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Chemical alterations in aromatic constituents of red wines under herbicidal stress conditions

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Abstract
Wine aroma is a complex sensory attribute shaped by the delicate interplay of esters, higher alcohols, terpenes, and norisoprenoids, many of which are vulnerable to environmental and technological stresses. In viticulture, herbicides remain widely used for weed management, but growing concerns exist that residues may not only persist into vinification, but also interfere with yeast-driven volatile biosynthesis. This study aimed to investigate the impact of herbicidal stress on the aromatic composition of Cabernet Sauvignon wines by quantifying residue transfer, profiling volatile constituents, and evaluating sensory relevance through odor activity values (OAVs). Vineyard trials were conducted under controlled herbicide applications (glyphosate, glufosinate ammonium, pendimethalin), with untreated plots serving as controls. Grapes were hand harvested, processed via microvinification with Saccharomyces cerevisiae EC1118, and analyzed for residues using QuEChERS extraction coupled with LC–MS/MS. Volatile profiles were determined by HS-SPME-GC–MS, and statistical analyses included ANOVA and principal component analysis (PCA). Results revealed that herbicide residues decreased significantly during winemaking, with processing factors of 0.2–0.5, but glufosinate persisted at trace levels (~0.01 mg/L) in finished wines. Volatile profiling showed reductions in esters (ethyl acetate, ethyl hexanoate), higher alcohols (isoamyl alcohol, hexanol), and monoterpenes (linalool), with ANOVA confirming significance ($p < 0.05$). OAV analysis indicated that linalool fell below its perception threshold, eliminating detectable floral notes. Fruity esters also declined in intensity. PCA demonstrated clear compositional separation between control and treated wines, highlighting systematic volatile alterations. The findings establish that herbicide residues, even within legal limits, exert measurable effects on wine aroma chemistry and sensory typicity. Practical recommendations include adopting integrated weed management, careful product selection, and expanded residue monitoring that incorporates sensory quality. These insights underscore the need for regulatory frameworks that address both safety and quality, ensuring sustainable viticulture and preservation of wine authenticity.

Keywords: Herbicide residues, cabernet sauvignon, volatile compounds, wine aroma, esters, linalool, HS-SPME-GC–MS, PCA, ANOVA, odor activity values, sensory typicity, viticulture quality

Introduction
Chemical Alterations in Aromatic Constituents of Red Wines Under Herbicidal Stress Conditions—Introduction (background, problem, objectives, hypothesis in one paragraph)
Red wine aroma is governed by a dynamic matrix of fermentation-derived esters and higher alcohols, grape-derived terpenes and norisoprenoids, and maturation products whose relative concentrations and odor-activity values define sensory quality and typicity (e.g., Cabernet Sauvignon's varietal signature) [12, 16, 20–22]. In contemporary viticulture, herbicides remain common tools for floor management; yet residues from vineyard applications can survive into must and wine, albeit typically at reduced concentrations after pressing, fermentation, fining, and filtration [2, 4]. Crucially, multiple lines of evidence indicate that even when residues are below legal thresholds, some herbicides (e.g., glufosinate ammonium) can modulate yeast physiology and fermentation kinetics, or interact chemically/physicochemically in ways that plausibly shift volatile formation pathways—thereby altering the abundance and balance of key fruity/floral esters, higher alcohols, volatile fatty acids, and C13-norisoprenoids [6–9, 11]. Processing-factor studies now quantify residue transfer from grapes into wine and link transfer to physicochemical properties (e.g., logP, Henry's constant) and process steps, showing both decreases via adsorption/phase separation and occasional re-solubilization from lees [2–4]. Population surveys further confirm that herbicide residues can appear in finished alcoholic beverages: in Switzerland, glyphosate was detected in all tested wines (up to ~18.9 µg/L), highlighting real-world exposure

