DOI: https://doi.org/10.20870/oeno-one.2026.60.3.9886

OPEN ACCESS

ENOLOGY ORIGINAL RESEARCH ARTICLES

Impact of vintage and winemaking practices on the volatile fingerprint of young wines from Moriles Alto, within Montilla-Moriles PDO

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Article number: 9886

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Associate editor:
Alice Vilela

Received:
02 February 2026

Accepted:
14 August 2026

Published:
21 September 2026

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ABSTRACT

The volatile composition of Pedro Ximénez (PX) wines from three wineries of a traditional and high-quality zone within the Montilla-Moriles Protected Designation of Origin (PDO) in Spain was quantified over two consecutive vintages to evaluate the influence of vintage and winery-specific practices on *terroir* differentiation. Chemical parameters and volatile compounds (major and minor) were quantified using gas-chromatographic and chemometric techniques. Two-way ANOVA revealed that vintage and winery exerted a strong effect on the content of volatile compounds, indicating the influence of abiotic factors and winemaker technological decisions. Also, the significant vintage × winery interactions confirmed the multifactorial nature of the wine's aromatic profile. Common oenological parameters are insufficient for *terroir* characterisation. Nevertheless, the contents of some major volatiles, such as acetaldehyde, ethyl acetate, isobutanol, 2-methyl-1-butanol, ethyl lactate, diethyl succinate, and 2-phenylethanol, have demonstrated their ability to authenticate wines by vintage and winery. The Principal Component Analysis (PCA) carried out on the major volatile compounds showed a well-defined structuring by winery along PC1 (40.18 % total variance), with additional separation by vintage along PC2 (31.72 % variance), revealing a year-dependent differentiation. Moreover, the PCA obtained with the contents of 51 minor volatiles quantified explained 70.68 % of the total variance (PC1 = 54.03 %, PC2 = 16.65 %). PC1 was defined by negative loadings of esters (*e.g.*, ethyl octanoate and ethyl decanoate) and wines from 2022 clustered around negative PC1 values. Positive PC1 loadings corresponded to γ-butyrolactone, 4-ethylguaiacol, long-chain alcohols, and lactones, which were more abundant in the 2021 wines. PC2 differentiated wines by winery due to contributions from furanic and carbonyl compounds (positive loadings) as well as compounds such as acetophenone, ethyl benzoate, and decanal (negative loadings). The compound-specific behaviour observed indicates that no single marker can describe the effect of each factor on the volatile composition. These results provide a detailed chemical baseline to differentiate wines from Moriles Alto and highlight the value of this molecular profiling as a tool for understanding how the studied factors modulate wine composition within *terroirs* from a specific traditional PDO winemaking region.

KEYWORDS: wine, volatilome, chemometrics, vintage, winery, *terroir*, zonation

INTRODUCTION

Winegrowing zonation, the systematic delineation of vineyard areas according to environmental characteristics and human practices, has evolved from an empirical practice into a scientific discipline that bridges tradition and innovation. By identifying how grapevine, landscape, and climate interact to define *terroir*, zonation provides winemakers with a structured framework to understand and preserve typicity. In a context of continuous climate change, this analytical approach supports both resilience and quality assurance. Spain, with its remarkable diversity of climates and soils, exemplifies how traditional winegrowing areas can integrate scientific tools to maintain their regional identity.

Wine quality is inherently linked to its geographical origin, reflecting the combined influence of natural and human factors. Abiotic factors, such as climate, soil composition, texture, and topography, interact with biotic factors like grape variety and microbiota to define *terroir* as an ecological and cultural system that imparts unique characteristics to its wines (Barham, 2003; Belda *et al.,* 2017; OIV, 2025a; van Leeuwen & Seguin, 2006; van Leeuwen *et al.,* 2020). However, scientific studies often overlook the significant effects of human action. As consumers increasingly value origin and identity, wines driven by *terroir* have gained international recognition, reinforcing the importance of regional heritage and sustainable production (Trubek, 2008; Teissedre *et al.,* 2022).

The conceptualisation of *terroir* has transitioned to quantitative, data-driven models. Early climatic indices have been superseded by sophisticated systems, such as multicriteria climatic classification to evaluate viticultural suitability (Tonietto & Carbonneau, 2004). Currently, geospatial mapping, remote sensing, and multivariate statistics facilitate high-resolution zoning by integrating complex environmental, agronomic, and microbiological variables. Within this quantitative framework, chemical profiling, underpinned by chemometric tools, is indispensable for verifying the authenticity and traceability of PDO wines.

Among Spain's diverse viticultural landscapes, the Montilla-Moriles PDO (Andalusia, Southern Spain) stands as a compelling case study of how scientific characterisation complements centuries-old practices (Osta-Fort, 2002; Fuentes-Espinosa *et al.,* 2025a; Fuentes-Espinosa *et al.,* 2025b; Alcalá-Jiménez *et al.,* 2025). Within this PDO, the Moriles Alto subzone is characterised by its *albariza* soils with soft, calcium carbonate-rich marls, which exhibit exceptional water retention and low organic matter content. Combined with a dry Mediterranean climate, these soils favour the cultivation of the Pedro Ximénez (PX) grape variety, which can achieve high sugar concentrations while maintaining balanced acidity (Palma-López *et al.,* 2024). These factors have historically allowed the production of special wines through biological ageing without ethanol fortification, thereby expressing the regional authenticity and distinctive character of their wines (Montilla-Moriles, 2025b; Muñoz-Castells *et al.,* 2025).

While industry professionals often rely on tradition and subjective perceptions to define a wine's *sense of place*, researchers emphasise that our quantitative understanding of *terroir* remains exploratory. Specifically, the relationship between a site's natural endowments and the resulting wine profile is still being characterised (White, 2020; van Leeuwen *et al*., 2023). In this context, recent work by Benito-Castellanos *et al*. (2026) concludes that the concept of 'microbial t*erroir*' is highly dynamic at the winery level, making its extension to broader regional scales a complex challenge. Furthermore, despite advances in environmental and spatial analysis, the integration of chemical and sensory data into comprehensive *terroir* research remains limited. In this regard, volatile composition is an essential tool for understanding the chemical basis of aroma and its relationship with vineyard conditions and winemaking practices (Francis & Newton, 2005; Gómez-Míguez *et al.,* 2007).

PX is the predominant white grape variety cultivated in the Montilla-Moriles PDO; environmental gradients and traditional practices, alongside vintage conditions, strongly influence PX grape composition and fermentation-derived volatiles (Fuentes-Espinosa *et al.,* 2025a; Fuentes-Espinosa *et al.,* 2025b). However, these volatile profiles are heavily modulated by technological interventions, including yeast activity and fermentation temperature (Osorio-Alises *et al.,* 2024; Priyadharshini *et al.,* 2025). Consequently, distinguishing the contributions of non-modifiable environmental factors versus human-driven oenological decisions remains a significant scientific hurdle.

This research evaluates the influence of vintage and winemaking practices on the volatile fingerprinting of wines from three *Pago* estates (single-vineyard production) located in the Moriles Alto subzone, within the Montilla-Moriles PDO. This work aims to identify structured patterns that contribute to a better understanding of the relationship between wine characteristics and production area.

