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Volatile compounds as indicators of terroir differentiation in Moldovan Feteasca Neagra Wines

Meiling Yao[1], Fei Wang[1], Gheorghe Arpentin[2], Chun Xiao[3]

[1] Technical University of Moldova, bd. Stefan Voda 168, Chisinau, Republic of Moldova
[2] Deparement R&D+I, Purcari Wineries Group, str. Calea Iesilor, Chisinau, Republic of Moldova
[3]Center of International Cooperation Service, Ministry of Agriculture and Rural Affairs, People's Republic of China

Abstract. This study examined volatile compounds in Feteasca Neagra wines from seven vineyards across three PGI regions in Moldova using GC-IMS. 33 volatile compounds were identified, including terpinolene (202.74-480.18 μg/L), typically abundant in white aromatic grapes but less common in red varieties. LDA revealed strong regional differentiation, confirming unique chemical fingerprints among vineyard sites. OAV analysis identified 15 key volatile compounds influencing sensory attributes, while PLSR examined the impact of terroir factors. Regression models for dimethyl sulfide (DMS, $R^2 = 0.97$, $p = 0.0009$) and terpinolene ($R^2 = 0.87$, $p = 0.01636$) showed that terroir affects the aroma profile of Feteasca Neagra wine in Moldova. DMS levels were significantly lower at higher altitudes ($p < 0.01$). Additionally, the interaction between altitude and heat index ($p < 0.01$) suggests that a high heat index at high altitudes promotes DMS precursor accumulation. Terpinolene had a significant negative correlation with the average minimum temperature during the ripening period. These findings provide insights into terroir's influence on the volatile composition of Fetească Neagră wines, supporting site-specific factors' role in shaping wine aroma and regional identity.

1. Introduction

Aroma is one of the most important indicators for evaluating wine quality, and it directly influences consumers' sensory experiences and preferences. Wine aroma can be categorized into primary aroma (varietal aroma), secondary aroma (fermentation aroma), and tertiary aroma (aging aroma). The primary aroma mainly originates from volatile compounds naturally present in grapes, including higher alcohols, esters, terpenes, pyrazines, phenols, aldehydes, and thiols [1].

A variety of factors, including grape genotype, light exposure, temperature fluctuations, vineyard management practices, and yeast strains influence the composition and concentration of volatile aromatic compounds [2]. These factors affect the metabolic processes of grapes during ripening and continue to shape the aromatic profile of the wine during fermentation. For example, in warm climates, elevated berry temperatures can significantly reduce the concentration of terpenes and norisoprenoids in wine[3].

In recent years, a growing body of research has demonstrated that terroir factors significantly influence the accumulation of volatile organic compounds (VOCs) in grapes and wines, contributing to aroma features with strong regional identity [4]. However, the relationship between pedoclimatic factors (e.g., soil and climate) and wine aroma remains complex yet fully understood, requiring further investigation using advanced analytical techniques.

Against this backdrop, Fetească Neagră emerges as an ideal model for terroir-expression studies. As one of the most representative indigenous red grape varieties of Eastern Europe (notably of Romania and the Republic of Moldova), Fetească Neagră originates from the Carpathian-Danube region and holds significant historical and cultural importance. Despite its winemaking potential and symbolic value, studies on its aromatic profile and relationship with terroir remain scarce, particularly in Moldova, where there has been no systematic investigation of how Fetească Neagră wines differ in volatile composition across various Protected Geographical Indication (PGI) zones.

This study applies GC-IMS to analyze the volatile profiles of Fetească Neagră wines produced under identical vinification protocols from seven vineyards located in three PGI regions of Moldova. The objectives are to:

Investigate the differences in aroma composition of Fetească Neagră wines under contrasting terroir conditions;

- Identify representative VOCs associated with terroir;
- Elucidate key environmental drivers of aroma expression;
- And provide a scientific basis for the flavor management, quality authentication, and regional branding of this local grape variety.

2. Material and Method

In this study, seven representative Fetească Neagră vineyards were selected from three PGI regions of the Republic of Moldova: Codru, Ștefan Vodă, and Valul lui Traian. These vineyards were chosen to represent a wide range of soil types, climatic conditions, and microhabitats to investigate the influence of terroir factors on the quality of Fetească Neagră wines. Climate and soil-related data, such as annual precipitation and temperature accumulation, were provided by the National Office for Vine and Wine of Moldova (ONVV).