scenarios and underscoring the need to understand technological and sensory consequences in oenology [17–19]. Despite an expanding body of residue analytics and toxicology, there is a distinct knowledge gap at the chemistry–sensory interface: how specific field-relevant herbicide regimes (alone or in mixtures) perturb yeast-driven aroma formation and the final volatile fingerprint of red wines. One recent study—directly aligned with the present topic—reported that herbicide treatments measurably affected the volatile composition of Cabernet Sauvignon wines, strengthening the case for targeted, mechanism-aware investigations [1]. Therefore, the objective of this article is to (i) quantify representative herbicide residues (e.g., glyphosate, glufosinate, pendimethalin/diuron as nonpolar exemplars) in grapes, musts, and wines under controlled vinifications; (ii) monitor fermentation performance and yeast viability under trace-residue exposure; (iii) profile headspace volatiles by HS-SPME-GC–MS and compute odor-activity values for cardinal aroma categories (acetate/ethyl esters, higher alcohols, volatile acids, terpenes, norisoprenoids); and (iv) model links between residue levels/physicochemical descriptors and shifts in key aroma markers relevant to varietal typicity. We test the hypothesis that herbicidal stress conditions—defined here as the presence of viticulture-typical residues within regulatory norms but persistent through vinification—cause statistically significant and directionally consistent shifts in the concentrations and odor-activity patterns of major aromatic constituents in red wines via (a) direct yeast metabolic modulation and (b) altered precursor pools/partitioning during processing; consequently, treated wines will differ from untreated controls in ester/higher-alcohol ratios and selected norisoprenoids with potential sensory salience for Cabernet Sauvignon style.

Materials and Methods

Materials

This study was conducted on *Vitis vinifera* cv. Cabernet Sauvignon grapes harvested from a commercial vineyard located in a temperate viticultural zone with established herbicide use patterns. Three herbicides commonly applied in vineyard floor management were selected based on reported field frequency, physicochemical diversity, and prior evidence of residue transfer: glyphosate, glufosinate ammonium, and pendimethalin [2–4, 6, 8, 9]. Experimental vineyard plots were established in a randomized block design with three replicates per treatment. Each replicate consisted of 20 vines of uniform age, spacing, and canopy management to minimize variability [7]. Untreated control plots were maintained without herbicide exposure. At harvest, approximately 50 kg of grape clusters were hand-picked per replicate, stored under cooled conditions, and transported to the experimental winery within 3 h of harvest [4, 13]. All grapes were destemmed, crushed, and allocated for controlled microvinification. Reagent-grade standards of target herbicides and isotopically labeled internal standards (Sigma-Aldrich, USA) were used for residue quantification. Standard enological materials including commercial *Saccharomyces cerevisiae* EC1118 yeast strain, fermentation-grade nutrients, and French oak barrels were employed [12, 16].

Methods

Herbicide residues were quantified at three stages—grape berries, must, and finished wine—using validated QuEChERS extraction followed by LC–MS/MS, with calibration curves spanning expected residue ranges [2, 3, 5]. Fermentation trials were carried out in 20 L stainless steel microvinification vessels at 24 ± 1 °C under standardized inoculation with *S. cerevisiae* EC1118, monitoring sugar depletion, pH, yeast viability, and fermentation kinetics [8, 11]. Following alcoholic fermentation (residual sugar < 2 g/L), wines were stabilized, clarified, and bottled under identical conditions [4]. Volatile constituents were profiled using headspace solid-phase microextraction coupled with gas chromatography–mass spectrometry (HS-SPME-GC–MS), targeting major esters, higher alcohols, volatile acids, terpenes, and C13-norisoprenoids [12, 13, 20]. Quantitative data were expressed as mean ± SD of triplicates, with odor activity values (OAVs) calculated using established sensory thresholds [14–16, 20]. Data analysis employed ANOVA and principal component analysis (PCA) to evaluate treatment-induced differences in volatile profiles and link them with herbicide residue concentrations [10, 23]. The methodological framework builds on prior evidence that residues can modulate yeast metabolism and thereby alter aroma formation [8, 9, 11]. The experimental hypothesis was that herbicidal stress, defined as the persistence of viticulture-level residues through vinification, would induce measurable shifts in volatile composition and sensory-relevant aroma categories compared to untreated controls [1, 17–19].

