beverages

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Varietal Terpenoids and C_{13}-Norisoprenoids in Alentejo Varietal Red Wines Across Two Vintages by HS-SPME–GC–TOFMS

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Abstract

This study characterizes the volatile organic compound (VOC) profiles of young monovarietal red wines from five cultivars widely grown in Alentejo, Portugal—Aragonez, Castelão, Merlot, Syrah, and Trincadeira, across two consecutive vintages (2021 and 2022), in a total of 20 samples. Understanding how grape variety and harvest year shape VOC composition is essential for defining varietal typicity, supporting authenticity assessments, and guiding quality-driven enological practices. VOCs were extracted using headspace solid-phase microextraction (HS-SPME) and analyzed by gas chromatography coupled to time-of-flight mass spectrometry (GC–TOFMS), providing a semi-quantification of 142 volatile compounds across nine chemical families. Statistical analyses, including ANOVA and Principal Component Analysis (PCA), unveiled significant effects of variety, vintage, and their interactions on VOC composition, enabling clear discrimination of the wines. Notably, terpenes and C_{13}-norisoprenoids exhibited strong varietal differentiation patterns: Castelão and Trincadeira showed higher relative proportions of monoterpenes like linalool, β-citronellol, and geraniol, whereas Aragonez presented increased sesquiterpene levels including β-bisabolene and α-muurolene, and Syrah and Merlot displayed more diverse terpenoid patterns. Despite their lower abundance, C_{13}-norisoprenoids, particularly *trans*-β-ionone, consistently differentiated wines across vintages. A 100% ribbon chart of the main terpenoids and C_{13}-norisoprenoids highlighted genotype-driven and vintage-independent patterns, underscoring their potential as robust markers of Portuguese red wines. This study presents a high-resolution HS-SPME–GC–TOFMS chemometric workflow for the profiling and classification of young Alentejo red wines, highlighting consistent varietal patterns in terpene and C_{13}-norisoprenoid distributions under the studied conditions.

Keywords: volatile organic compounds; HS-SPME; GC–TOFMS; terpenes; C_{13}-norisoprenoids; Portuguese wines; varietal typicity

Received: 26 January 2026
Revised: 10 March 2026
Accepted: 18 March 2026
Published: 1 April 2026

1. Introduction

Aromatic quality is a central sensory attribute that strongly determines modern consumers' perception, identity, and acceptance of wines. In an increasingly competitive and experience-driven marketplace, consumers value not only flavor but also authenticity, typicity, and geographical origin. Within this context, wine aroma, predominantly

https://doi.org/10.3390/beverages12040041

shaped by volatile organic compounds (VOCs), plays a decisive role in varietal characterization, perceived quality, and market differentiation [1,2]. Wine volatile compounds arise from multiple biochemical and technological sources, including grape precursors, yeast metabolism during fermentations, and post-fermentation transformations such as esterification or oxidation. In addition to grape-derived precursors, spontaneous alcoholic fermentations can further influence volatile compounds through native yeast and bacterial communities. These microorganisms affect the formation of higher alcohols, esters, volatile acids and carbonyls compounds. Non-inoculated fermentations may therefore enhance terroir expression, as microbial ecology interacts with cultivar and must composition to generate specific aromatic profiles under similar winemaking conditions [3]. Among the thousands of compounds identified in wines, only a small fraction have sensory thresholds low enough to perceptibly influence the aroma profile; these are commonly referred to as impact odorants [4]. These key compounds include esters, higher alcohols, norisoprenoids, and terpenes, and, in some cases, volatile thiols, which together contribute to the aromatic complexity of wines, depending on grape variety and winemaking conditions. Recent studies show that consumers value specific aromatic profiles that evoke fresh fruit, floral notes, and authentic varietal characteristics, often linked to esters, higher alcohols, terpenes, and thiol compounds [5]. Terpenoids and C_{13}-norisoprenoids are widely recognized as key contributors to varietal aroma and geographical differentiation in wines because many originate directly from grape metabolism and possess very low odor thresholds. Monoterpenes such as linalool, geraniol, and α-terpineol, together with C_{13}-norisoprenoids like β-ionone, have been widely reported as markers of cultivar typicity and regional differentiation when interpreted within multivariate analytical frameworks [6].

The correlation between volatile composition and consumer preference is particularly evident in young varietal wines, where primary compounds remain more expressive and less masked by the aromas of aging [2]. Terpenoids and C_{13}-norisoprenoids have long been established as key contributors to varietal aroma and geographical differentiation in red wines, primarily due to their grape-derived origin and low odor thresholds. Recent studies on Italian appellations have further confirmed their discriminative power among cultivars and denominations. Terpenoids and C_{13} norisoprenoids have emerged as particularly informative markers of varietal and geographical origin in red wines, with recent work on Italian appellations demonstrating their discriminative power among cultivars and denominations [7].

Accurate identification and quantification of these compounds are vital; however, the chemical diversity and low concentrations of these compounds, coupled with the intricate wine matrix, challenge analytical and interpretative methods. The high-dimensional data generated by advanced analytical techniques such as headspace solid-phase microextraction (HS-SPME) coupled to gas chromatography-mass spectrometry (GC-MS) or comprehensive two-dimensional gas chromatography (GC×GC) present significant challenges for data interpretation [8]. The chemical diversity and low concentrations of many wine volatile compounds generate complex, high-dimensional datasets that are difficult to interpret using conventional analytical approaches alone. Consequently, chemometric methods, which integrate chemical measurements with multivariate statistical analysis, have become essential tools for exploring wine volatile profiles. By facilitating dimensionality reduction, pattern recognition, and predictive modeling, chemometrics enables deeper insights into aroma profiles and their sensory relevance [9]. In recent years, non-targeted HS-SPME–GC–MS approaches combined with multivariate statistics have become central to the characterization and authentication of wines, as they efficiently handle high-dimensional VOC datasets and capture subtle varietal and terroir effects. PCA and supervised methods such as PLS-DA are now widely applied to differentiate monovarietal wines and to model the impact of viticultural and technological factors on aroma composition.

Wine aroma results from the combined contributions of several VOC families, mainly esters, higher alcohols, fatty acids, carbonyls, volatile phenols, lactones, terpenes, and C_{13}-norisoprenoids [4,10]. Their relative levels depend on grape variety, vintage, and vinification. Modern HS-SPME–GC/MS techniques allow semi-quantitative profiling of these families and, when combined with chemometrics, support discrimination by variety and vintage based on peak-area patterns rather than absolute concentrations, provided matrix effects and response-factor differences are acknowledged [11,12].

Given the growing emphasis on typicity, authenticity, and Protected Designation of Origin (PDO) valorization in the European wine market, understanding the volatile signatures of Portuguese monovarietal red wines is both scientifically and economically relevant. In this context, the present study investigates the volatile organic compound profiles of five important red grape cultivars cultivated in the Alentejo region (Aragonez, Castelão, Merlot, Syrah, and Trincadeira). Using HS-SPME coupled with GC–TOFMS and multivariate statistical analysis, the study aims (i) to characterize the VOC composition of these monovarietal wines and evaluate their potential for varietal discrimination, and (ii) to assess the interannual consistency of terpenoids and C_{13}-norisoprenoids as candidate chemical markers across two consecutive vintages. In the Portuguese context, GC-based profiling has been successfully applied to define regional aromatic matrices and varietal signatures, demonstrating that volatile fingerprints can capture both cultivar and appellation effects [13]. Studies on Douro wines by Martins et al. [14] and Vinho Verde wines by Oliveira et al. [15] have connected monoterpenes and C_{13}-norisoprenoids to regional differences, supporting their use as markers of typicity and offering a framework for understanding the volatile profiles of Alentejo monovarietal red wines explored in this work.