2.1. Sampling

The grapes used in this study were manually harvested at their optimal technological ripeness (sugar content of 255 g/L and titratable acidity of 4.65 g/L as tartaric acid) under strictly hygienic conditions. Samples were collected from seven distinct vineyards representing three PGI regions in the Republic of Moldova: Codru, Ștefan Vodă, and Valul lui Traian. These vineyards were selected to capture a broad range of terroir conditions, including variations in soil type, topography, elevation, and vine training systems. Detailed characteristics of the sampled vineyards are as follows:

In the IGP Valul lui Traian region:

- Alexandru Ioan Cuza: Feteasca Neagra planted in 2014, using rootstock 5BB, trained in a bilateral Cazenave system, located at N45.6454, E28.4629. The vineyard is situated on a southwest-facing slope at an altitude of 70.50 meters, with a slope gradient of 1º-5º.
- Leova: Feteasca Neagra planted in 2009, using rootstock 5BB, trained in a unilateral arched Guyot system, located at N46.4877, E28.2867. The vineyard lies on a west-facing slope, at 163.34 meters above sea level, with a slope of 1º-3º.
- Bugeac: Feteasca Neagra planted in 2008, using rootstock 5BB, trained in a unilateral arched Guyot system, located at N46.3975, E28.7169. It is located on a southwest-facing hillside at an altitude of 151.56 meters, with a slope between 1º and 5º.

In the IGP Ștefan Vodă region:

- Purcari: Feteasca Neagra planted in 2010, using rootstock SO4, trained in a bilateral Royat system, located at N46.5192, E29.8686. The vineyard lies on an east-facing slope at 120.94 meters elevation, with a gradient between 1º and 7º.

In the IGP Codru region:

- Speia: Feteasca Neagra planted in 2015, using rootstock SO4, trained in a standard bilateral Guyot system, located at N46.9884, E29.2683. The site is a gently sloping hillside with eastern exposure, 133.31 meters above sea level, and a slope of 3º–10º.
- Nisporeni: Feteasca Neagra planted in 2006, using clone rootstock 5BB, trained in a unilateral arched Guyot system, located at N47.0759, E28.1389. It is positioned on a southwest-facing slope, at 121 meters altitude, with a slope between 7º and 10º.
- Mircești: Feteasca Neagra planted in 2014, using clone rootstock SO4, trained in a unilateral arched Guyot system, located at N47.4008, E27.9883. The vineyard lies on an east-facing slope, at a high elevation of 304.10 meters, with a steep gradient of 5º - 20º.

2.2. Microvinification

Grapes were stored at 10 °C post-harvest and processed using a standardized microvinification protocol. Each 3 kg batch received 100 mg/dm^3 potassium metabisulfite to inhibit microbes, followed by must clarification and inoculation with 80 mg/dm^3 commercial yeast. Fermentation involved twice-daily pigeage and remontage, with density monitored twice daily. At 1010-1020 density, 25 mg/dm^3 lactic acid bacteria were added for malolactic fermentation. Wines were pressed when residual sugar dropped below 3 g/dm^3, then racked, bottled, and stabilized with 50 mg/dm^3 PMS.

2.3. Volatile compounds analysis[1]

Volatile compounds in Fetească Neagră wines from seven Moldovan vineyards were analyzed using GC-IMS (FlavourSpec®, G.A.S., Germany) with a static headspace sampler. Each 1 g wine sample was spiked with 100 ppm internal standard (2-methyl-3-heptanone), incubated at 60 °C for 20 min, and analyzed in triplicate. GC used an MXT-WAX column with a 40 min gradient; IMS employed a tritium source and nitrogen drift gas. Data were processed with VOCal software; compound

[1] GC-IMS analyses were performed at the analytical laboratory of Hanon Instruments Co., Ltd. (Shandong, China).

concentrations were calculated using internal standard ratios, and odor activity values (OAVs) were computed to assess sensory relevance. Results linked volatile profiles to regional terroir.