MATERIALS AND METHODS

1. Location of wineries and vineyards, soil type, and climatic conditions

Three traditional wineries recognised for the high quality of their products were selected from the "Moriles Alto" subzone, classified as a premium wine-growing area within the Montilla-Moriles PDO in Andalusia, southern Spain (Montilla-Moriles, 2025b). The wineries are located at the following GPS coordinates: Lagar Casablanca (LC): 37°26′31″ N, 4°36′41″ W (425 m above sea level); Bodegas El Monte (EM): 37°26′23″ N, 4°37′14″ W (425 m ASL), and Bodegas Doblas-Martos (DM): 37°26′09″ N, 4°36′44″ W (385 m ASL). Each winery maintains its own vineyards nearby, where only PX grapes are grown for traditional wine production. This grape variety is well adapted to the soil and climate conditions of this subzone. The dominant pruning system used in these vineyards is the vertical trellis, although the traditional head pruning is used for the most ancient vines (Palma-López *et al.,* 2024).

The Moriles Alto subzone is characterised by its white, calcareous "albariza" soils, whose parent materials are marls and limestones, with high calcium carbonate content and low levels of organic matter, non-crystalline Fe oxides and available Fe content. The most representative soils of this subzone are those of Lagar Casablanca, classified as *Calcixerepts* according to the Soil Surveys Staff (USDA, 2022) by Palma-López *et al.* (2024).

The geographical region within which the wineries are located has a Csa climate type, according to the Köppen classification system, characterised by hot, dry summers and mild, wet winters. This climate type is identified by average monthly temperatures above 10 °C for at least four months, with the warmest month exceeding 22 °C. Data provided by the nearest state meteorological agency (AEMET, 2023) indicated that, for 2021 and 2022, the average maximum temperatures were 24.6 °C and 25.8 °C, respectively, with absolute maxima of 46.1 °C and 43.1 °C. The average minimum temperatures were 11.5 °C and 12.2 °C, with absolute minima of –3.5 °C and 0.3 °C. Total annual precipitation for these years was 330 and 427 mm, respectively.

2. Traditional vinification processes and wine samples

PX grapes were harvested manually from two consecutive vintages (2021 and 2022) in September, when they reached a sugar content above 250 g L^{-1} in order to yield wines with an alcohol content of 14.5 % *v/v* or higher. Wines were produced using grapes from vineyards whose vines were between 20 and 25 years old. The grape yield was about 6300 kg/ha in the 2021 harvest and 5400 kg/ha in the 2022 harvest. These values are below the maximum yield of 7000 kg/ha established for this grape variety within the zone by the regulations of Montilla-Moriles (2025a), consistent with traditional practices aimed at maximising grape quality through yield restriction. Vinification was carried out on an industrial scale at each winery using traditional practices, also according to these PDO rules, which include the extraction of 70 L of must per 100 kg of grapes by gentle pneumatic pressing, followed by pH adjustment with tartaric acid to approximately 3.3 and a settling period of 24 to 48 hours. Alcoholic fermentation was conducted at a controlled temperature of 18 ± 2 °C in 50 – 80 hL stainless steel containers with indigenous yeasts, using the traditional *pied de cuve* method. This procedure allows the natural selection of yeasts present in the grape microbiome of each vineyard, and no active dry yeasts were used as a starter culture. The *pied de cuve* methodology ensures that suitable yeast strains linked to each vineyard are responsible for fermentation. Additional grape must fractions were incrementally added during the exponential growth phase of yeast activity in alcoholic fermentation. The advantages of this method include improved yeast growth, nutrient complementation, and maintenance of the fermentation activity until the containers reach full capacity. Typically, this method ensures the complete fermentation of sugars and the production of dry wines with a high alcohol content; however, the alcoholic fermentation process generally lasts about one month.

From each year, three biological replicates of young wines, taken from three independent tanks (4500 – 6000 L), were collected per winery and vintage following the natural cold stabilisation and clarification period (post-winter). Samples (750 mL per replicate) were transported to the laboratory in portable coolers with ice and stored at 3 ºC until analysis, which was conducted within one week. All analyses were performed in triplicate.

3. Analysis of main wine parameters

The primary characterisation of wines was based on the analysis of standard oenological parameters, including ethanol content, total and volatile acidity, pH, density, and residual sugars. These parameters were quantified according to the analytical methods recommended by the International Organisation of Vine and Wine (OIV, 2025b). Lactic and malic acid contents were determined using the Merck reflectometric method with a Reflectoquant™ system (Merck®, Darmstadt, Germany) according to the procedure provided with the equipment. Absorbances at 280 and 420 nm were measured in a Cary 60 UV-Vis spectrophotometer (Agilent Technologies, Santa Clara, CA, USA).

4. Analysis of major volatile compounds and polyols by gas chromatography

Major volatile compounds, present in wines at concentrations above 10 mg L^{-1} (10 ppm), include the alcohols methanol, 1-propanol, isobutanol, 2- and 3-methyl-1-butanol, and 2-phenylethanol; the carbonyl compounds acetaldehyde and acetoin; and the esters ethyl acetate, ethyl lactate, and diethyl succinate. In addition, the polyols 2,3-butanediol (*levo* and *meso* isomers) and glycerol were also quantified. All these fourteen compounds were determined by direct injection of wine following previously published methodology (Peinado *et al.,* 2004; Fuentes-Espinosa *et al.,* 2025a; Fuentes-Espinosa *et al.,* 2025b; Alcalá-Jiménez *et al.,* 2025). Briefly, the sample preparation consisted of adding 1 mL of an internal standard solution (4-methyl-1-butanol at 1.018 g L^{-1}) to 10 mL of wine and 0.2 g of pure calcium carbonate. After sonication and centrifugation, 0.7 µL of the supernatant was directly injected into the injector (in split mode) of an Agilent 6890 GC equipped with an FID and a CP-WAX 57 CB (60 m, 0.25 mm, 0.40 µm capillary column), both from Agilent (Santa Clara, CA, USA). Target compounds were quantified using calibration curves obtained by subjecting standard solutions of pure compounds whose concentrations vary from 0 to 5 times the average values described in the literature, and using the same procedure as the samples (Peinado *et al.,* 2004). The chemicals were provided by Merck and Sigma-Aldrich.

5. Analysis of minor volatile compounds by stir-bars-solid-extraction and gas chromatography-mass spectrometry

The methodology used for identification and quantification of minor volatile compounds (which are those with a content of less than 10 mg L^{-1}) was described in detail in a previous work (Palenzuela *et al.,* 2023). The volatiles were extracted using

a Polydimethylsiloxane (PDMS) coated stir bar (Twister® 10 mm, 0.5 mm film thickness from Gerstel, Mülheim an der Ruhr, Germany). The Twister® was placed in a 10 mL vial containing 1.0 mL wine, 0.1 mL of internal standard solution (0.4116 g L^{-1} of hexyl butyrate in absolute ethanol), and 8.9 mL of a pH 3.5 buffered solution prepared with 12 % *v/v* ethanol, 2.6 g L^{-1} tartaric acid and 2.2 g L^{-1} potassium bitartrate. After stirring for 120 min at 1200 rpm and 20 °C, using a Variomag multipoint magnetic stirrer (Thermo Fisher Scientific, Waltham, MA, USA), the Twister was rinsed with distilled water, dried, and transferred to a thermal desorption unit (TDU) coupled to the GC for desorption and to transfer the volatiles to an HP-5MS fused silica column (60 m, 0.25 mm, 0.25 µm) from Agilent Technologies (Santa Clara, CA, USA).