2. Materials and Methods

2.1. Wine Samples

Wines were produced at the University of Évora experimental winery from Aragonez, Castelão, Merlot, Syrah, and Trincadeira grapes harvested from the same vineyard blocks in 2021 and 2022, under identical soil, climate, and cultivation conditions. Grapes were destemmed, crushed, and vinified independently by variety in 50 L stainless steel tanks. For each grape variety and vintage, two independent vinifications were performed under identical conditions and considered as biological replicates. Only SO_2 was added at 50 mg L^{-1} at the beginning of fermentation, using a commercial 6% sodium bisulfite solution (SAI, SOLFOX 6, CE 231-870-1). Grapes were destemmed, crushed, and vinified independently by variety in 50 L stainless steel tanks, using spontaneous alcoholic fermentation without yeast inoculation and without sugar or acidity corrections. Only SO_2 was added at 50 mg L^{-1} at the beginning of fermentation, using a commercial 6% sodium bisulfite solution (SAI, SOLFOX 6, CE 231-870-1).

Fermentations were conducted under spontaneous conditions, without inoculation of commercial yeast or lactic acid bacteria. During alcoholic fermentation, musts were punched down twice daily and when reducing sugars fell below 3 g L^{-1}, free-run wines were separated from the pomace without pressing. Malolactic fermentation subsequently occurred spontaneously in 25 L glass vessels under identical wine cellar conditions for all varieties and vintages.

Fermentations were punched down twice daily, and when reducing sugars fell below 3 g L^{-1}, free-run wines were separated from the pomace without pressing; malolactic fermentation then proceeded in 25 L glass vessels. After completion of malolactic fermentation, wines were racked off the lees, sampled, and stored at −32 °C until analysis. For each variety and vintage, two independent vinifications were carried out and treated as biological replicates.

2.2. Climatic Conditions

Meteorological data for the 2021 and 2022 agricultural years (October–September), including monthly average temperatures (Tavg), maximum temperatures (Tmax), and minimum temperatures (Tmin), as well as precipitation, were obtained from the Mitra weather station through the ICTERRA Meteorological Data Portal (Évora, Portugal). The climate data are presented graphically in Figure 1. In 2021, average monthly temperatures ranged from about 10 °C during winter (December–January) to above 23 °C in summer (July–August). During the ripening period, Tmax exceeded 33–35 °C, while Tmin stayed above 14–15 °C. Precipitation was concentrated between October and February, with monthly values often exceeding 100 mm, whereas spring and summer rains were minimal (June–August), resulting in a significant water deficit during ripening. The 2022 growing season followed a similar pattern but had higher thermal intensity, with summer Tmax values exceeding 35 °C and elevated Tmin levels. Precipitation was more irregular, with peaks in March and lower rainfall in late spring and summer, which further intensified water stress during berry growth and ripening. Overall, both harvests experienced high thermal accumulation combined with limited water availability, typical of the Alentejo region's climate and known to influence grape ripening dynamics and composition.

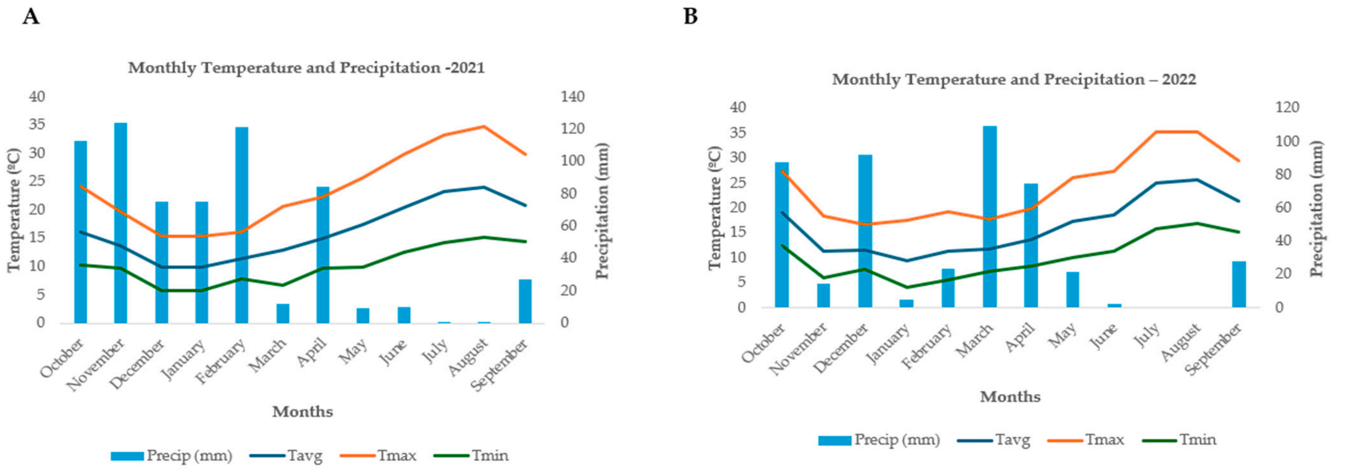

Figure 1. Monthly variation in average (Tavg), maximum (Tmax), and minimum (Tmin) temperatures (°C) and precipitation (mm) during the 2021 (**A**) and 2022 (**B**) agricultural years (October–September) recorded at the Mitra weather station (Évora, Portugal). Precip = precipitation, Tavg = mean temperature average, Tmax = mean maximum temperature, Tmin = mean minimum temperature.

2.3. Enological Parameters

Grape classical parameters such as °Brix, pH, and titratable acidity (TA, g/L of tartaric acid), along with the classical parameters of wines, such as alcoholic degree (°A), pH, titratable acidity (TA, g/L of tartaric acid), volatile acidity (VA, g/L of acetic acid), total and free SO_2 were analyzed by International Organisation of Vine and Wine (OIV) OIV methods [16].

2.4. Volatiles Extraction by HS SPME

HS-SPME sampling was performed using CAR/DVB/PDMS fibers, 1 cm, 50/30 µm film thickness (df), supplied by Supelco (Bellefonte, PA, USA). Before use, the fiber was conditioned according to the manufacturer's recommendations, and fiber blanks were run every 6 samples to ensure the absence of contaminants and carryover. Wine samples (5.0 mL) and 0.6 g of sodium chloride were placed into a 20.0 mL headspace vial sealed with a PTFE/silicone septum and a screw cap. Vials were placed in a SepSolve Analytical Sample Preparation Robot (Peterborough, UK), equilibrated for 5 min at 30 °C, and then extracted for 15 min at the same temperature. Thermal desorption of the analytes was carried out by exposing the fiber in the GC injection port at 260 °C for 3 min in splitless mode.