2.4. Statistic

All statistical analyses in this study were conducted using the R programming language (version 4.4.3). Analysis of variance (ANOVA) was used to test for significant differences among different groups of samples, and the Tukey's Honest Significant Difference (Tukey HSD) test was applied for multiple comparisons and group differentiation. Linear Discriminant Analysis (LDA) was applied to assess whether the volatile compound profiles of Fetească Neagră wines produced from different vineyards could be distinguished despite identical vinification protocols. This supervised method allowed for dimensionality reduction while maximizing class separability based on vineyard origin. In addition, Spearman correlation analysis was performed to evaluate nonlinear relationships between variables. Partial Least Squares Regression (PLSR) was applied to investigate the relationship between volatile compounds and the different groups of wine samples. The statistical significance level for all analyses was set at $p < 0.05$.

3. Results and Discussion

This subsection focuses on the chemical composition of Fetească Neagră wines, particularly the volatile organic compounds (VOCs) that shape their aromatic profile. Through GC-IMS analysis and OAV-based interpretation, combined with regression models, it examines how terroir-related factors including pedoclimatic conditions and viticultural practices contribute to the formation of distinctive aroma-relevant compounds.

3.1. The volatile organic compounds (VOCs) of Fetească Neagră wine

A total of 33 volatile organic compounds were detected, encompassing various chemical classes, including esters, alcohols, aldehydes, acids, ketones, terpenes, and sulfur-containing compounds.

Table 1. The content of (µg/L) volatile organic compounds (VOCs) of Fetească Neagră wine.

	Mircești	**Nisporeni**	**Speia**	**Cuza**	**Bugeac**	**Leova**	**Purcari**
Acetic acid	6240.7±338.24b	7207.77±508.34ab	7416.91±895.43ab	7451.37±465.51ab	8792.02±1014.88a	8098.95±336.85a	7262.73±603.75ab
(E)-2-Hexenol	344.22±8a	312.95±17.58a	269.17±4.52b	313.27±19.27a	267.61±6.71b	185.49±10.78d	218.94±2.84c
Z-3-Hexenol	354.57±5.68ab	365.51±13.28a	327.69±18.44ab	342.94±17.13ab	344.29±25.81ab	284.56±2.74c	313.97±0.7bc
1-Hexanol	742.35±40.11e	851.98±14.3cd	904.27±8.93c	821.05±19.29d	1119.81±16.88a	984.98±24.98b	880.83±38.14cd
3-Methyl-1-butanol	23848.41±493.76c	26006.53±438.83b	25724.42±82.35bc	25047.86±352.17bc	28334.9±1444.52a	25194.75±379.08bc	26723.57±1019.79ab
1-Penten-3-ol	105.85±2.87a	79.92±2.84de	82.3±1.33d	94.96±3.1bc	85.81±3.32cd	98.06±5.97ab	71.07±3.31e
1-Butanol	2543.99±49.75e	3793.06±51.43bc	3149.15±23.28d	4741.81±91.57a	3913.4±60.03b	3942.79±136.89b	3619.31±111.27c
2-Methyl-1-propanol	26666.19±345.65bc	27544.12±425.45bc	27600.45±146.16bc	26047.42±225.15c	30387.18±1396.71a	28468.94±642.38ab	28675.44±981.23ab
1-Propanol	8703.35±52.96d	11382.57±213.92bc	11002.05±137.93c	11315.28±135.68bc	12793.11±761.44a	11897.31±200.29abc	12057.17±445.23ab
Ethanol	214922.3±1882.3b	232032.54±6058.37b	233573.75±6165.19b	235788.72±5465.51b	260976.21±18453.57a	222633.54±3134.57b	230722.5±8670.48b
Octanal	73.02±3.63a	48.27±1.41cd	41.22±3.45d	54.4±1.77bc	58.89±4.43b	48.15±6.1cd	47.13±1.72cd
Pentanal	58.48±0.81e	212.18±8.51cd	192.42±4.36d	285.91±2.31b	227.89±13.07c	331.58±2.03a	224.94±9.63c
Ethyl propanoate	1238.61±15.5d	2406.2±50.33b	2103.34±26.21c	2683.03±43.01a	2327.36±166.67bc	2674.37±28.3a	2333.03±116.63b
Propanal	471.71±13.71a	204.59±18.91c	221.31±8.51c	237.02±15.74c	311.52±2.42b	326.58±26.16b	322.56±15.79b
Ethyl octanoate	1096.77±84.17e	1571.59±57.42bc	1670.05±30.86b	2422.21±47.13a	1311.57±86.68d	1460.57±50.79cd	1122.39±82.42e
Ethyl lactate	5208.78±160.56c	5623.82±247.91abc	5511.45±370.74bc	6482.67±387.71ab	6711.65±902.02a	5474.84±164.34bc	6052.74±274.12abc
Ethyl hexanoate	1306.3±1.84f	1980.53±12.54c	2215.21±9.76b	2800±17.55a	1823.25±75.67d	1821.62±53.55d	1602.47±55.99e
Isoamyl acetate	11470.27±137.11b	12002.9±27.71ab	12601.91±64.86ab	12944.03±144.9a	13009.58±944.88a	11868.79±246.12ab	11543.74±461.48b

