compounds for the control group and the four experimental variants. Statistically significant differences ($p < 0.05$) between the variants are indicated by different superscript letters. Bold p-values indicate statistically significant differences at $p < 0.05$. GSH-IDY, glutathione-rich inactivated yeast; n.d., not detectable.

The overall limited influence of the experimental factors on ester concentrations may be partly due to the relatively short bottle ageing period of five months. Esters are known to degrade over time via oxidation or hydrolysis, processes that can be mitigated by substances such as glutathione, which preserve ester stability and sensory integrity (Roussis *et al.*, 2009). Oxygen exhibited only minor effects under the present conditions but appeared to contribute to the formation of certain esters, such as diethyl succinate and ethyl octanoate. These observations are consistent with previous studies, which report that oxygen additions during red wine fermentation can lead to modest increases in specific esters, particularly short-chain ethyl and acetate esters (Day *et al.*, 2021). However, the magnitude of these changes is typically limited, with some reports even indicating slight reductions depending on grape variety and oxygen dosage (Picariello *et al.*, 2020).

8. Free C_{13}-norisoprenoids

Table 9 presents the quantified concentrations of C_{13}-norisoprenoids. These chemically diverse compounds—including ketones, aldehydes, and alcohols—have a 13-carbon backbone. Among the most prominent representatives are β-damascenone, associated with apple, rose, dried fruit, honey, and exotic floral notes, and β-ionone, which contributes to raspberry-like, violet, and other floral or fruity aromas (Sefton *et al.*, 2011; Robinson *et al.*, 2014). These compounds enhance the fruity character of wines and can mask herbaceous or green-grassy notes originating from methoxypyrazines or C_6 alcohols (Pineau *et al.*, 2007; Escudero *et al.*, 2007).

The highly significant effects of oak chips, GSH-IDY, and oxygen on vitispirane concentrations are consistent with the literature: researchers have described complex interactions between wine matrix components and the release or preservation mechanisms of norisoprenoids. Rodríguez-Bencomo *et al.* (2011) reported that tannins and the polymerisation of phenolic compounds may influence the release of norisoprenoid ketones, including vitispirane. The elevated vitispirane concentration observed in BF_V4 may be attributed to phenolic compounds present in oak and their potential interactions.

The strong effect of GSH-IDY on β-damascenone is particularly noteworthy, as glutathione may protect norisoprenoids from oxidation through its free thiol group (Papadopoulou & Roussis, 2008). BF_V3 had the highest β-damascenone concentration, highlighting the stabilising role of GSH-IDY, while oak chips and oxygen had no significant influence. These results contrast with those of Rodríguez-Bencomo *et al.* (2011), who reported reduced norisoprenoid release at higher tannin levels. This discrepancy may be due to differences in phenolic composition or storage conditions.

The β-ionone concentrations differed significantly among the variants, although none of the tested factors showed a significant effect. Therefore, these variations may be attributed to uncontrolled variables or analytical uncertainty.

The consistent concentrations of TDN across all variants and the absence of significant factor effects confirm the chemical stability of this norisoprenoid under the present experimental conditions. TDN is commonly associated with kerosene-like aromas and typically increases in aged wines, suggesting a distinct dynamic of release and preservation (Gök, 2015).

In summary, the results underscore the central role of GSH-IDY in stabilising β-damascenone and the multifactorial influences of oak chips, GSH-IDY, and oxygen on the vitispirane concentration, while β-ionone and TDN remain largely unaffected.

TABLE 9. The content of free C_{13}-norisoprenoids and their modulation in Blaufränkisch wines after the application of selected additives and treatment aids.

Free C_{13}-norisopenoids [µg/L]	Significance of the experimental factors			Control and experimental variants [$\bar{x} \pm \sigma$] (n = 3)				
	Oak chips 80 g/100 kg Incanto Natural®	**GSH-IDY** 30 g/100 kg ProBlanco®	**O_2** 15 mg/kg MicroOx®	**Control**	**BF_V1** (+) Oak chips (–) GSH-IDY (–) O_2	**BF_V2** (+) Oak chips (+) GSH-IDY (+) O_2	**BF_V3** (+) Oak chips (+) GSH-IDY (–) O_2	**BF_V4** (+) Oak chips (–) GSH-IDY (+) O_2
Vitispirane [p < **0.001**]	***	***	***	$4.2^{a} \pm 0.1$	$4.7^{b} \pm 0.1$	$4.6^{b} \pm 0.1$	$4.1^{a} \pm 0.0$	$4.9^{c} \pm 0.0$
ß-Ionone [p < **0.001**]	n.s.	n.s.	n.s.	$0.6^{c} \pm 0.2$	$0.4^{ab} \pm 0.0$	$0.3^{a} \pm 0.0$	$0.5^{bc} \pm 0.0$	$1.0^{d} \pm 0.0$
TDN [p = 0.759]	n.s.	n.s.	n.s.	$0.1^{a} \pm 0.0$	$0.1^{a} \pm 0.0$	$0.1^{a} \pm 0.0$	$0.1^{a} \pm 0.0$	$0.1^{a} \pm 0.0$
ß-Damascenone [p < **0.001**]	n.s.	***	n.s.	$2.6^{a} \pm 0.1$	$2.6^{a} \pm 0.1$	$2.8^{b} \pm 0.0$	$3.0^{c} \pm 0.0$	$2.7^{a} \pm 0.0$
Σ Free C_{13}-norisoprenoids [p < **0.001**]	-	-	-	$7.5^{a} \pm 0.3$	$7.8^{a} \pm 0.2$	$7.8^{a} \pm 0.1$	$7.6^{a} \pm 0.0$	$8.7^{b} \pm 0.1$