2.5. Volatiles Analysis by GC–TOFMS

Volatile analysis was performed on an Agilent 8890 GC system coupled to a BenchTOF-Select detector (Markes International, Bridgend, UK) and equipped with a CTC Analysis autosampler PAL-System (SepSolve Analytical, Zwingen, Switzerland). Data was acquired and analyzed with TOF-DS 4.1 from Markes International. Chromatographic separation was performed in a DB-WAX capillary column (60 m × 0.25 mm i.d. and 0.25 µm df) supplied by Agilent (Santa Clara, CA, USA). The oven temperature program started at 50 °C, held for 4 min, raised to 4° C min^{-1} up to 240 °C, and finally 4° C min^{-1} to 250 °C, and held for 5 min. Helium was used as the carrier gas under constant flow mode (1.2 mL min^{-1}), corresponding to an initial column head pressure of 24.09 psi. The MS transfer line and source temperatures were set at 250 °C. Electron ionization (EI) at 70 eV mass spectra of the analytes was recorded in the full scan, from 30 to 400 Da.

Volatile compounds were tentatively identified by comparing their mass spectra with NIST mass spectral library (NIST MS Search Program Version 2020) and by calculating linear retention indices (LRIs) using an injected C_8–C_{20} *n*-alkane series, then matching LRIs against literature values for wine matrices.

VOCs were semi-quantified as relative peak areas by calculating the percentage of their peak area relative to the total chromatographic peak area, and the results were expressed as percentages (%). All wines were analyzed in triplicate. Representative total ion chromatograms (TICs) obtained through HS-SPME–GC–TOFMS for all analyzed wine samples are provided in the Supplementary Information (Figures S1–S10). These cover the five grape varieties (Aragonez, Castelão, Merlot, Syrah, and Trincadeira) across the two vintages studied (2021 and 2022).

2.6. Statistical Analysis

The statistical analysis of the data was performed using IBM SPSS Statistics, Version 29 (IBM Corp., Armonk, NY, USA), which was used to conduct the Principal Component Analysis (PCA). PCA was performed on autoscaled data (mean-centered and unit variance scaled) to standardize variables prior to multivariate analysis. To evaluate the effects of year (Y), grape variety (V), and their interaction (Y × V) on the volatile composition of the five varietal wines, an analysis of variance (ANOVA) was carried out using NCSS Statistical Software (2016) (NCSS, LLC, Kaysville, UT, USA). Differences were considered statistically significant at $p < 0.05$, $p < 0.01$, and $p < 0.001$, and post hoc comparisons were made using the Tukey–Kramer test. Visualization of relative abundances across varieties on volatile compounds was performed using OriginPro 2023b SR1 (OriginLab, Northampton, MA, USA), which was used to create the 100% ribbon chart.

3. Results and Discussion

3.1. Oenological Parameters of Wines

The analysis of must and wine composition across the two vintages (2021 and 2022) is presented in Table 1. Overall, must and wine parameters fell within the typical range reported for red wines from warm regions. Sugar content, represented by °Brix, is very similar between varieties over the two vintages. The higher °Brix and acidity values observed in Merlot and Syrah reflect their intrinsic capacity for sugar accumulation under the edaphoclimatic conditions of the studied region. These findings align with previous studies highlighting the role of genotype in determining grape maturity indices [17,18]. Wines presented alcohol content (% *v*/*v*) ranging from 12.6 to 14.75 (% *v*/*v*), lower levels of reducing sugars, total acidity higher than 3.5 g/L (tartaric acid), mandatory by law, and volatile acidity lower than 1.2 g/L (acetic acid). We should emphasize that alcoholic fermentation was done without yeast inoculation and without any correction for sugar or acidity. Merlot and Syrah wines showed higher alcohol and acidity, as expected, while Castelão and Aragonez wines showed lower total acidity. Although pH and volatile acidity values remained within typical ranges for young red wines, the slightly higher pH levels observed in some varieties in 2022 may have implications for long-term stability. Elevated pH reduces the antimicrobial effectiveness of free SO_2 and may increase susceptibility to microbial spoilage and chemical instability if not properly managed [19]. Environmental stresses such as higher temperatures and reduced precipitation can influence grape organic acid metabolism, accelerating malic acid degradation and altering overall acid balance, which, in turn, affects wine pH and titratable acidity [20]. Wine pH also plays a central role in shaping microbial community dynamics during vinification [21]. The 2022 growing season featured higher Tmax values and less late-season precipitation compared to 2021, conditions known to speed up malic acid breakdown and alter berry acid balance. Heat and water stress can greatly affect organic acid metabolism in grape berries, resulting in lower acidity and higher pH in the wines produced. These findings align with broader evidence that links increased temperature and drought stress to changes in grape composition and wine chemistry [22].

Table 1. General composition of musts and wines (mean values) of Aragonez, Castelão, Merlot, Syrah, and Trincadeira over two consecutive vintages (2021–2022).

	Aragonez		**Castelão**		**Merlot**		**Syrah**		**Trincadeira**	
Musts										
Parameters	**2021**	**2022**	**2021**	**2022**	**2021**	**2022**	**2021**	**2022**	**2021**	**2022**
Date of vintage	25/08	02/09	30/8	26/08	25/08	26/08	25/08	02/09	25/08	08/09
°Brix	21.7	21.0	22.4	22.1	25.2	24.85	23.4	23.0	22.5	22.6
pH	3.32	3.49	3.31	3.7	3.08	3.17	3.28	3.4	3.15	3.62
%Probable alcohol	12.4	12.0	12.9	12.7	14.8	14.55	13.6	13.25	12.9	13.0
Total acidity	4.04	4.08	5.35	3.7	7.35	6.59	9.95	5.87	5.51	4.71
Wines										
Free SO_2 (mg/L)	8.0	4.5	4.36	4.47	6.5	8.5	6.5	5.0	6.3	5.2
Total SO_2 (mg/L)	29.5	20.5	28.0	11.5	14.0	33.5	19.0	21.5	39.5	24.5
Alcoholic (% *v*/*v*)	14.4	12.9	12.6	13.75	14.75	14.7	14.1	13.55	12.95	14.65
Reducing sugars (g/L)	1.3	1.0	1.5	1.9	2.7	1.5	1.5	1.3	1.1	3.3
Total acidity (g/L tartaric acid)	5.0	5.32	4.36	4.47	8.08	7.78	6.63	6.73	6.66	6.19
pH	4.04	3.79	3.92	4.03	3.2	3.33	3.5	3.69	3.51	3.58
Volatile acidity (g/L acetic acid)	0.64	0.54	0.47	0.63	0.68	0.72	0.63	0.82	0.57	0.9

Total acidity is expressed in g/L tartaric acid; Volatile acidity is expressed in g/L acetic acid.

3.2. Volatile Organic Compounds

HS-SPME extraction followed by GC–TOFMS analysis enabled the semi-quantitative profiling of 142 VOCs across the five varieties and two vintages. Compounds were grouped into eight chemical families (Table 2) with esters (71 compounds) and higher alcohols (30) predominating in number and total relative abundance, in agreement with their recognized role as the main contributors to young red wine fruity and fermentative notes.

Table 2. VOCs were tentatively identified in Aragonez, Castelão, Merlot, Syrah, and Trincadeira wines, with ANOVA results.