Ethyl 3-methylbutanoate	1935.71±52.27c	2742.33±72.88a	2347.13±65.11b	2356.73±61.1b	2247.79±228.07b	2270.13±69.19b	2667.56±117.27a
Ethyl 2-methylbutanoate	2201.72±41.14c	2917.84±58.93a	2714.88±72.51ab	2376.72±63.03c	2457.52±243.52bc	2478.77±55.87bc	2905.8±123.65a
Ethyl butanoate	2140.65±34.3d	3105.73±38.7b	3123.93±39.87b	4331.05±67.68a	3190.11±244.94b	2987.39±60.35b	2577.23±105.63c
2-Methylpropyl acetate	3114.8±53.8e	3651.84±44.38bc	3558.15±38.41cd	3331.31±46.75de	3889.65±238b	4439.7±72.57a	3614.15±148bcd
Propyl acetate	55.43±1.4d	240.7±2.21b	188.92±4.86c	401.12±8.34a	251.91±11.51b	415.13±10.28a	257.88±10.44b
Ethyl isobutyrate	6122.59±75.06ab	6411.87±66.26ab	6348.74±83.89ab	5961.75±74.82b	5989.34±434.43b	6645.66±87.76a	6630.39±278.06a
Ethyl acetate	24360.81±101.75c	29325.3±427.18b	29107.01±370.02b	30481.29±322.74ab	32334.03±2491.84a	30873.9±399.91ab	29535.57±1159.86ab
Methyl acetate	3584.75±29.11c	4362.61±53.34b	4568±43.78b	4239.25±34.03b	5349.06±407.9a	4556.22±48.61b	4591.7±178.21b
Acetophenone	426.38±31.6c	559.5±44.87bc	583.73±62.16abc	566.08±23.81bc	743.45±63.98a	587.17±62.58abc	615.57±88.94ab
2-Pentanone	174.35±1.08a	86.88±4.92cd	77.8±2.44d	145.43±9.9b	101.21±5.31c	147.77±5.15b	100.63±5.91c
Acetone	2749.65±61.36	2469.34±84.8	2414.26±74.8	2749.48±43.66	2870.16±496.12	2433.79±22.66	2455.67±107.26
Dimethyl sulfide	549.28±9.27d	1172.28±22.58b	744.69±10.54cd	1684.67±17.56a	1365.62±218.36b	1205.42±38.05b	914.71±39.65c
Terpinolene	480.18±34.74a	237.49±21.79bc	202.74±8.45c	426.23±32.28a	278.11±24.76b	245.9±24.98bc	205.28±15.91c
gamma-Butyrolactone	606.8±15.35b	854.26±14.93ab	854.93±95.72ab	718.5±52.7ab	1000.68±193.91a	841.15±17.4ab	914.75±164.67a
3-Hydroxy-2-butanone	124.72±3.31a	79.95±7.48bc	66.9±6.5c	97.39±10.67b	85.03±11.74bc	82.58±7.9bc	76.31±0.89bc

Acetic acid is the main component of volatile acidity, and its concentration in all samples remained below the safety threshold established by the OIV (1.2 g/L) for red wines. Bugeac and Leova exhibited relatively higher levels but without negatively affecting the sensory quality of the wines. The concentration of higher alcohols varied among samples, with Bugeac showing the highest content (338,222.31 µg/L) and Mircești the lowest (278,231.23 µg/L), which may be associated with stronger or weaker fruity aromas and body structure. Cuza and Purcari displayed higher concentrations of esters, mainly ethyl acetate and ethyl hexanoate, contributing to fruity notes, while Mircești had significantly lower ester content. Under the applied GC-IMS analytical conditions, dimethyl sulfide (DMS) was the only sulfur-containing compound detected. Its concentrations were remarkably high, ranging from 549.28 µg/L in Mircești to 1,684.67 µg/L in Cuza, which substantially exceeds the levels typically reported in the literature for wines (generally below 100 µg/L). Given the known limitations of GC-IMS in distinguishing structurally similar volatile sulfur compounds, the identification of this peak may be subject to interference. Future studies should include GC-MS analysis or comparison with authentic standards to further confirm the identity of the compound.