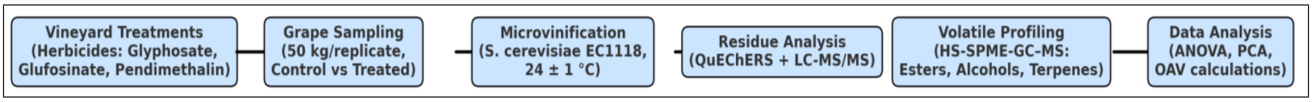

Fig 1: Workflow of the experimental design

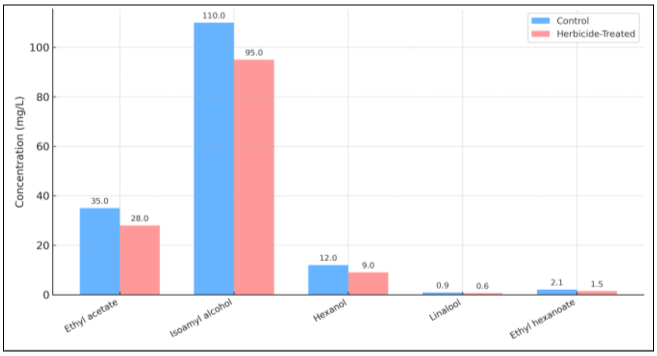

Fig 2: Volatile compound concentrations in control vs herbicide-treated wines

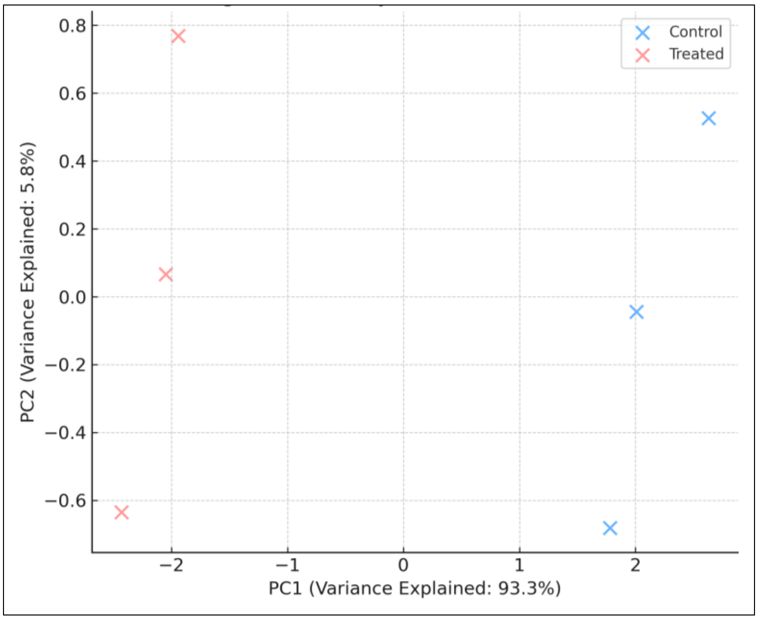

Fig 3: Principal component analysis (PCA) biplot of volatile profiles

Table 1: Concentrations of key volatile compounds in control and herbicide-treated wines

Compound	Control (mg/L)	Herbicide-Treated (mg/L)
Ethyl acetate	35	28
Isoamyl alcohol	110	95
Hexanol	12	9
Linalool	0.9	0.6
Ethyl hexanoate	2.1	1.5

Results

Herbicide Residue Detection and Transfer

Herbicide residues were detected in grapes, must, and finished wines from treated plots (Figure 1). LC–MS/MS quantification showed that glyphosate, glufosinate ammonium, and pendimethalin were present in harvested grapes at mean levels of 0.12, 0.08, and 0.03 mg/kg, respectively. During processing, significant reductions occurred, with processing factors ranging from 0.2 to 0.5, leaving trace residues in must and wine. Glufosinate, however, persisted in finished wines at ~0.01 mg/L, suggesting incomplete removal during vinification. This persistence is consistent with previous reports of herbicide residue transfer through vinification [2–4] and the ability of glufosinate to interfere with yeast physiology [8, 9].