Esters	RT	Compound Name	LRI Lit	Most Abundant Ions m/z	ANOVA		
					Y	V	Y × V
E1	5.8811	Ethyl Acetate	888	43/45/61	n.s	***	***
E2	7.6366	Propanoic acid, ethyl ester	953(±8)	57/74/102	n.s	**	n.s
E3	7.8734	Propanoic acid, 2-methyl-, ethyl ester	961(±6)	43/71/116	n.s	***	n.s
E4	8.1439	Propyl ethanoate	973(±11)	43/61/73	*	***	**
E5	9.3677	Isobutyl acetate	1012(±8)	43/56/73	n.s	***	n.s
E6	10.1746	Butanoic acid, ethyl ester	1036(±8)	43/71/88	n.s	**	n.s
E7	10.7335	Butanoic acid, 2-methyl-, ethyl ester	1052(±8)	41/57/74/85	*	***	**
E8	11.347	Butanoic acid, 3-methyl-, ethyl ester	1068(±8)	40/57/70/85	n.s	n.s	n.s
E9	11.4694	Acetic acid, butyl ester	1074(±8)	56/61/73	n.s	n.s	n.s
E10	13.382	1-Butanol, 3-methyl-, acetate	1123(±8)	43/55/70	n.s	***	n.s
E11	13.8545	Pentanoic acid, ethyl ester	1134(±7)	41/57/70	n.s	***	n.s
E12	14.9384	2-Butenoic acid, ethyl ester	1160(±5)	69/99	n.s	***	n.s
E13	15.9322	1-Butanol, 3-methyl-, propanoate	1185(±7)	43/57/70	***	n.s	n.s
E14	15.9611	Pentanoic acid, 4-methyl-, ethyl ester	1190(±9)	41/70/101	n.s	n.s	n.s
E15	16.1183	Propanoic acid, 2-methyl-, 3-methylbutyl ester	1192(±5)	43/55/70/89	n.s	***	n.s
E16	17.5985	Hexanoic acid, ethyl ester	1233(±9)	43/88/99	n.s	***	**
E17	18.7812	Butanoic acid, 3-methylbutyl ester	1259(±5)	43/71/89	n.s	n.s	n.s
E18	19.0365	Acetic acid, hexyl ester	1273(±7)	43/56/69	n.s	**	n.s
E19	19.2731	Hex-5-enoic acid, ethyl ester	1271(±1)	41/55	n.s	n.s	n.s
E20	20.2245	3-Hexenoic acid, ethyl ester	1290(1)	41/69	n.s	n.s	n.s
E21	20.6584	3-Hexen-1-ol, acetate	1316(±6)	43/67/82	***	***	***
E22	20.6907	Hexanoic acid, propyl ester	1316(±9)	43/61/99	n.s	n.s	n.s
E23	21.2267	Heptanoic acid, ethyl ester	1331(±8)	43/60/88/101	n.s	***	*
E24	21.5576	Propanoic acid, 2-hydroxy-, ethyl ester	1347(±9)	45	n.s	***	n.s
E25	21.6386	2-Hexenoic acid, ethyl ester	1340(±13)	68/81	n.s	***	n.s
E26	21.8935	Hexanoic acid, 2-methylpropyl ester	1350(±4)	56/71/99	***	***	***
E27	22.6296	Acetic acid, heptyl ester	1377(±8)	43/56/70	n.s	***	*
E28	23.1734	Octanoic acid, methyl ester	1385(±7)	74/87	n.s	***	**
E29	23.6629	3-Hexen-1-ol, propanoate	1390(±10)	67/82	***	***	***
E30	24.6864	Octanoic acid, ethyl ester	1435(±7)	43/57/73	*	***	n.s
E31	25.4792	Isopentyl hexanoate	1451(±5)	43/70/99	n.s	n.s	n.s
E32	25.5063	Propanoic acid, 2-hydroxy-, 2-methylpropyl ester	1455(±3)	45/57	n.s	***	n.s
E33	26.3647	7-Octenoic acid, ethyl ester	1478±7(5)	41/55/70	n.s	n.s	n.s
E34	27.4222	Propyl octanoate	1510(±5)	43/102/127	n.s	n.s	n.s
E35	27.9431	Nonanoic acid, ethyl ester	1532(±7)	43/61/73	n.s	**	n.s
E36	28.1901	Pentanoic acid, 2-hydroxy-4-methyl-, ethyl ester	1547(±1)	43/69/87	n.s	***	n.s
E37	28.4477	2-Methylpropyl octanoate	1548(±4)	41/73/101	***	***	***
E38	28.9829	3-(Methylthio)propanoic acid ethyl ester	1562(±7)	61/74	n.s	n.s	n.s
E39	28.9307	Isoamyl lactate	1580(±20)	45/55/70	n.s	***	n.s
E40	29.7634	Decanoic acid, methyl ester	1593(±9)	43/74/87	n.s	***	n.s
E41	30.6197	2-Furancarboxylic acid, ethyl ester	1618(±6)	68/95/112	n.s	***	n.s
E42	31.0483	Decanoic acid, ethyl ester	1639(±8)	88	*	***	n.s
E43	31.6474	Octanoic acid, 3-methylbutyl ester	1658(±6)	43/57/71	*	***	**
E44	32.011	Benzoic acid, ethyl ester	1658(±11)	51/77	n.s	n.s	n.s
E45	32.1796	Butanedioic acid, diethyl ester	1681(±9)	101/129	n.s	***	n.s
E46	32.589	Ethyl trans-4-decenoate—4-Decenoic acid, ethyl ester	1683(±4)	41/55/69/88	*	***	n.s
E47	32.744	*trans*-Geranic acid methyl ester	1677(±2)	41/69/114	**	n.s	n.s
E48	33.4995	Decanoic acid, propyl ester	1724(±4)	43/61	n.s	n.s	n.s
E49	33.9836	Undecanoic acid, ethyl ester	1739(±12)	41/55/88	n.s	**	*
E50	34.3888	2-Methylpropyl decanoate	1750(±1)	43/57	*	**	**
E51	35.1702	Methyl salicylate	1765(±21)	65/92/120	n.s	***	n.s
E52	35.3612	Benzeneacetic acid, ethyl ester	1783(±10)	91	n.s	***	n.s
E53	35.4329	Formic acid, 2-phenylethyl ester	1771(±11)	91/104	n.s	***	n.s
E54	36.1803	Acetic acid, 2-phenylethyl ester	1813(±15)	43/91/104	n.s	***	n.s
E55	36.7909	Dodecanoic acid, ethyl ester	1843(±8)	88/101	n.s	***	n.s
E56	37.2996	Pentadecanoic acid, 3-methylbutyl ester	1863(±5)	43/70	n.s	***	n.s
E57	38.047	Ethyl 3-hydroxyoctanoate	1884(±4)	43/71/117	*	*	n.s
E58	38.3576	Butanedioic acid, ethyl 3-methylbutyl ester	1901(±9)	70/101/129	n.s	***	n.s
E59	39.4574	Ethyl tridecanoate	1947(±7)	88/101	n.s	***	n.s
E60	40.0414	β-Phenylethyl butyrate	1958±17	71/104	n.s	n.s	n.s
E61	41.7164	Isopropyl myristate	2027(±7)	43/60/102	n.s	**	n.s
E62	42.0173	Tetradecanoic acid, ethyl ester	2050(±12)	88/101	n.s	***	n.s