The only terpene compound identified was terpinolene, with concentrations in Fetească Neagră wines significantly higher than those typically reported for other red grape varieties, particularly in the Mircești and Cuza vineyards, highlighting a potential varietal specificity and terroir influence.

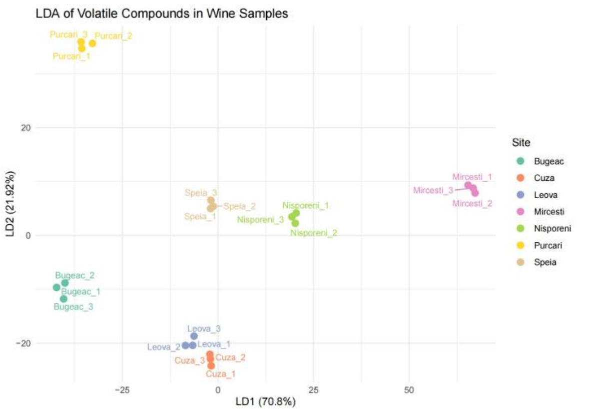

Figure 1. LDA Analysis based on the results of volatile compounds.

The LDA plot (Figure 1) demonstrates that, despite identical vinification protocols, the volatile compound profiles of Fetească Neagră wines varied according to vineyard origin. Samples from sites such as Mircești and Purcari exhibited clear separation along LD1, indicating that terroir-related factors significantly influence aroma composition and allow for effective differentiation between vineyard sources.

3.2. OAV analysis

Although numerous volatile compounds were detected in each wine sample, not all significantly influence the overall aromatic characteristics of the wine [4].

By evaluating the contribution of different volatile compounds to the olfactory profile of the wines, this study calculated the Odor Activity Value (OAV) based on the analytically determined mean concentrations and published perception thresholds [5-7]. Detailed information is presented in the table below.

In general, only compounds with an OAV greater than 1 are considered to significantly impact the wine aroma. The results (Table 2) show that a total of 15 aroma compounds had OAV values above 1, suggesting that these key compounds play an essential role in defining the aromatic characteristics of the wine.

Table 2. The odour threshold of VOC and its OAV value (>1) range in wine samples.

	Odour Threshold (μg/L)	OAV value
Ethyl octanoate	580	1.89~ 4.18
Terpinolene	200	1.01~2.40
Octanal	30	1.31~2.43
Ethyl hexanoate	5	261.26~560.02
Isoamyl acetate	30	382.33~432.20
Ethyl 3-methylbutanoate	3	645.23~914.08
Ethyl 2-methylbutanoate	18	122.31~162.10
Ethyl butanoate	20	107.03~216.53
2-Methylpropyl acetate	1600	1.95 ~2.77
Ethyl isobutyrate	15	397.41~443.00
Ethyl propanoate	1080	1.15~2.48
Ethanol	95000	2.26~2.74
Ethyl acetate	12300	1.98~2.62
Propanal	81	2.52~5.82
Dimethyl sulfide	27	20.34~62.39

Among the 15 aroma compounds with OAV values greater than 1, ethyl esters were predominant, with 8 exceeding the odor threshold. Notably, ethyl 3-methylbutyrate and ethyl hexanoate exhibited the highest OAVs in Speia and Purcari, respectively, indicating strong potential contributions to fruity aroma intensity. Ethyl butanoate and ethyl 2-methylbutyrate consistently exceeded thresholds across all samples, suggesting a general role in enhancing perceived fruitiness.

Dimethyl sulfide (DMS) also showed OAVs above 1 in all regions, ranging from 20.34 in Mircești to 62.40 in Purcari. Although often below its individual detection threshold, DMS is known to enhance fruitiness through synergistic interactions with esters, which may contribute to aromatic intensity in regions like Cuza and Purcari.

Propanal, a low-threshold aldehyde, exhibited elevated concentrations and OAVs in Mircești, indicating a potential association with green or herbaceous sensory notes.