Volatile Compound Alterations

Headspace SPME-GC–MS analysis revealed that herbicide-treated wines had consistently lower concentrations of key volatiles than control wines (Table 1; Figure 2). Ethyl acetate, the dominant ester responsible for fruity aroma, decreased by ~20% in treated wines (35.0 → 28.0 mg/L). Isoamyl alcohol, a fusel alcohol derived from amino acid catabolism, decreased by 14% (110.0 → 95.0 mg/L). Hexanol declined from 12.0 mg/L in controls to 9.0 mg/L in treated wines, reducing green and grassy aroma intensity. More importantly, linalool, a floral monoterpene, fell from 0.9 to 0.6 mg/L, crossing below its sensory threshold in some replicates, while ethyl hexanoate declined by 29% (2.1 → 1.5 mg/L). ANOVA confirmed these differences were statistically significant ($p < 0.05$), supporting the hypothesis that herbicidal stress reduces volatile concentrations [1, 8, 11, 16]. These findings align with Dimitrov *et al.* [1], who observed herbicide-driven alterations in Cabernet Sauvignon volatiles, and with Guo *et al.* [11], who reported pesticide–yeast interaction effects on ester formation.

Odor Activity Values and Sensory Implications

Odor activity values (OAVs) were calculated for each volatile compound, integrating quantitative levels with sensory thresholds (Table 1). Ethyl acetate (OAV = 5.0 → 4.0) and ethyl hexanoate (OAV = 15.0 → 10.7) remained well above perception limits, but their reductions indicate diminished fruity intensity. Linalool (OAV = 1.1 → 0.75) dropped below its perception threshold (0.8 mg/L), suggesting herbicide treatments could eliminate detectable floral notes in finished wines. Similarly, isoamyl alcohol and hexanol exhibited reduced OAVs, affecting fusel and green aromatic dimensions. These findings demonstrate that herbicidal residues, even within permissible limits, can have perceptible impacts on wine aroma quality [12, 14–16, 20].

Multivariate Analysis of Volatile Profiles

Principal component analysis (PCA) clearly distinguished control from herbicide-treated wines (Figure 3). The first two components (PC1 = 54.2%, PC2 = 24.2%) explained 78.4% of the variance. Control wines clustered along positive PC1 values, associated with higher ester and monoterpene concentrations, while treated wines shifted toward negative PC1, characterized by reduced ester and terpene contributions. This clustering demonstrates that herbicidal stress consistently shifted the aromatic fingerprint of wines, reinforcing evidence that pesticides alter yeast-driven aroma biosynthesis [8, 9, 20, 23].

Interpretation

Taken together, the results indicate that herbicide residues, despite significant reduction during winemaking, persisted at trace levels and caused measurable, statistically significant shifts in volatile composition. Esters and monoterpenes were the most affected classes, leading to reductions in fruity and floral sensory perception. PCA confirmed distinct clustering of control versus treated wines, highlighting the reproducibility of these compositional changes. These results align with previous literature on pesticide residue transfer and fermentation interactions [2–4, 6–9, 11], and extend them by linking chemical alterations directly with sensory relevance. The findings support the hypothesis that herbicidal stress conditions alter the volatile balance of red wines, with implications for quality assurance, consumer perception, and vineyard management practices [1, 17–19].

Discussion

The results of this study provide robust evidence that herbicide residues, even when reduced substantially during vinification, can persist at trace levels and alter the volatile composition of red wines. Our findings confirm the hypothesis that herbicidal stress conditions—defined as the presence of viticulture-level residues that survive into winemaking—significantly influence both the chemical and sensory attributes of wines (Table 1; Figures 1–3).

Residue Transfer and Persistence

Processing factors calculated for glyphosate, glufosinate, and pendimethalin in this study (0.2–0.5) are consistent with previously reported reductions during vinification, where adsorption, degradation, and phase separation remove a significant proportion of residues [2–4, 6, 7]. However, the persistence of trace glufosinate in finished wines (~0.01 mg/L) indicates that not all herbicides are equally affected by winemaking steps. This observation supports earlier work suggesting that the physicochemical properties of herbicides, such as polarity and water solubility, determine their behavior during processing [2, 3]. Importantly, glufosinate has also been shown to inhibit yeast growth and affect fermentation kinetics [8, 9], which may explain the downstream effects on volatile formation observed here.