Gas chromatography was performed on an Agilent 7890A GC coupled to a 5975C MSD detector and a Gerstel MPS autosampler, controlled by ChemStation v.02.02.143 and the Maestro v.1.2 software, from ChemStation International, Inc., Dayton, OH, USA, and Gerstel Mülheim an der Ruhr, Germany, respectively. Compound identification was carried out by comparing mass spectra of each compound with those of the NIST08 and Wiley7 libraries and further confirmed using the mass spectra of pure compounds. Quantification was based on calibration curves obtained from standard solutions of pure compounds, with concentrations ranging from 0 to 5 times the average values described in the literature and detailed in a previous article by Palenzuela *et al.,* 2023. The chemicals used as standards were supplied by Sigma-Aldrich and Merck.

6. Statistical analysis of the data sets

Data matrices obtained for wine characterisation and volatile compounds (major and minor) were subjected to a two-way Analysis of Variance (ANOVA) to evaluate the effects of vintage, winery and their interaction. Fisher's Least Significant Difference (LSD) test was used for mean comparison ($p \leq 0.05$) using IBM SPSS Statistics software v. 29.0.2.0 (IBM Corp., Armonk, NY, USA). Furthermore, a Multivariate Analysis of Variance (MANOVA) and a Principal Component Analysis (PCA) were performed using Statgraphics Centurion (v. 16.1.11) (Statgraphics Technologies, Inc., The Plains, Virginia, USA). For PCA, data were auto-scaled (normalised) to reduce dimensionality and identify key volatile compounds with discriminant power regarding winery and vintage influence.

RESULTS

1. Chemical characterisation of wines

Table 1 summarises the average values, standard deviations and HGs at the $p \leq 0.05$ significance level for the wine samples from the three wineries and two vintages. All wine samples met the Montilla-Moriles PDO normative for Fino-type wines (Montilla-Moriles, 2025a). The vintage was the dominant factor influencing general parameters, particularly ethanol content (higher in 2021) and absorbance at 420 nm (higher in 2022). In contrast, the winery factor, likely reflecting winemaking practices, primarily affected total acidity, volatile acidity and absorbance at 280 nm, with the exception of LC winery, where this index remained stable across years

The high variability in malic and lactic acid concentrations is attributable to both initial grape composition and the elective occurrence of malolactic fermentation (MLF). Because the bacteria responsible for MLF are highly susceptible to pH and ethanol values, the process is significantly modulated by discretionary oenological practices. Two-way ANOVA (vintage, winery, and interaction) confirmed that most variables, except density, were significantly influenced by the interaction ($p \leq 0.001$) between vintage and winery (Table S1), aligning with previous studies in young PX wines from other production zones in the same PDO (Fuentes-Espinosa *et al*., 2025a; Fuentes-Espinosa *et al*., 2025b; Alcalá-Jiménez *et al.*, 2025).

2. Major volatile compounds and polyols

Table 2 lists the average concentrations and standard deviations of 11 volatile compounds and 3 polyols, as well as the HGs at $p \leq 0.05$. Several of these compounds exhibit significant statistical differences among wineries and vintages.

Acetaldehyde, acetoin, and ethyl lactate emerged as main discriminators for the DM winery, where the higher concentrations, together with greater volatile acidity, suggest a partial MLF and different microbial management. Regarding vintage 2022, wines were characterised by higher levels of 1-propanol, isobutanol, 2-methyl-1-butanol, and glycerol, likely due to warmer conditions and higher sugar content in the musts.

Ethyl acetate and diethyl succinate levels varied significantly across samples and were strongly influenced by winery and vintage.

Differences in the concentrations of *levo*- and *meso*-2,3 butanediol, aligned with acetoin levels, reflecting specific yeast responses to redox conditions.

2.1. Two-way ANOVA results

The results in Table S2 indicate that while most alcohols and esters were significantly affected by both vintage and winery, methanol and 2-phenylethanol contents were mainly influenced by the winery. The interaction between both factors highlights how environmental conditions and vinification technologies collectively shape the fermentative profile of PX wines in this zone.

2.2. PCA of major volatile compounds and polyols

PCA results (Figure 1 and Table S4 explained 71.9 % of the total variance, effectively differentiating samples by winery and vintage. PC1 (40.18 %) primarily separated wines based on fermentation management, being positively correlated with acetaldehyde, acetoin, ethyl lactate, diethyl succinate, and 2-phenylethanol, and negatively with isobutanol. PC2 (31.72 %) was mainly influenced by 2-methyl-1-butanol

with positive loading, and ethyl acetate and the two isomers of 2,3-butanediol with negative loading, reinforcing that the interaction between microclimate and winemaking technology defines the profile of major volatiles and polyols in PX wines.

3. Minor volatile compounds

The concentrations and standard deviations of 51 minor volatile compounds, grouped by chemical families, are listed in Table 3. All of them exhibit concentrations at ppb levels (μg L^{-1}), with the exception of γ-butyrolactone. These volatiles are mainly formed by yeast metabolism during alcoholic fermentation through several biochemical pathways involving amino acid catabolism, fatty acid synthesis, and esterification. Due to their low odour detection thresholds and pleasant nuances, they have a significant influence on wine aroma (Palenzuela *et al.*, 2023; Carpena *et al.*, 2021; He *et al.*, 2023).

Esters were the most abundant group in this minor volatile fraction, comprising 26 compounds (8 acetates of higher alcohols, 13 ethyl esters of medium- and long-chain fatty acids, and 5 other esters), all of which showed a significant increase in the 2022 vintage. Specifically, the higher levels of C_6-C_{16} ethyl esters and acetates (*e.g.*, isoamyl acetate and 2-phenylethyl acetate) in 2022 point to enhanced lipid metabolism and fermentative activity of yeasts under the stress conditions caused by the high sugar content of the musts and subsequent high alcohol production.

Regarding other chemical families, higher alcohols such as hexanol, octanol, and decanol appeared at moderate concentrations, likely linked to the action of lipoxygenase (LOX) enzymatic activity during grape processing (Piombino *et al.*, 2022). Within the lactones, γ-butyrolactone was detected in all samples and dominated this family. In contrast, furanic and carbonyl compounds (the latter comprising aldehydes and ketones with six to ten carbon atoms) exhibit the highest variability among wineries. This variation was probably driven by differences in LOX activity, oxygen management, antioxidant protection and clarification procedures during vinification in each facility, as well as the pre-harvest grape dehydration, which is required to obtain traditional wines in this zone. Finally, minor terpenes were also detected, reflecting limited yeast biotransformation of grape-derived precursors during fermentation.

3.1. Two-way ANOVA results

The ANOVA (Table S3) confirmed that while almost all 51 minor volatiles were significantly influenced by both winery and vintage factors, furfural was the only compound unaffected by vintage.

TABLE 1. Average values and standard deviations of the oenological parameters in wines from three wineries of the higher-quality zone Moriles Alto in the DOP Montilla-Moriles.