The three columns to the right of the parameter column present the results of the three-way analysis of variance, indicating the main effects of oak chips, GSH-IDY, and oxygen (** $p < 0.01$; *** $p < 0.001$; n.s., not significant). The adjacent columns to the right contain the quantified concentrations ($\bar{x} \pm \sigma$) of individual compounds for the control group and the four experimental variants. Statistically significant differences ($p < 0.05$) between the variants are indicated by different superscript letters. Bold p-values indicate statistically significant differences at $p < 0.05$. GSH-IDY, glutathione-rich inactivated yeast.

9. Principal component analysis

To provide a comprehensive visual representation of the dataset, a PCA was performed on the analytical data. The Kaiser–Meyer–Olkin (KMO) measure of sampling adequacy was 52.35 %, which supports the suitability of PCA for the data (Leyer & Wesche, 2007).

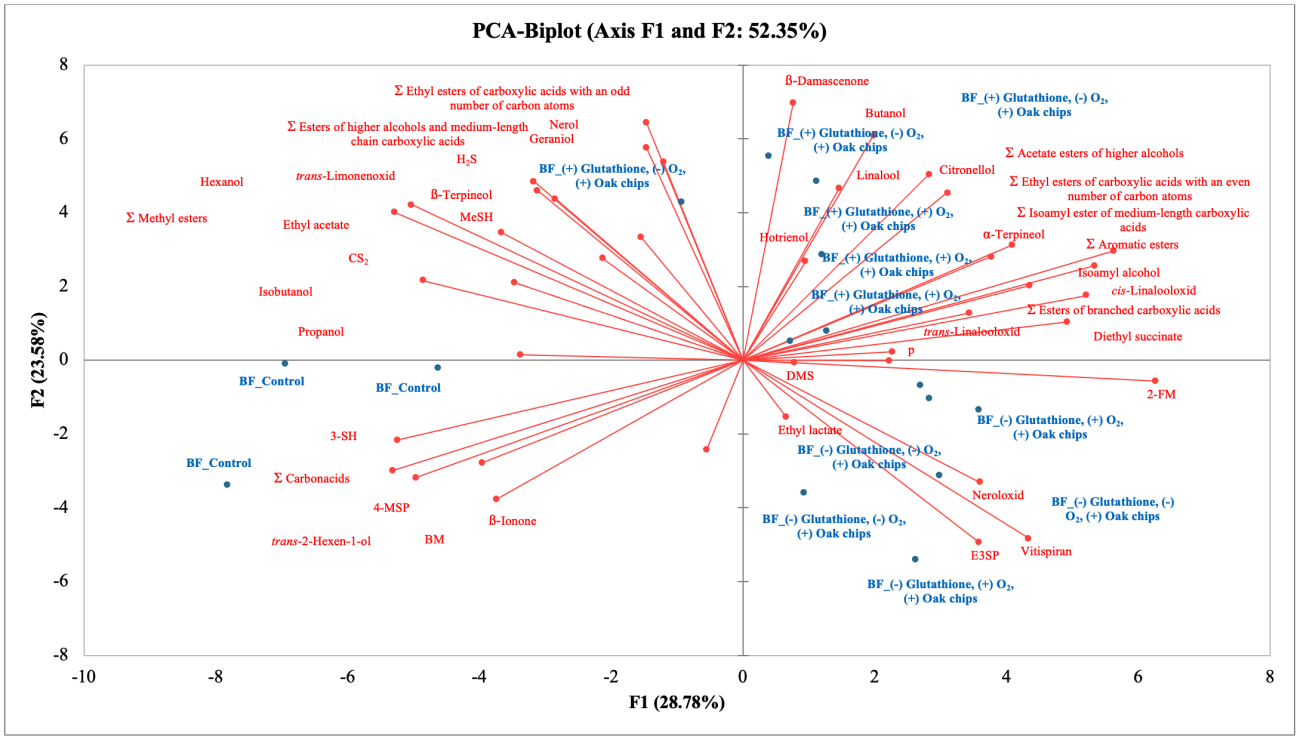
Figure 2 illustrates the relationship between the control and experimental variants and the quantified aroma compounds, with several variables presented as grouped parameters. The first two principal components (F1 and F2) accounted for nearly 53 % of the total variance, with F1 and F2 explaining 23.58 % and 28.78 %, respectively. The PCA revealed distinct positive correlations between specific analytical compounds and the individual treatment variants.