Table 2. *Cont.*

Esters	**RT**	**Compound Name**	**LRI Lit**	**Most Abundant Ions *m*/*z***	**ANOVA**		
					Y	**V**	**Y × V**
E63	42.4605	Isoamyl laurate	2062(±5)	70	n.s	***	n.s
E64	44.145	2-Propenoic acid, 3-phenyl-, ethyl ester	2128(±17)	77/103	n.s	***	*
E65	44.4653	Pentadecanoic acid, ethyl ester	2148±11	88/101	n.s	**	n.s
E66	46.8082	Hexadecanoic acid, ethyl ester	2251(±10)	43/88/101	n.s	***	n.s
E67	47.4098	Ethyl 9-hexadecenoate	2283(±9)	55/69	n.s	***	n.s
E68	50.5289	Butanedioic acid, 1-ethyl ester	2368(±9)	55/73/101	n.s	***	*
E69	51.2215	Octadecanoic acid, ethyl ester	2451(±13)	88/101	n.s	***	n.s
E70	51.6546	Ethyl (9Z)-octadec-9-enoate	2471(±12)	41/69	n.s	n.s	n.s
E71	52.6364	Ethyl (9Z,12Z)-octadeca-9,12-dienoate	2521(±10)	41/67/95	*	***	n.s
Alcohols							
A72	12.1919	1-Propanol, 2-methyl-	1092(±9)	43/55/74	n.s	***	n.s
A73	14.1041	1-Butanol	1142(±11)	43/56	n.s	***	n.s
A74	14.7696	1-Penten-3-ol	1158(±9)	57	***	***	***
A75	16.5054	1-Butanol, 2-methyl-	1208(±5)	41/59/70	***	n.s	n.s
A76	16.5193	1-Butanol, 3-methyl-	1209(±9)	43/55/70	n.s	***	n.s
A77	18.0928	3-Buten-1-ol, 3-methyl-	1248(±8)	41/56/68	n.s	**	n.s
A78	20.2447	1-Butanol, 2-ethyl-	1310(±8)	43/55/70	**	***	***
A79	20.4455	1-Pentanol, 4-methyl-	1315(±14)	41/56/69	n.s	***	n.s
A80	20.6345	2-Heptanol	1320(±8)	41/55/83	n.s	n.s	n.s
A81	20.8975	1-Pentanol, 3-methyl-	1326(±9)	43/56/69	n.s	***	**
A82	21.8123	1-Hexanol	1355(±7)	43/56/69	n.s	***	n.s
A83	22.2016	3-Hexen-1-ol	1367(±7)	41/55/67	n.s	**	n.s
A84	22.6163	1-Propanol, 3-ethoxy-	1373(±7)	45/59/75/86	***	***	***
A85	22.927	3-Hexen-1-ol	1382(±9)	41/55/67/82	*	***	***
A86	23.216	3-Octanol	1393(±5)	55	***	***	***
A87	25.1327	1-Octen-3-ol	1450(±7)	43/57/72	n.s	***	*
A88	25.2732	1-Heptanol	1453(±9)	41/56/71	n.s	***	*
A89	25.5392	5-Hepten-2-ol, 6-methyl-	1465 (±6)	53/67/69	***	***	***
A90	27.3229	2-Nonanol	1521(±11)	45/55/69	n.s	***	n.s
A91	30.3305	5-Octen-1-ol	1615(±1)	41/67/82	*	***	***
A92	31.6082	1-Nonanol	1660(±7)	41/56/70/84	n.s	n.s	n.s
A93	32.346	3-Nonen-1-ol	1682 (7)	41/55/68/81	n.s	**	n.s
A94	33.3645	1-Propanol, 3-(methylthio)	1719(±9)	58/106	n.s	n.s	n.s
A95	33.6864	2-Undecanol	1718(±6)	45	n.s	***	***
A96	34.5214	1-Decanol	1760 (±9)	56/70/83	n.s	***	n.s
A97	34.6472	7-Octen-1-ol, 3,7-dimethyl	1768(±1)	41/69/82	**	***	n.s
A98	37.7817	Phenylmethanol)	1870(±13)	51/79/91	*	***	n.s
A99	38.6951	2-Phenylethanol	1907(±15)	65/91	n.s	***	n.s
A100	39.9424	1-Dodecanol	1969(±9)	55/69/83	n.s	***	n.s
A101	40.1736	Ethanol, 2,2′-oxybis-	1972(±10)	45/75	***	***	***
A102	44.9058	1-Tetradecanol	2165(±10)	55/69/83	n.s	***	n.s
Aldehydes							
Al103	19.6471	Octanal	1289(±9)	43/56/84	n.s	*	n.s
Al104	23.3322	Nonanal	1391(±8)	41/57/70	n.s	**	**
Al105	26.7947	Decanal	1498(±8)	43/57/70	n.s	*	n.s
Al106	30.6195	1-Cyclohexene-1-carboxaldehyde, 2,6,6-trimethyl-	1611(±17)	67/81/109	n.s	***	n.s
Al107	31.2632	2-Decenal, (E)-	1644(±11)	41/70/83	***	***	***
Al108	31.3781	Benzeneacetaldehyde	1641(±13)	65/91/120	n.s	***	n.s
Carboxylic acids							
AC109	25.795	Acetic acid	1449(±13)	43/60	n.s	***	n.s
AC110	29.3058	Propanoic acid, 2-methyl	1570(±13)	43/73	n.s	***	n.s
AC111	32.2986	Butanoic acid, 3-methyl	1666(±11)	60/87	n.s	***	n.s
AC112	37.1596	Hexanoic acid	1846(±12)	60/73/87	n.s	***	n.s
AC113	42.5579	Octanoic acid	2060(±15)	55/60/73	n.s	***	n.s
AC114	45.0756	Nonanoic acid	2170(±17)	43/60/73	n.s	*	n.s
AC115	47.4768	Decanoic acid	2276(±15)	43/60/73	n.s	***	n.s
AC116	51.9679	Dodecanoic acid	2496(±10)	55/73	n.s	***	n.s
Ketones							
K117	8.3209	2,3-Butanedione	979(±10)	43/86	**	***	***
K118	11.0992	2,3-Pentanedione	1058(±8)	43/57/100	***	***	***
K119	18.3421	3-Octanone	1253(±11)	43/57/72/85	***	***	***
K120	19.5159	Acetoin 3-hydroxy-2-butanone	1285(±12)	43/45/73	***	*	n.s
K121	27.8359	3(2H)-Thiophenone, dihydro-2-methyl	1527(±15)	60/116	n.s	n.s	n.s
K122	29.8711	2-Undecanone	1598(±7)	43/58/71	***	***	***
K123	31.5779	Acetophenone	1647(±13)	51/77/105	***	***	***
Lactones							
La124	33.6983	2(3H)-Furanone, 5-ethoxydihydro	1728(2)	58/85	*	n.s	n.s
La125	38.9081	2(3H)-Furanone, 5-butyldihydro	1910(±19)	85	***	***	***
Phenols							
Ph126	64.7245	Benzeneethanol, 4-hydroxy-	2999(±13)	138	n.s	***	n.s
Ph127	48.1399	2,4-Di-tert-butylphenol	2321(±6)	57/191	***	***	***