Parameters	LC2021	LC2022	EM2021	EM2022	DM2021	DM2022	HGs
Ethanol (% *v/v*)	15.0 ± 0.1 b	14.5 ± 0.1 a	15.0 ± 0.1 b	14.5 ± 0.1 a	15.0 ± 0.1 b	14.5 ± 0.1 a	2
pH	3.46 ± 0.00 c	3.35 ± 0.00 b	3.20 ± 0.01 a	3.54 ± 0.00 d	3.54 ± 0.01 d	3.57 ± 0.00 e	5
Volatile acidity (g L^{-1})	0.47 ± 0.02 e	0.24 ± 0.00 a	0.33 ± 0.00 c	0.30 ± 0.00 b	0.62 ± 0.02 f	0.38 ± 0.02 d	6
Total acidity (g L^{-1})	4.18 ± 0.02 a	4.76 ± 0.00 c	5.38 ± 0.02 e	4.17 ± 0.00 a	5.10 ± 0.04 d	4.61 ± 0.00 b	5
Malic Acid (g L^{-1})	0.70 ± 0.00 d	0.74 ± 0.02 d	0.63 ± 0.01 c	0.58 ± 0.07 bc	0.32 ± 0.01 a	0.52 ± 0.05 b	4
Lactic Acid (g L^{-1})	0.57 ± 0.01 d	0.48 ± 0.04 c	0.30 ± 0.00 a	0.41 ± 0.02 b	0.96 ± 0.01 e	2.31 ± 0.05 f	6
Density (g L^{-1})	987 ± 0 a	988 ± 0 b	987 ± 0 a	987 ± 0 a	987 ± 0 a	987 ± 0 a	2
Reducing sugars (g L^{-1})	0.7 ± 0.0 a	0.96 ± 0.00 b	0.7 ± 0.0 a	0.96 ± 0.00 b	1.3 ± 0.1 c	1.68 ± 0.00 d	4
Absorbance at 280 nm	8.1 ± 0.1 a	8.0 ± 0.1 a	9.1 ± 0.1 c	11.3 ± 0.1 e	8.4 ± 0.1 b	9.6 ± 0.01 d	5
Absorbance at 420 nm	0.162 ± 0.001 a	0.199 ± 0.001 c	0.198 ± 0.001 b	0.253 ± 0.001 f	0.209 ± 0.001 d	0.234 ± 0.000 e	6

Different letters (a, b, c, d, e, f) in the same row indicate different Homogeneous Groups (HGs) at the $p < 0.05$ significance level. Identification of wine samples from 2021 and 2022 vintages: "Lagar Casablanca" LC2021, LC2022; "Bodegas El Monte" EM2021, EM2022; and "Bodegas Doblas Martos" DM2021, DM2022.

TABLE 2. Average values and standard deviations of the major volatile compounds and polyols in wines from three wineries of the higher quality zone Moriles Alto in the DOP Montilla-Moriles.

Compounds (mg L^{-1})	CAS	LC2021	LC2022	EM2021	EM2022	DM2021	DM2022	HGs
Carbonyls (2)								
Acetaldehyde	75-07-0	189 ± 19 c	94 ± 3 a	178 ± 11 c	134 ± 6 b	352 ± 9 e	217 ± 12 d	5
Acetoin	513-86-0	46 ± 4 a	43 ± 2 a	53 ± 5 b	40 ± 2 a	85 ± 6 d	68 ± 3 c	4
Alcohols (6)								
Methanol	67-56-1	68 ± 4 a	73 ± 1 a	82 ± 4 b	74 ± 2 a	73 ± 7 a	82 ± 5 b	2
1-Propanol	71-23-8	26 ± 1 a	52 ± 2 d	37 ± 1 b	46 ± 1 c	38.0 ± 0.9 b	48 ± 2 c	4
Isobutanol	78-83-1	31 ± 1 d	30 ± 1 d	21 ± 1 b	22.9 ± 0.5 c	20.8 ± 0.3 bc	19.0 ± 0.5 a	4
2-Methyl-1-Butanol	137-32-6	35 ± 1 c	38.3 ± 0.4 d	32 ± 0.6 b	28 ± 1 a	32.0 ± 0.3 b	35.7 ± 0.6 c	4
3-Methyl-1-Butanol	123-51-3	204 ± 9 b	265 ± 4 de	254 ± 6 d	185 ± 5 a	226 ± 2 c	268 ± 8 e	5
2-Phenylethanol	60-12-8	22 ± 2 a	28 ± 1 bc	33 ± 3 c	26 ± 1 ab	46 ± 4 d	47 ± 3 d	4
Ethyl esters (3)								
Ethyl Acetate	141-78-6	69 ± 2 ab	67 ± 1 a	76 ± 3 c	123.7 ± 0.6 e	71 ± 2 b	89 ± 2 d	5
Ethyl Lactate	97-64-3	50 ± 4 b	13.1 ± 0.1 a	41 ± 2 b	17 ± 1 a	189 ± 18 d	100 ± 9 c	4
Diethyl Succinate	123-25-1	6.7 ± 0.5 c	4.7 ± 0.2 ab	5.7 ± 0.2 b	4.3 ± 0.3 a	17 ± 1 e	10.0 ± 0.5 d	5
Polyols (3)								
2,3-Butanediol (*levo*)	24347-58-8	465 ± 53 a	758 ± 67 b	1,185 ± 85 c	1,579 ± 127 d	2,000 ± 83 c	839 ± 39 b	4
2,3-Butanediol (*meso*)	5341-95-7	165 ± 19 a	238 ± 21 b	368 ± 22 c	516 ± 42 d	398 ± 27 c	277 ± 11 b	4
Glycerol	56-81-5	5,612 ± 797 a	9,901 ± 532 b	11,583 ± 633 c	14,132 ± 1057 d	11,354 ± 1254 bc	12,868 ± 506 cd	4

Different letters (a, b, c, d, e, f) in the same row indicate different Homogeneous Groups (HGs) at the $p < 0.05$ significance level. Identification of wine samples from 2021 and 2022 vintages: "Lagar Casablanca" LC2021, LC2022; "Bodegas El Monte" EM2021, EM2022; and "Bodegas Doblas Martos" DM2021, DM2022.

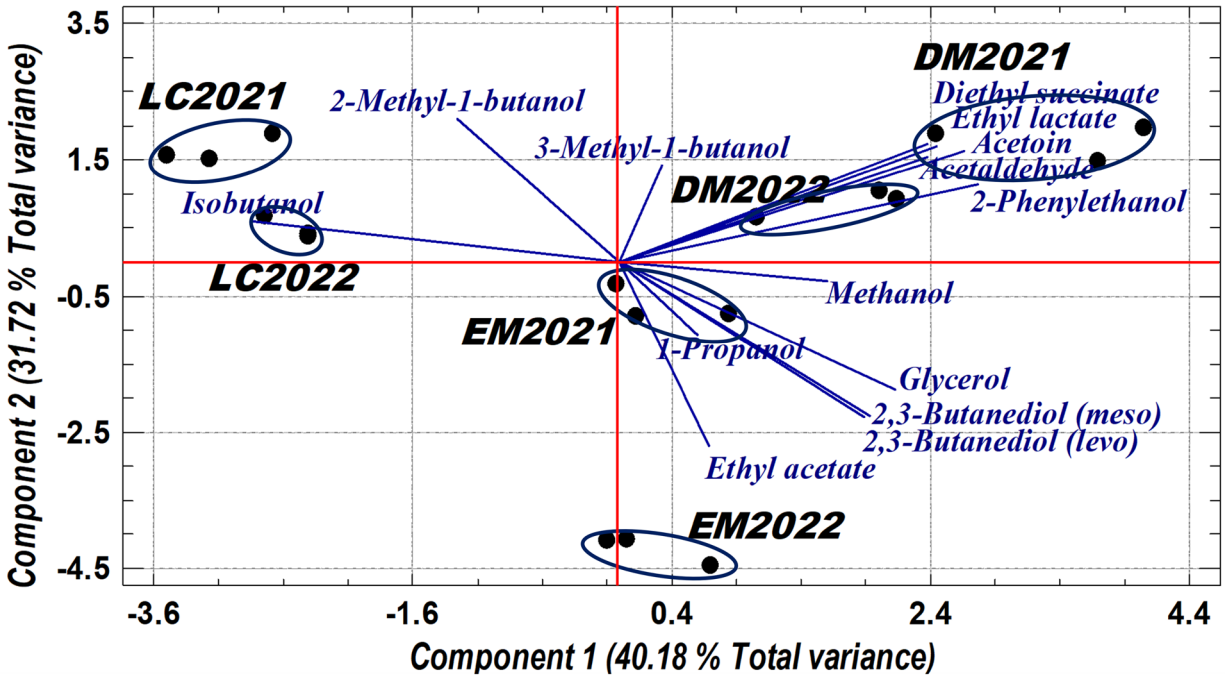


FIGURE 1. Principal Components Analysis biplot obtained from the major volatiles and polyols content of wines of Moriles Alto zone produced in the 2021 and 2022 vintages.