In the upper right quadrant, BF_V2 and BF_V3, both combining oak chips and GSH-IDY, showed strong positive correlations with butanol, acetate esters, even-chain ethyl esters, isoamyl esters, and aromatic esters, as well as with isoamyl alcohol, ethyl propanoate, DMS, and diethyl succinate. These compounds are known contributors to fruity, floral, and complex wine aromas (Rapp & Versini, 1995; Francis & Newton, 2005). As fermentation-derived volatiles, esters and higher alcohols are critical to wine flavour and aroma (Valero *et al.*, 2002; Ribéreau-Gayon *et al.*, 2006). Their positive association with these treatments may be attributed to the synergistic effects of oak and GSH-IDY, the latter potentially enhancing ester stability and concentration via antioxidant mechanisms (Rodríguez-Bencomo *et al.*, 2014; Bahut *et al.*, 2020). Linalool, α-terpineol, citronellol, and hotrienol, all monoterpenes associated with citrus and rose notes, also showed positive correlations with these variants (Kritzinger *et al.*, 2013). Notably, β-damascenone, which contributes to red fruit aromas, was positively associated with these treatments, suggesting a potential protective effect of GSH-IDY (Escudero *et al.*, 2007; Pineau *et al.*, 2007).

In the upper left quadrant, a biological replicate of BF_V3 correlated positively with odd-chain ethyl esters, esters of higher alcohols, and medium-length fatty acid esters, as well as with geraniol, nerol, hexanol, and *trans*-Limonene oxide compounds known to enhance floral and fruity notes and contribute to sensory complexity (Francis & Newton, 2005). These correlations may also be attributed to the antioxidant capacities of GSH-IDY and oak (Papadopoulou & Roussis, 2008; Andújar-Ortiz *et al.*, 2012). Additionally, volatile sulfur-containing compounds such as MeSH, H_2S, and CS_2 also correlated positively. These compounds can enhance complexity at low levels but cause sensory faults at higher concentrations (Siebert *et al.*, 2009; Solomon *et al.*, 2010).

In the lower left quadrant, all biological replicates of the control were associated with compounds such as β-ionone, 3-SH, 4-MSP, BM, and *trans*-2-hexen-1-ol. Both 3-SH and 4-MSP are thiols linked to blackcurrant, grapefruit, and tropical fruit aromas (Tominaga & Dubourdieu, 2003). The positive correlation between these thiols and *trans*-2-hexen-1-ol is particularly noteworthy. During fermentation, *trans*-2-hexen-1-ol can react with H_2S and glutathione to form intermediates such as Glut-3MHal (Clark & Deed, 2018). The strong correlation of the control with 3-SH and 4-MSP may be partly explained by higher levels of *trans*-2-hexen-1-ol, which is reduced significantly in treatments involving oak chips—potentially limiting thiol precursor availability.

FIGURE 2. Principal component analysis biplot correlating the individual analytical results of Blaufränkisch wines treated with oak chips, glutathione-rich inactivated yeast (GSH-IDY), and oxygen.



In the lower right quadrant, the biological replicates of BF_V1 and BF_V4 (both without GSH-IDY) were associated with ethyl lactate, vitispirane, nerol oxide, E3SP, and 2-FM. The absence of reduced glutathione, known for its antioxidant properties, may have facilitated oxidative processes, leading to increased formation of oxidised monoterpene derivatives such as nerol oxide (Papadopoulou & Roussis, 2008; Rodríguez-Bencomo *et al.*, 2014). The presence of E3SP suggests that its formation may not be exclusively modulated by glutathione but could also be influenced by H_2S and other factors (Clark & Deed, 2018; Harsch *et al.*, 2013). The positive correlation with 2-FM is likely attributable to the use of oak chips (Blanchard *et al.*, 2001).

CONCLUSION

The results of this study demonstrate that oak chips and GSH-IDY exert a significant influence on the aroma composition of Blaufränkisch wines, whereas oxygen played a subordinate role under the given experimental conditions. Oak chips reduced the concentrations of C_6 alcohols such as *trans*-2-hexen-1-ol while simultaneously promoting the formation of 2-FM and selected esters. GSH-IDY exhibited a stabilising effect on certain acetate, ethyl, and isoamyl esters. Monoterpenes and C_{13}-norisoprenoids remained largely stable in absolute terms and appeared to be predominantly varietal in origin, although slight protective effects were observed in variants treated with GSH-IDY.

Overall, the findings highlight the potential of targeted oenological interventions—such as the use of oak chips and GSH-IDY—to influence the volatile composition of Blaufränkisch wines in ways that may affect their aromatic expression. Sensory analyses are required to fully evaluate the practical and perceptual impact of these treatments and to establish evidence-based recommendations for winemaking practice.

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