Table 2. *Cont.*

Esters	**RT**	**Compound Name**	**LRI Lit**	**Most Abundant Ions *m*/*z***	**ANOVA**		
					Y	**V**	**Y × V**
Terpenes							
T128	28.2497	Linalool	1547(±7)	43/55/71	**	***	***
T129	31.8714	(E)-β-Farnesene	1665(±6)	41/69/93	n.s	***	**
T130	32.7579	α-Terpineol	1697(±10)	59/81/121/136	*	***	n.s
T131	33.5912	α-Muurolene	1719(±32)	105/119/161	***	***	***
T132	33.6297	β-Bisabolene	1728(±11)	69/93/109	n.s	***	***
T133	34.6463	β-Citronellol	1765(±9)	41/69	n.s	***	**
T134	35.6182	2,6-Octadien-1-ol, 3,7-dimethyl	1797(±11)	41/69/93	*	***	n.s
T135	36.8544	Geraniol	1847(±10)	41/69	*	**	**
T136	38.8649	α-Calacorene	1919(±21)	142/157	n.s	***	n.s
T137	41.7874	1,6,10-Dodecatrien-3-ol, 3,7,11-trimethyl-	2034(±14)	41/69/93	n.s	***	n.s
T138	49.0029	2,6,10-Dodecatrien-1-ol, 3,7,11-trimethyl-	2350(±8)	41/69	n.s	**	n.s
		C_{13}-norisoprenoids					
NI139	34.2571	Naphthalene, 1,2-dihydro-1,1,6-trimethyl (TDN)	1732(±16)	142/157	n.s	*	**
NI140	36.2999	2-Buten-1-one,1-(2,6,6-trimethyl-1,3-cyclohexadien-1-yl)	1823(±14)	69/105/121	n.s	**	n.s
NI141	46.3117	Naphthalene, 1,6-dimethyl-4-(1-methylethyl)-	2233(±23)	152/168	n.s	***	n.s
NI142	39.3694	*trans*-β-Ionone	1941(±17)	135/177	n.s	***	**

Y: Year; V: Variety; RT: Retention time (min); LRI lit: Linear retention index values from the literature; Statistically significant at * $p \leq 0.05$, ** $p \leq 0.01$, and *** $p \leq 0.001$, n.s: Not significant, respectively.

The combined use of HS-SPME and GC–ToFMS for wine VOC profiling has been extensively validated in the literature. For example, Weldegergis et al. [10] employed this methodology to capture the complexity of red wine aroma profiles, while Pereira et al. [3] and Santos et al. [23] applied it to white wines. These studies reinforce the suitability of the analytical protocol used in the present investigation.

To provide a broader view of volatile compound distribution, a graphical analysis is presented to compare the families of compounds across the different wines. Figure 2 illustrates the total peak area values of volatile compounds grouped by chemical family for the varieties analyzed over two years. According to these results, esters and alcohols were consistently the predominant compounds across all varieties and vintages. Aragonez wines show higher levels of esters and lower levels of alcohols in both vintages, whereas the opposite is observed in Merlot and Trincadeira wines. Ester compounds are widely recognized for conferring fresh and fruity aromas [6,24], and the major factors affecting ester formation during alcoholic fermentation include yeast strain, fermentation conditions such as temperature, oxygen availability, sugar content, and nutrient availability. In fact, Aragonez grapes were the ones presenting lower sugar content, and Merlot grapes were the ones with higher sugar content.

Trincadeira exhibits a slightly higher relative content of terpenes, and Merlot and Syrah exhibit significantly higher amounts of norisoprenoids, reinforcing their potential for complex aromatic expression. These patterns echo those observed by Mele et al. [6], emphasizing the impact of variety and vintage on terpene expression. The Principal Component Analysis (PCA) results further reinforce these observations, demonstrating distinct clustering of Syrah and Trincadeira based on their volatile profiles, driven by the elevated presence of these compound families. Syrah presented balanced proportions between alcohols and esters, suggesting moderate fermentation-derived aroma intensity. This type of profile has been associated with more subtle and integrated aromatic expression, as demonstrated by Cosme et al. [24].

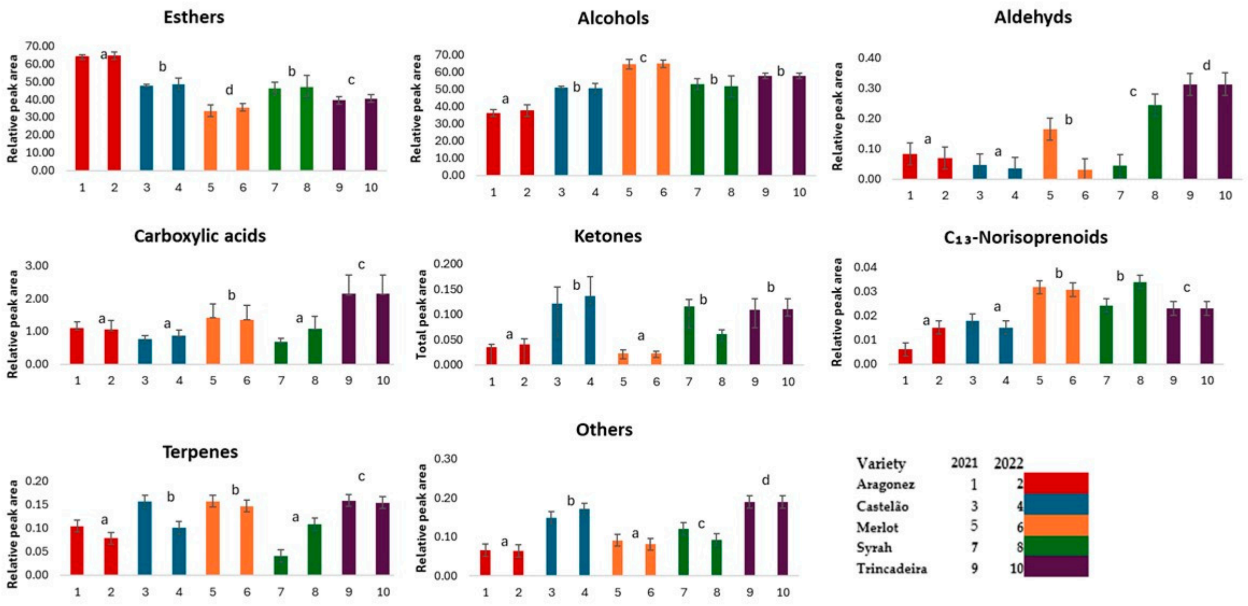

Figure 2. Distribution of volatile compound families by variety and vintage, expressed as relative percentage of total chromatographic peak area (A%). Different letters indicate significant differences between wine varieties ($p < 0.05$).

Ketones and acids appeared in lower amounts but were relatively consistent across varieties, indicating minor yet stable contributions to the aromatic matrix, corroborating the findings of Diéguez et al. [25] who studied the aromatic composition of the *Vitis vinifera* grape Albariño and emphasized the influence of esters, alcohols, and C6 compounds on the varietal aroma profile, supporting the contribution of subtle volatile components to sensory complexity. This graphic visualization complements the statistical data from Table 2 and strengthens the interpretation of varietal and interannual consistency. The distribution also highlights that the uniqueness of Trincadeira and Merlot is largely associated with higher relative contributions of terpenoids and C_{13} norisoprenoids in these wines, mirroring findings in Italian red wines, where monoterpenes and norisoprenoids were key loadings driving varietal and geographical discrimination [7]. The Principal Component Analysis (PCA) biplots (Figure 3) were performed exclusively using volatile organic compounds that exhibited statistically significant differences among grape varieties (ANOVA, Table 2), enabling dimensionality reduction while preserving the most discriminative chemical information. The first two principal components accounted for 82.53% of the total variance, 64.18% for PC1 and 18.35% for PC2, indicating strong explanatory power and highlighting the major variability trends within the dataset.