Terpinolene, though rarely detected above threshold levels in red wines, was quantified at notable concentrations in Fetească Neagră, particularly in Mircești and Cuza. This suggests a possible varietal or terroir-related origin and highlights its potential role as a distinguishing aromatic marker in these regions.

3.3. PLSR

Based on the OAV and sensory analysis, it can be observed that the fruity aroma of Fetească Neagră wines is primarily determined by ethyl ester compounds. At the same time, dimethyl sulfide (DMS) contributes to the overall aromatic complexity [8], and propanal imparts subtle vegetal or herbaceous notes. Additionally, terpinolene appears to be an aroma compound characteristic of this grape variety. Given that the levels and expression of these key compounds vary significantly from one vineyard plot to another, this subsection examines how terroir influences their formation and expression.

In the analysis dedicated to propanal, terroir factors significantly correlated with its OAV value were selected based on Spearman correlation coefficients. These include P_2O_5 content (phosphorus availability), the drought index (DI), and the ripening period thermal amplitude (RMT). These factors show both theoretical and statistical correlations with propanal concentration. However, subsequent stepwise regression analysis failed to produce a significant predictive model, suggesting that the expression of propanal may be influenced by multiple nonlinear factors or complex interactions between variables that cannot be captured through a simple or linear combination. Therefore, the regression modeling proceeded only with the analysis of DMS and terpinolene.

Based on Spearman correlation coefficients, the terroir factors that showed a significant correlation ($p < 0.05$) with the Odor Activity Value (OAV) of terpinolene were selected, namely:

VM_1: average minimum temperature during the ripening period

VM_4: average temperature during the ripening period

Subsequently, a stepwise regression method was applied, and the model parameters were estimated using the Ordinary Least Squares (OLS) method. The final regression model obtained is as follows:

$$OAV_{Terpinolene}=76.48-8.54*VM_1+0.24VM_4, \quad (1)$$

where:

$R^2 = 0.8721$, indicating that the model explains 87.21% of the variation in OAV values for terpinolene;

Adjusted $R^2 = 0.8081$, suggesting the model is robust;

F-statistic = 13.64 with a p-value of 0.01636, indicating that the overall model is statistically significant ($p < 0.05$).

For the DMS, based on the Spearman correlation analysis, altitude (Alt) and the heat index (HI) were identified as the main terroir variables. The final regression model obtained is as follows:

$$OAV_{DMS}=-23.58-1.49*Alt+0.00081(Alt*HI), \quad (2)$$

where:

$R^2 = 96.95\%$ and adjusted $R^2 = 95.43\%$, indicating a very high degree of model fit;

F-statistic = 63.66, with a p-value of 0.0009279, showing that the model is highly significant overall ($p < 0.01$).

Altitude (Alt) shows a significant negative correlation with DMS ($p < 0.01$), suggesting that DMS content tends to be lower in higher-altitude regions. A potential reason for this result may be that the lower temperature in high-altitude areas inhibit S-methylmethionine (SMM) degradation, thereby reducing DMS formation.

The interaction term (Alt × HI) is also significant ($p < 0.01$), indicating that in high-altitude regions, the influence of the heat index (HI) on DMS increases. A potential cause of the phenomenon may be that grape maturity tends to be lower under high-altitude conditions, and a higher thermal index may promote the accumulation of DMS precursors.

4. Conclusion

A total of 33 volatile organic compounds (VOCs) were identified using GC-IMS, including terpinolene, which is relatively uncommon in red wines. LDA analysis revealed that Feteasc ă Neagr ă wines could be effectively differentiated by vineyard origin, highlighting terroir’s significant role in shaping aroma composition even under standardized vinification protocols. Notably, samples from Mircești and Purcari showed clear separation in the LDA score plot, indicating distinct aromatic profiles.

Odor activity value (OAV) analysis identified 15 compounds with OAVs greater than 1, most of which were ethyl esters, contributing to fruity aromas. In addition, dimethyl sulfide (DMS) and propanal were identified as key modulators of the overall aroma profile.

Partial least squares regression (PLSR) analysis suggested that the distribution of these key volatiles was influenced by pedoclimatic factors. Specifically, terpinolene negatively correlated with the minimum temperature during ripening, suggesting that cooler conditions may favor its accumulation. Conversely, DMS levels were found to decrease with elevation and to be positively associated with the heat index (HI), indicating a stronger expression of DMS in warmer, lower-altitude sites.

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