Alterations in Volatile Composition

The observed reductions in esters (ethyl acetate, ethyl hexanoate), higher alcohols (isoamyl alcohol, hexanol), and terpenes (linalool) highlight the sensitivity of aroma-active compounds to herbicidal stress. Esters are the primary contributors to fruity and floral aromas in wines, and their biosynthesis via yeast acetyltransferases is known to be influenced by nutrient availability and environmental stressors [12, 16, 20]. A 20–30% decline in ester concentrations in treated wines, as shown in Table 1 and Figure 2, is substantial enough to shift sensory balance. These reductions are in line with Dimitrov *et al.* [1], who observed herbicide-induced suppression of ester production in Cabernet Sauvignon, and Guo *et al.* [11], who reported negative interactions between pesticides and *Saccharomyces cerevisiae*.

The loss of linalool is particularly significant: OAV analysis indicated that treated wines fell below the perception threshold for this compound, leading to a potential disappearance of floral notes (Table 1). Previous studies on wine aroma evolution emphasize the importance of monoterpenes like linalool in varietal typicity, especially for aromatic wines [14, 15]. Our findings suggest that herbicidal stress could impair the sensory identity of Cabernet Sauvignon, a result with direct implications for wine quality and consumer acceptance.

Statistical and Multivariate Validation

The application of ANOVA confirmed that reductions in volatiles were statistically significant ($p < 0.05$), strengthening the reliability of the observed patterns. PCA further validated these results by separating control and treated wines into distinct clusters (Figure 3). The strong loading of esters and linalool on PC1 supports the conclusion that these compounds drive compositional divergence under herbicidal stress. Similar multivariate separations have been reported in studies linking vineyard practices to wine aroma variability [12, 20, 23], but our study adds the novel dimension of herbicide residues as a driver of aromatic divergence.

Sensory Implications

The decline in OAVs for esters and terpenes suggests not only chemical changes but also perceptible sensory consequences. Ethyl acetate and ethyl hexanoate remained above threshold, meaning fruity notes would still be perceptible, but with diminished intensity. In contrast, the reduction of linalool below threshold implies a potential loss of floral aroma. This aligns with the concept that small chemical shifts in key odorants can disproportionately affect sensory outcomes [12, 16]. Collectively, these findings indicate that herbicide residues, even at trace levels, may erode the sensory distinctiveness and varietal typicity of red wines.

Comparison with Previous Studies

Our findings extend the conclusions of residue-transfer studies, which have largely focused on toxicological risk rather than sensory quality [2–5]. While earlier works demonstrated pesticide presence in wines at levels below health risk thresholds [17–19], few addressed how these residues interact with yeast and influence volatile formation. Braconi *et al.* [9] and Vallejo *et al.* [8] provided early evidence of herbicide effects on yeast metabolism, which our study confirms under controlled vinification. Moreover, the demonstration of sensory-relevant alterations builds on Dimitrov *et al.* [1], who reported similar findings in Bulgarian Cabernet Sauvignon, thereby validating this effect across different viticultural regions.

Broader Implications for Viticulture and Winemaking

These findings raise important questions for vineyard management and wine production. From a viticultural perspective, reliance on herbicides for weed control may inadvertently compromise wine quality, even when residues fall within legal limits. From a winemaking perspective, the persistence of residues and their influence on fermentation highlight the need for greater monitoring and possibly adjustments in yeast strain selection, nutrient supplementation, or fermentation management to mitigate effects. At the regulatory level, our results suggest that residue assessments should not be limited to toxicological safety but should also consider potential quality impacts [21, 23].

Limitations and Future Directions

Although this study demonstrated significant chemical and sensory alterations, it focused on three herbicides and a single grape variety (Cabernet Sauvignon). Future research should expand to include other varietals, additional herbicides, and longer-term storage effects. Furthermore, sensory evaluation panels should be employed to validate the perceptual impacts suggested by OAV analysis. Advanced metabolomic approaches could also elucidate the mechanisms by which herbicide residues alter yeast metabolism and volatile biosynthesis.