The PCA biplots effectively highlight distinct clustering of grape varieties based on their volatile organic compounds (VOCs), strongly supporting the influence of grape variety on volatile composition under the experimental conditions studied. Aragonez, Merlot, Castelão, Trincadeira, and Syrah wines exhibit clear spatial separation, which corresponds with previously documented chemical trends indicative of varietal typicity. Notably, Merlot and Castelão wines are positioned along the positive axis of PC1, while Trincadeira shows higher PC2 values.

The clear distinction between Aragonez, Trincadeira, and Merlot wines provides strong evidence of compositional differences arising from varietal characteristics. Clustering patterns suggest that the unique volatile profiles of these grapes are not only indicative of their individual chemical composition but also contribute significantly to their sensory distinction. This correlation between VOC profiles and sensory attributes has been elucidated in several studies, demonstrating the vital role these compounds play in determining the overall quality and identity of young single varietal wine [26,27].

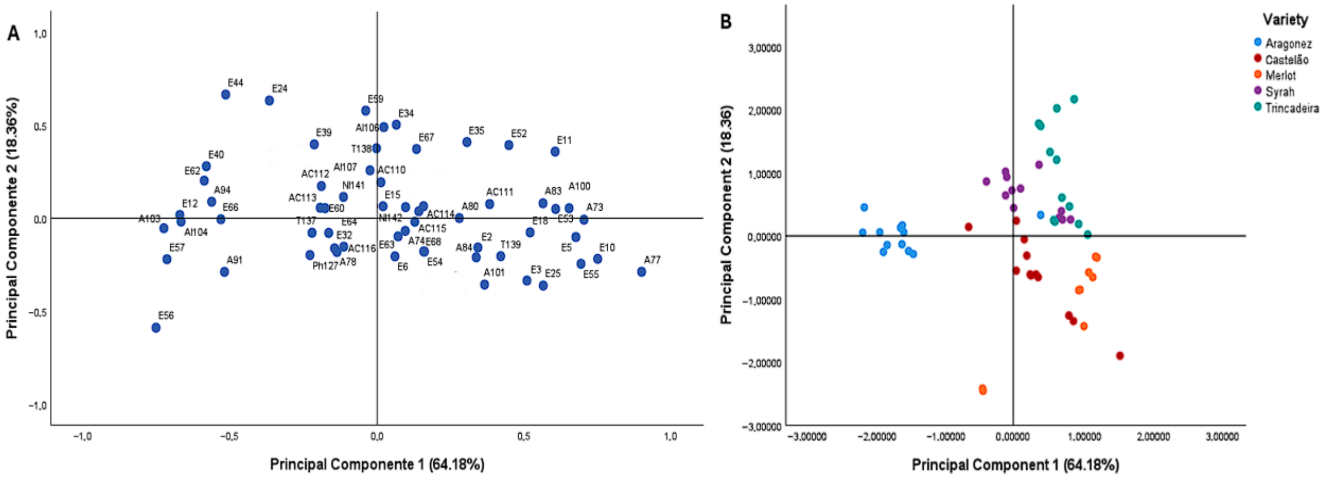

Figure 3. Principal Component Analysis (PCA) based on statistically significant volatile organic compounds (ANOVA, Table 2) of five monovarietal wines. (**A**) Score plot of volatile compounds projected onto the first two principal components: PC1 (64.18%) and PC2 (18.35%). (**B**) PCA clustering of wine samples according to grape variety: Aragonez (blue), Castelão (dark red), Merlot (orange), Syrah (purple), and Trincadeira (green). For compound identification (**A**), see Table 2.

This statistical separation confirms that volatile compound profiles contribute to varietal typicity and likely sensory distinctiveness in young monovarietal wines. Therefore, integrating chemical composition data with multivariate statistical tools, such as PCA, provides a robust framework for characterizing varietal differences and interpreting enological variability. Building upon the overall VOC distribution patterns described above, the following section focuses specifically on the terpene and C_{13}-norisoprenoid fraction due to their recognized relevance in varietal differentiation.

3.3. Varietal Profiles and Interannual Consistency

Table 2 highlights compounds with high statistical significance (ANOVA) and indicates apparent differences in volatile compound content among varieties. For most of the compounds identified in the analyzed samples, a statistical level ($p < 0.001$) was observed for the variety factor. In fact, variety was the factor that most influenced the varietal composition of wines, rather than the vintage year. Several volatile organic compounds (VOCs) demonstrated statistically significant differences among the five red grape varieties studied (Aragonez, Castelão, Merlot, Syrah, and Trincadeira), providing crucial markers for varietal discrimination. The compounds most frequently showing significant varietal effects included specific esters, higher alcohols, terpenes, and C_{13}-norisoprenoids, each linked to distinct aromatic attributes in wine.

For instance, mid- and long-chain ethyl esters, which are considered key contributors to fruity aroma, frequently exhibited clear varietal differentiation, consistent with patterns seen in the quantification of total esters in these wines. Some higher alcohols also varied across grape varieties, potentially influencing fullness and aromatic complexity. In addition, terpenes such as linalool and related compounds, which are characteristic of floral and citrus notes, showed significant variation across varieties, underscoring their importance as typicity markers in grape characterization. C_{13}-norisoprenoids, known for supporting nuanced fruity and floral profiles, further contributed to varietal discrimination despite their lower overall abundance.

https://doi.org/10.3390/beverages12040041

Building on the compound analysis, the assessment of varietal profiles and their stability over two vintages revealed meaningful trends in the expression of key volatile compounds. The present study provides varietal results for Aragonez, Castelão, Merlot, Syrah, and Trincadeira, expressed as mean ± standard deviation, highlighting interannual patterns in the behavior of volatile compounds (Table S1, Supplementary Files). The data presented in Table S1 demonstrate that several compounds maintained consistent concentrations across years, notably in Trincadeira and Merlot, reinforcing their potential as varietal markers. For example, Trincadeira consistently showed high levels of ethyl acetate (compound **1**), hexanoic acid, propyl ester (compound **22**), and 2-hexenoic acid, ethyl ester (compound **25**), associated with fruity and fresh odors. The findings are consistent with research by Weldegergis et al. [10] and Tufariello et al. [9], highlighting the dependability of alcohols and esters as markers for distinguishing varieties among young red wines.

Merlot also showed stability in the expression of medium-chain esters, such as hexanoic acid ethyl ester (compound **16**) and 2-Phenylethanol (compound **99**), supporting previous observations by Francis and Newton [28] on the role of these volatiles in enhancing perceived aromatic complexity. The interannual persistence of these markers suggests a genetic basis in biosynthetic capacity, rather than exclusive dependence on environmental factors. In contrast, Aragonez and Castelão showed more balanced profiles with fewer standout compounds across years. Despite environmental variation, the relative stability of volatile composition across 2021 and 2022 vintages emphasizes the role of grape genotype in determining aromatic profiles; this observation supports the chemometric use of volatile data for vintage-independent classification models, as highlighted by Perestrelo et al. [29].