DM: Doblas Martos, EM: El Monte, and LC: Lagar Casablanca. The points represent the scores of the wine samples, and the vectors represent each compound's contribution to each principal component.

TABLE 3. Average values and standard deviations of 51 minor volatiles quantified (μg L^{-1}) in wines from three wineries of the Moriles Alto higher quality zone. (Part 1/2).

Compounds (μg L^{-1})	CAS	LC2021	EM2021	DM2021	LC2022	EM2022	DM2022	HGs
Acetates (8)								
Butyl Acetate	123-86-4	3.8 ± 0.2 a	4.5 ± 0.4 b	9.5 ± 0.2 d	4.4 ± 0.0 b	3.5 ± 0.2 a	5.7 ± 0.3 c	4
Isoamyl Acetate	123-92-2	1,826 ± 58 a	3,422 ± 137 c	2,573 ± 106 b	6,696 ± 265 d	6,587 ± 187 d	7,342 ± 480 e	5
Hexyl Acetate	142-92-7	35 ± 1 a	84 ± 3 b	59 ± 1 ab	282 ± 25 c	328 ± 17 d	277 ± 24 c	4
Octyl Acetate	112-14-1	3.2 ± 0.3 b	3.4 ± 0.1 bc	4.2 ± 0.5 d	3.67 ± 0.04 c	3.3 ± 0.1 bc	1.1 ± 0.1 a	4
Ethyl-phenyl Acetate	101-97-3	4.71 ± 0.09 a	2.04 ± 0.01 a	2.8 ± 0.3 a	97 ± 3 b	117 ± 3 c	100 ± 5 b	3
2-Phenyl-ethyl Acetate	103-45-7	1,008 ± 61 a	1,384 ± 63 b	2,743 ± 166 c	3,644 ± 110 d	4,352 ± 114 e	3,703 ± 225 d	5
Geranyl Acetate	105-87-3	7.28 ± 0.03 d	1.83 ± 0.05 a	3.1 ± 0.2 c	2.5 ± 0.1 b	2.28 ± 0.07 b	2.3 ± 0.1 b	4
(Z)-3-Hexenyl Acetate	3681-71-8	0.00 a	0.00 a	0.00 a	6.7 ± 0.1 c	6.6 ± 0.2 c	6.0 ± 0.1 b	3
Ethyl Esters (13)								
Ethyl Isobutyrate	97-62-1	50 ± 2 d	37 ± 3 b	40 ± 2 c	0.00 a	0.00 a	0.00 a	4
Ethyl Butyrate	105-54-4	245 ± 10 c	158 ± 12 a	192 ± 4 b	268 ± 24 d	191 ± 8 b	295 ± 11 e	5
Ethyl 2-methyl-butyrate	7452-79-1	7.0 ± 0.5 b	9.9 ± 0.7 d	8.6 ± 0.2 c	0.00 a	0.00 a	0.00 a	4
Ethyl 3-methyl-butyrate	108-64-5	14 ± 1 b	21 ± 1 d	18.1 ± 0.7 c	0.00 a	0.00 a	0.00 a	4
Ethyl Hexanoate	123-66-0	200 ± 17 a	453 ± 11 b	615 ± 5 c	1,180 ± 22 e	634 ± 3 c	1045 ± 11 d	5
Ethyl Heptanoate	106-30-9	0.29 ± 0.03 d	0.20 ± 0.02 b	0.26 ± 0.01 c	0.40 ± 0.03 e	0.22 ± 0.01 b	0.00 a	5
Ethyl Octanoate	106-32-1	417 ± 20 a	608 ± 29 b	762 ± 2 c	1,509 ± 53 e	1,084 ± 15 d	1,696 ± 78 f	6
Ethyl Decanoate	110-38-3	806 ± 23 a	906 ± 64 a	1,417 ± 121 b	4,704 ± 216 d	3,603 ± 196 c	4,740 ± 462 d	4
Ethyl Undecanoate	627-90-7	0.00 a	0.00 a	0.00 a	1.5 ± 0.0 d	0.87 ± 0.04 b	1.4 ± 0.1 c	4
Ethyl Dodecanoate	106-33-2	17.2 ± 0.7 a	122 ± 8 a	310 ± 11 b	3,421 ± 103 d	2,264 ± 100 c	3,659 ± 214 e	5
Ethyl Tetradecanoate	124-06-1	8.7 ± 0.6 a	8.0 ± 0.2 a	19 ± 3 b	58 ± 3 d	35 ± 1 c	76 ± 2 e	5
Ethyl Hexadecanoate	628-97-7	12.8 ± 0.2 a	28.9 ± 0.4 b	60 ± 1 c	87 ± 8 d	80 ± 4 d	113 ± 7 e	5
Ethyl Benzoate	93-89-0	0.57 ± 0.06 b	1.00 ± 0.03 d	0.00 a	0.00 a	0.94 ± 0.02 c	0.00 a	4
Other esters (5)								
Hexyl Hexanoate	6378-65-0	0.00 a	0.00 a	0.00 a	12.4 ± 0.2 c	10.5 ± 0.4 b	11.9 ± 0.6 c	3
Phenethyl Benzoate	94-47-3	3.28 ± 0.09 a	3.20 ± 0.05 a	3.2 ± 0.3 a	3.4 ± 0.1 ab	3.3 ± 0.1 a	3.5 ± 0.1 b	2
Phenethyl Butyrate	103-52-6	0.00 a	0.63 ± 0.05 b	1.57 ± 0.06 c	6.5 ± 0.3 f	2.1 ± 0.2 d	5.9 ± 0.3 e	6
Phenethyl Hexanoate	627-90-7	0.00 a	0.00 a	0.00 a	1.5 ± 0.0 c	0.35 ± 0.02 b	1.52 ± 0.01 c	3
(E)-Methyldihydrojasmonate	2630-39-9	0.70 ± 0.08 a	0.92 ± 0.01 ab	1.8 ± 0.1 c	1.9 ± 0.1 c	1.1 ± 0.2 b	2.1 ± 0.3 c	3
Higher Alcohols (5)								
Hexanol	111-27-3	2,359 ± 130 d	1,562 ± 96 b	1,319 ± 135 a	1,619 ± 158 bc	1,287 ± 86 a	1,796 ± 14 c	4
2-Ethyl-1-Hexanol	104-76-7	58 ± 5 c	60 ± 3 c	56 ± 5 c	24 ± 2 a	19 ± 3 a	43.4 ± 0.6 b	3
Octanol	111-87-5	53 ± 2 b	0.00 a	59 ± 4 b	122 ± 12 c	0.00 a	129 ± 11 c	3
Decanol	112-30-1	0.00 a	22 ± 2 d	17.5 ± 0.4 c	24 ± 1 de	14.1 ± 0.7 b	24.4 ± 0.4 e	5
Dodecanol	112-53-8	9.6 ± 0.6 a	11 ± 1 bc	11.8 ± 0.4 cd	10.6 ± 0.2 ab	14.61 ± 0.04 e	12.3 ± 0.5 d	5
Phenols (2)								
4-Ethyl Guaiacol	2785-89-9	614 ± 56 b	52 ± 1 a	1,213 ± 60 c	0.00 a	0.00 a	0.00 a	3
2-Methoxy-4-Vinyl-phenol	7786-61-0	0.00 a	43 ± 3 b	0.00	101 ± 5 d	97.5 ± 0.8 cd	94 ± 4 c	4