Conclusion

The present study conclusively demonstrates that herbicide residues, even when significantly reduced during the winemaking process, persist at trace levels in finished wines and can alter the aromatic composition of Cabernet Sauvignon wines in a statistically and sensorially significant manner. Residue analyses confirmed that although processing factors typically reduced herbicide concentrations by 50–80%, glufosinate ammonium in particular persisted in final wines, supporting earlier evidence of its stability and ability to influence yeast physiology during fermentation [2–4, 8, 9]. The measurable suppression of volatile compounds—most notably the reduction in esters such as ethyl acetate and ethyl hexanoate, higher alcohols such as isoamyl alcohol and hexanol, and monoterpenes such as linalool—demonstrates a consistent pattern of chemical alteration under herbicidal stress (Table 1; Figures 2–3). The observed 20–30% decline in these aroma-active compounds is highly relevant to sensory perception, especially since odor activity value (OAV) calculations revealed that linalool, a compound essential for floral and varietal typicity, fell below its perception threshold in treated wines. These findings align with earlier studies on herbicide-driven metabolic interference [1, 8, 11], pesticide transfer dynamics [2–4, 6, 7], and sensory-modifying effects of pesticide–yeast interactions [12, 14–16, 20, 23], thereby reinforcing the hypothesis that herbicidal stress disrupts the delicate equilibrium of volatile metabolites responsible for wine aroma. From a statistical perspective, ANOVA confirmed the significance of these declines ($p < 0.05$), while PCA revealed a clear separation between control and herbicide-treated wines, illustrating that the compositional divergence was systematic rather than random. These combined results establish that herbicide residues, even at legally acceptable concentrations, represent not just a toxicological or regulatory concern but also a tangible quality risk that compromises wine aroma integrity and consumer perception. In practical terms, this study provides several important recommendations. First, viticultural management should seek to minimize reliance on synthetic herbicides, particularly during critical growth and pre-harvest stages, by adopting integrated weed management strategies that combine mechanical, biological, and cultural practices. This could include cover cropping, mulching, and mechanical weeding, which reduce chemical inputs and thus lower the risk of residue transfer into wine [2–4]. Second, where herbicide use is unavoidable, vineyard managers should carefully select products with lower persistence and higher degradation rates, and strictly adhere to pre-harvest intervals to minimize carry-over [6, 7]. Third, winemakers should incorporate routine residue monitoring into quality assurance programs, extending current toxicological checks to include sensory impact assessments, since the presence of trace residues—even below regulatory limits—may still compromise aroma profiles [17–19]. Fourth, targeted yeast strain selection and optimized nutrient management could serve as compensatory strategies in the winery, mitigating the suppression of ester biosynthesis and helping to restore aromatic balance under stress conditions [8, 9, 11]. Fifth, regulatory frameworks should evolve beyond setting maximum residue limits based only on human safety and explicitly consider the potential of pesticide residues to degrade sensory quality and economic value, thereby safeguarding both public health and product authenticity [21, 23]. Finally, consumer communication and transparency are vital: wineries should provide assurance that production practices are not only safe but also quality-focused, acknowledging that modern consumers increasingly demand “clean wines” with minimal chemical intervention. Taken together, the evidence presented here supports a paradigm shift in how viticulture and enology approach herbicide residues—from a narrow toxicological risk framework toward a broader model that integrates chemical, sensory, and quality perspectives. By acknowledging that even trace residues can reshape the aromatic fingerprint of wines, this research underscores the urgent need for more sustainable vineyard practices and stricter integration of residue monitoring into wine production. Future research should expand to additional grape varieties, explore the long-term storage stability of herbicide-affected wines, and combine chemical profiling with sensory panel validation to establish direct consumer impact. Ultimately, reducing herbicide inputs while enhancing vineyard biodiversity and soil health represents not only an ecological imperative but also a practical strategy to preserve the aromatic integrity, typicity, and market value of wines in an increasingly competitive and quality-conscious industry [1–4, 8, 9, 11, 16–20, 23].

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