Different letters (a, b, c, d, e, f) in the same row indicate different Homogeneous Groups (HGs) at the $p < 0.05$ significance level. Identification of wine samples from 2021 and 2022 vintages: "Lagar Casablanca" LC2021, LC2022; "Bodegas El Monte" EM2021, EM2022; and "Bodegas Doblas Martos" DM2021, DM2022.

TABLE 3. Average values and standard deviations of 51 minor volatiles quantified (μg L^{-1}) in wines from three wineries of the Moriles Alto higher quality zone. (Part 2/2).

Compounds (μg L^{-1})	CAS	LC2021	EM2021	DM2021	LC2022	EM2022	DM2022	HGs
Lactones (3)								
γ-Butyrolactone	96-48-0	14,669 ± 382 c	14,227 ± 816 c	13,503 ± 541 c	11,950 ± 440 b	7,006 ± 191 a	12,072 ± 1,242 b	3
γ-Nonalactone	104-61-0	43 ± 3 c	28 ± 2 b	29 ± 3 b	17 ± 1 a	14 ± 1 a	14.7 ± 0.2 a	3
β-Damascenone	23726-93-4	27.1 ± 0.2 a	31 ± 2 a	45 ± 3 b	145 ± 7 d	112 ± 6 c	140 ± 14 d	4
Carbonyls (7)								
Hexanal	66-25-1	8.6 ± 0.5 c	6.9 ± 0.7 b	6.8 ± 0.3 b	5.4 ± 0.2 a	6.2 ± 0.2 b	8.1 ± 0.3 c	3
Benzaldehyde	100-52-7	0.00 a	0.00 a	0.00 a	2.7 ± 0.4 b	3.7 ± 0.6 c	0.00 a	3
Acetophenone	98-86-2	0.00 a	0.00 a	0.00 a	0.00 a	1.32 ± 0.02 b	0.00 a	2
Octanal	124-13-0	1.10 ± 0.04 a	1.27 ± 0.06 a	1.35 ± 0.08 a	2.0 ± 0.2 a	1.29 ± 0.03 a	3 ± 1 b	2
(E)-2-Octenal	2548-87-0	0.00 a	0.00 a	0.00 a	8.8 ± 0.1 b	0.00 a	0.00 a	2
(E)-2-Nonenal	18829-56-6	3.77 ± 0.03 b	0.00	4.2 ± 0.6 b	14 ± 1 d	10.4 ± 0.5 c	17 ± 1 e	5
Decanal	112-31-2	7.1 ± 0.2 a	7.8 ± 0.6 ab	9 ± 1 b	11.5 ± 0.4 c	14.2 ± 0.6 d	8.8 ± 0.6 b	4
Terpenes (5)								
(DL)-Limonene	138-86-3	0.00 a	0.00 a	0.00 a	3,501 ± 279 c	2,711 ± 98 b	0.00 a	3
(E)-Geranyl Acetone	3796-70-1	0.91 ± 0.09 a	1.6 ± 0.2 bc	1.9 ± 0.3 c	1.0 ± 0.3 a	0.85 ± 0.01 a	1.4 ± 0.2 b	3
(Z)-Geranyl Acetone	3879-26-3	1.98 ± 0.09 a	2.14 ± 0.02 a	2.4 ± 0.3 b	2.1 ± 0.1 a	2.05 ± 0.00 a	1.97 ± 0.05 a	2
(E)-Citral	141-27-5	0.00 a	7.9 ± 0.1 b	0.00 a	8.6 ± 0.3 c	0.00 a	11.7 ± 0.6 d	4
(Z)-Nerolidol	7212-44-4	0.00 a	0.20 ± 0.02 b	0.00 a	0.00 a	0.00 a	0.00 a	2
Furanic (3)								
Furfural	98-01-1	408 ± 21 a	609 ± 27 b	808 ± 59 c	758 ± 29 c	396 ± 15 a	749 ± 48 c	3
2-Furanmethanol	98-00-0	2.1 ± 0.2 a	4.7 ± 0.2 c	3.9 ± 0.3 b	8.2 ± 0.9 e	3.2 ± 0.1 b	6.5 ± 0.5 d	5
2-Acetylfuran	1192-62-7	0.00 a	0.00 a	0.00 a	25 ± 1 b	0.00 a	0.00 a	2

Different letters (a, b, c, d, e, f) in the same row indicate different Homogeneous Groups (HGs) at the $p < 0.05$ significance level. Identification of wine samples from 2021 and 2022 vintages: "Lagar Casablanca" LC2021, LC2022; "Bodegas El Monte" EM2021, EM2022; and "Bodegas Doblas Martos" DM2021, DM2022.

3.2. PCA of minor volatile compounds

To evaluate the complex biochemical relationships of the minor volatile compounds quantified and their dependence on vintage and winery practices, a PCA was performed. The model explained 70.68 % of the total variance (PC1 = 54.03 %; PC2 = 16.65 %). PC1 clearly differentiated the samples by vintage; the 2022 wines clustered on the negative side, driven by high negative loadings of ethyl and acetate esters (*e.g.*, ethyl octanoate, decanoate, and dodecanoate, and isoamyl and 2-phenylethyl acetates) together with several medium-chain fatty acid esters (Table S5). Conversely, the 2021 wines correlated with positive PC1 loadings, which are influenced mainly by γ-butyrolactone, 4-ethylguaiacol, long-chain alcohols, and lactones. Meanwhile, PC2 differentiated the wines according to winery technical variables (such as oxygen exposure, clarification and tank capacity) that modulate yeast redox metabolism. Positive contributions were associated with furanic and carbonyl compounds (furfural, octanol, and γ-butyrolactone), whereas acetophenone, ethyl benzoate, and decanal exerted negative loadings. Overall, the PCA underscores that both vintage and traditional winemaking practices are the primary drivers shaping the minor volatile profile.

4. Volatile fingerprinting by multivariate analysis (MVA)

The MVA fingerprints (Figures 3 and 4) provide a global visualisation of how yeast metabolism responded to the studied factors, revealing a clear separation between vintages.

The 2022 vintage was characterised by higher relative intensities of higher alcohols (isobutanol, 2- and 3-methyl-1-butanol), ethyl esters, acetates, and terpene derivatives, confirming a more vigorous fermentation and enhanced esterification pathways. In contrast, the 2021 wines showed a profile shifted toward higher relative values of acetaldehyde, ethyl lactate, glycerol, lactones, furanic compounds, and carbonyl derivatives, alongside a moderate increase in volatile

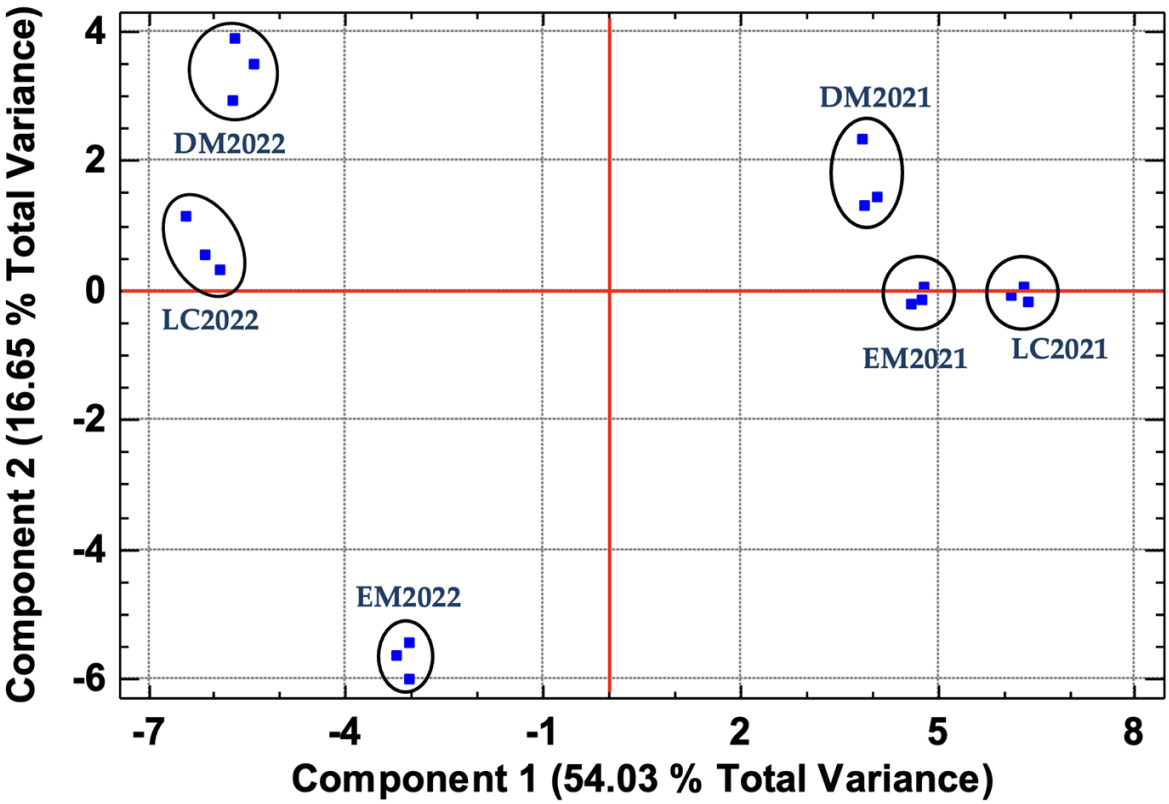


FIGURE 2. Sample scores of wines obtained by Principal Component Analysis of 51 minor volatile compounds in wines from the Moriles Alto zone produced in the 2021 and 2022 vintages.

DM: Doblas Martos, EM: El Monte, and LC: Lagar Casablanca. The points represent the scores of the wine samples.

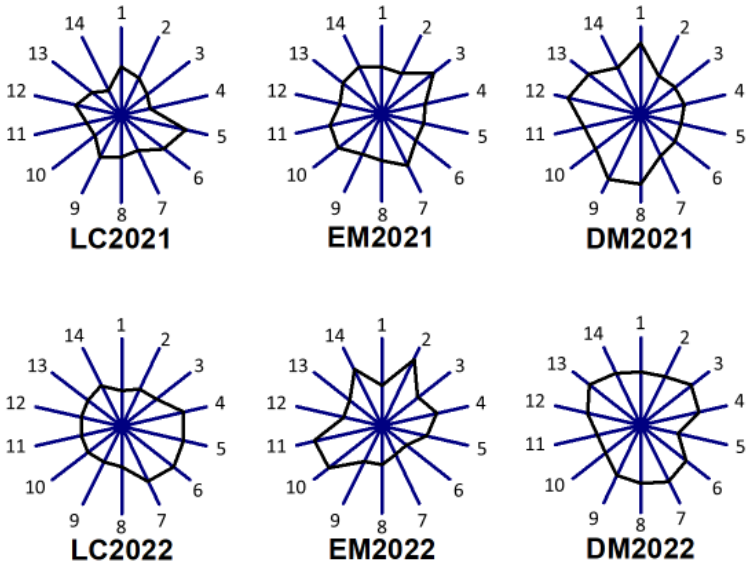


FIGURE 3. Fingerprinting obtained by a Multiple Variable Analysis of the average contents of wines from two vintages in the major volatile compounds and polyols.

1: acetaldehyde; 2: ethyl acetate; 3: methanol; 4: 1-propanol; 5: isobutanol; 6: 2-methyl-1-butanol; 7: 3-methyl-1-butanol; 8: acetoin; 9: ethyl lactate; 10: 2,3-butanediol (levo); 11: 2,3-butanediol (meso); 12: diethyl succinate; 13: 2-phenylethanol; 14: glycerol. Each sunray shows the value for each variable scaled from the mean minus three times its standard deviation at the origin to the mean plus three times its standard deviation at the extreme. DM: Doblas Martos, EM: El Monte, and LC: Lagar Casablanca.

phenols. This fingerprint suggests slower fermentation kinetics and stress-related pathways in yeast cells, as well as a partial shift towards oxidative metabolic routes potentially linked to variations in must clarification or air exposure.

In summary, while the vintage exerted the primary influence on both the major and minor volatile fractions, the winery practices acted as a secondary modulator, defining the specific distribution and unique "fingerprint" of the wines within each year. These metabolic patterns underscore the complex interplay between environmental conditions and the traditional vinification technology of the Montilla-Moriles PDO.

DISCUSSION

The quantitative analysis of wine volatile compounds has emerged as a powerful tool to explore the synergistic influence of environmental factors and winemaking practices associated with the concept of *terroir* (van Leeuwen *et al.*, 2020; Bedreag *et al.*, 2025). In traditional areas, controlled by the PDO regulations, this approach allows for a clear distinction between the contributions of vintage and winery-specific technology, ensuring the preservation of the identity of their wines, thereby deepening the understanding of their quality and typicity (van Leeuwen *et al.*, 2020; Miliordos *et al.*, 2024; Bedreag *et al.*, 2025). In this study, PCA identified that major volatile compounds and polyols accounted for 71.90 % of the total variance, a value consistent with other sub-zones within the same PDO and vintages (Fuentes-Espinosa *et al.*, 2025a; Fuentes-Espinosa *et al.*, 2025b). However, the Moriles Alto Quality Zone reveals a balanced differentiation where winery practices (40.18 %) and vintage (31.72 %) almost equally modulate the final profile. This highlights that in this specific zone, the winemaker's decisions are as influential as the climatic conditions.

For minor volatiles, the differentiation was even more pronounced. The vintage effect (54.03 % variance) was primarily driven by acetates and ethyl esters of C8, C10, and C12 fatty acids, confirming that climate significantly influences yeast-derived fermentative metabolites (Bedreag *et al.*, 2025; Eder *et al.*, 2024; Liu *et al.*, 2019; Roullier-Gall *et al.*, 2014; van Leeuwen *et al.*, 2020). Conversely, winery-specific practices (16.65 %) were reliably marked by furfural, octanol, and γ-butyrolactone, volatiles that remain stable regardless of the year and reflect the influence of technological heritage on the sensory properties of wine from each winery (Schmidtke *et al*., 2020).

Environmental conditions strongly influence the biosynthesis of grape aromatic precursors and subsequent yeast-derived metabolites during fermentation, allowing the wine volatiles to reflect *terroir* nuances (Bedreag *et al.*, 2025; Eder *et al.*, 2024; Liu *et al.*, 2019; Roullier-Gall *et al.*, 2014; van Leeuwen *et al.*, 2020). While soil properties and microbial communities characterise subzones within specific regions like Montilla-Moriles (Alcalá-Jiménez *et al.*, 2025; Fuentes-Espinosa *et al.*, 2025a; Fuentes-Espinosa *et al.*, 2025b; Osta-Fort, 2002; Palma-López *et al.*, 2024), annual climatic

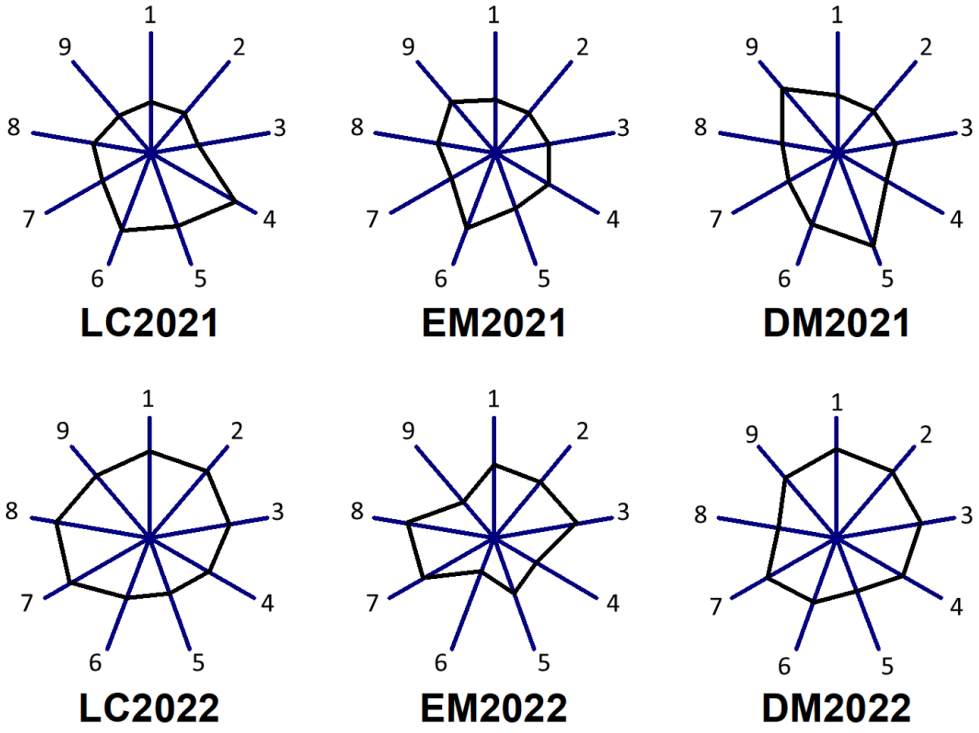


FIGURE 4. Fingerprinting obtained by a Multiple Variable Analysis of the average contents of wines from two vintages in minor volatile compounds grouped by chemical families.

1: ethyl esters. 2: other esters. 3: acetates. 4: higher alcohols. 5: volatile phenols. 6: lactones. 7: carbonyl compounds. 8: terpenes and derivatives. 9: furanic compounds. Each sunray shows the value for each variable scaled from the mean minus three times its standard deviation at the origin to the mean plus three times its standard deviation at the extreme. DM: Doblas Martos, EM: El Monte, and LC: Lagar Casablanca.

variations significantly affect fermentative yeast diversity. Indeed, the absence of stable, year-round strains across vintages suggests that a repeatable microbial signature cannot be solely responsible for *terroir* specificity (Benito-Castellanos *et al.*, 2026). Previous studies in neighbouring subzones have shown that chemical fingerprints can reveal *terroir*-related differences even when the vintage exerts a dominant influence on the overall composition (Bedreag *et al.*, 2025; Liu *et al.*, 2019), and that such chemical variations often correlate with sensory perception (Miliordos *et al.*, 2024; Ohwofasa *et al.*, 2024).

The interplay between production region, microbial patterns, and quality outcomes is driven by the interaction of three primary abiotic determinants: climate, edaphic (soil) conditions, and topography, alongside the human dimension of viticulture.

Therefore, a comprehensive definition of *terroir* within traditional PDO must holistically integrate abiotic determinants with human dimensions, including winery-specific practices and traditional winemaking techniques (Bokulich *et al.*, 2013; Schmidtke *et al.*, 2020). Integrating environmental and chemical data through high-resolution metabolomics clarifies how vintage and technological factors interact, identifying which volatile compounds remain recognisable over time despite climatic or technological shifts (Visconti *et al.*, 2024).

For the Montilla-Moriles winegrowing area, and specifically prominent zones like Moriles Alto, this approach provides regulatory councils and winemakers with objective, marker-based evidence to reinforce geographical indications, optimise technological decisions to maintain distinctive wine styles and ultimately enhance the economic valuation of these traditional high-quality assets.

CONCLUSIONS

This study demonstrates that the volatile profile of young Pedro Ximénez wines from the Moriles Alto zone, sharing identical cultivar and abiotic factors, is significantly shaped by both vintage and winery-specific practices. Vintage emerged as the primary driver, modulating volatile compounds, highlighting how interannual meteorological variability impacts grape composition and fermentation. Concurrently, within the two vintages studied, preliminary evidence suggests that winery practices impart distinct metabolic profiles to each producer. Despite interannual variations, multivariate analysis identified stable volatile patterns that consistently differentiated wines by winery over time. These findings demonstrate that winemaking decisions and traditional practices are integral components of high-quality *terroirs* and must be given greater weight in their definition. Lastly, this work establishes a chemical framework that provides a scientific basis for future zoning criteria, adaptive viticulture strategies, and the preservation of traditional winemaking expertise and product typicity in traditional PDO regions.

HIGHLIGHTS

Vintage is the primary driver of volatile fingerprinting in young Pedro Ximénez wines.

Winery-specific practices create a recognisable chemical signature.

Multivariate patterns successfully differentiate wines by *terroir (Pago)* despite annual climatic shifts.

The volatile profile provides a scientific basis for *terroir* zoning in traditional PDOs.

ACKNOWLEDGEMENTS

The authors are grateful for the support, wine samples, and information provided by the owners and winemakers from Bodegas Doblas Martos; Bodegas El Monte and Lagar Casablanca, vineyards and wineries. All of them are located in the “Moriles Alto” top-quality area of Montilla-Moriles Protected Denomination of Origin (Córdoba, Andalusia, Spain). This research was funded by JUNTA ANDALUCIA (SPAIN) Program: Specialised Transfer Actions Universities-CEIS-RIS3- FEDER. Singular Project AGROMIS-ceiA3, SUBSECTOR: TRADITIONAL WINES OF ANDALUCIA. Call 2020: Grants for R+D+I projects in the field of Innovation Ecosystems of the International Centres of Excellence (PY2020 ECOSIS INNOV CEIS) – Grant number PYC20 RE 068 UCO.

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