These findings show that, although many VOCs are present in all samples, only a subset achieves concentrations and distribution patterns that serve as reliable varietal markers, particularly those with low sensory thresholds and high aromatic impact. The ANOVA results support this, highlighting the role of these compounds in explaining the varietal clustering observed in both PCA and chemical family bar plots.

3.4. C13-Norisoprenoids and Terpenes as Chemical Markers in Young Red Wines

C_{13}-norisoprenoids and terpenes represent a class of compounds with extreme sensory relevance in wines, even when present at trace concentrations [6]. Although these compounds are frequently associated with aromatic white wines, their presence in young red wines has also attracted attention, as they can serve as varietal markers even in non-aromatic varieties [30]. To provide additional context regarding the potential sensory relevance of the compounds discussed, characteristic aroma descriptors for the main terpenoids and C_{13}-norisoprenoids identified in this study were compiled from literature sources and are summarized in Table S2 [12,31–33]. The 100% ribbon chart (Figure 4) provides an insightful analysis of the relative distributions of terpenes and C_{13}-norisoprenoids in our five young varietal red wines, Aragonez, Castelão, Merlot, Syrah, and Trincadeira, harvested in the 2021–2022 vintages. Each variety exhibits distinct aromatic signatures that reflect the complex interactions between its genetic background and the metabolic pathways involved in terpene and C_{13}-norisoprenoid biosynthesis. These findings align with recent reports on Italian monovarietal reds, in which monoterpenoids and C_{13} norisoprenoids were decisive for varietal and regional discrimination [7]. Linalool, β-citronellol, and geraniol stand out as key components within the monoterpene fraction across all cultivars, confirming their established roles as vital floral and citrus aroma contributors in *Vitis vinifera* wines [34,35]. Among the evaluated varieties, Castelão and Trincadeira show the highest relative levels of monoterpenes, supporting the observation that non-Muscat varieties can exhibit relatively higher proportions of monoterpenes, compounds widely reported in the literature as aroma-active due to their low odor thresholds, frequently in the µg L^{-1} or sub-µg L^{-1} range. Although no sensory evaluation or odor activity value (OAV) calculation was performed in the present study, such low thresholds suggest that even modest concentrations may influence aroma perception [36]. Conversely, Aragonez displayed a more balanced distribution of terpene subclasses, with significant contributions from sesquiterpenes such as β-bisabolene and α-muurolene [37,38]. This nuanced profile outlines the varietal diversity and the inherent aroma complexity of these wines.

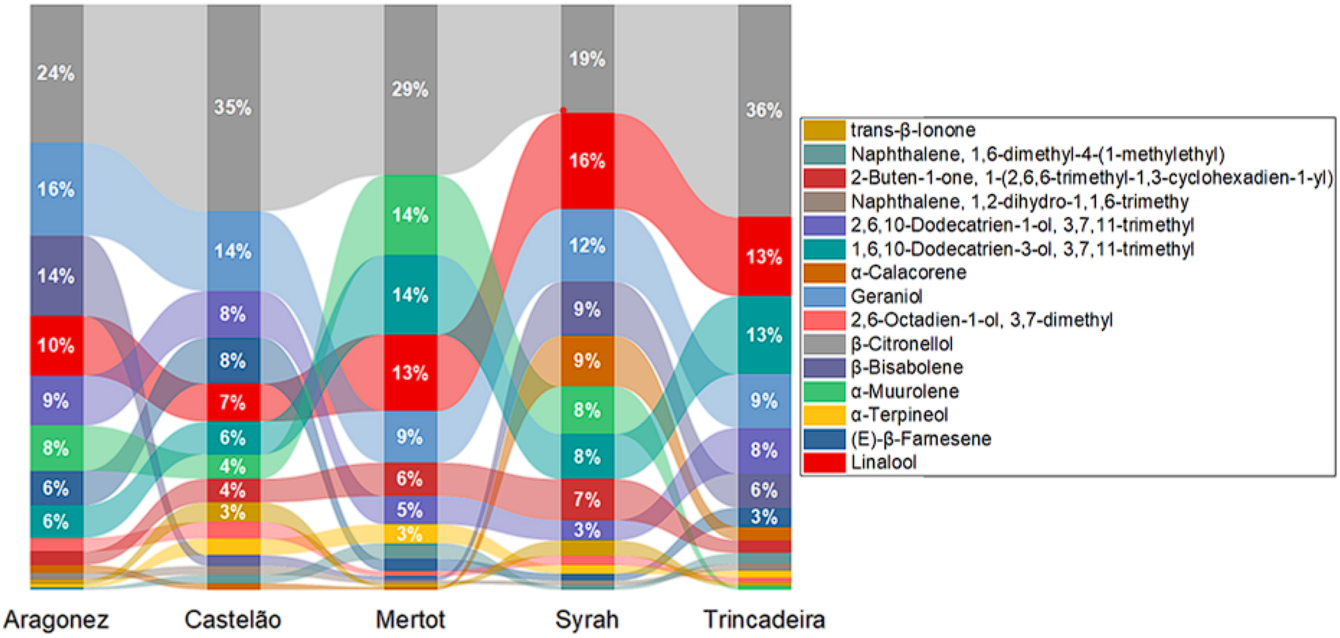

Figure 4. One-hundred percent ribbon chart illustrating the relative distribution of major terpenes and C_{13}-norisoprenoids in young monovarietal red wines (Aragonez, Castelão, Merlot, Syrah, and Trincadeira) from the 2021 and 2022 vintages. Each percentage represents the relative contribution of each individual terpene and C_{13}-norisoprenoid to the total terpenoid + C_{13}-norisoprenoid fraction within each grape variety. A total of 15 compounds were included in the analysis.

Merlot and Syrah present intermediate yet distinct aromatic characteristics. Specifically, Merlot showcases elevated levels of β-citronellol alongside notable sesquiterpenes like α-calacorene, while Syrah's profile is characterized by a more complex interplay of linalool, geraniol, β-bisabolene, and α-calacorene, aligning with the well-documented complexity of Syrah wines [31,36]. While C_{13}-norisoprenoids, including *trans*-β-ionone and various derivatives, constitute a smaller fraction of the total aroma compounds, their significance is underscored by their extremely low odor thresholds [39,40]. Their consistent presence across all varieties highlights their chemical persistence and statistical relevance for varietal differentiation, with heightened relative proportions observed in Aragonez and Merlot. This supports their relevance as discriminant volatile compounds, although their direct sensory contribution was not evaluated in the present study [41,42].

From a vintage perspective, the relative ordering of dominant compounds within each cultivar mainly remained stable between 2021 and 2022. Although absolute concentrations may fluctuate with climatic conditions, the percentage distributions depicted in the ribbon chart and Table S1 indicate a consistent varietal effect within the vineyard and vinification conditions studied, while recognizing that broader environmental variables may influence volatile expression. The warmer, drier conditions recorded in 2022 may also have influenced secondary metabolite pathways involved in terpene and C_{13}-norisoprenoid formation. Elevated temperatures are known to accelerate carotenoid degradation in grape berries, potentially enhancing the formation of norisoprenoid precursors. Likewise, heat and water stress can modulate terpene biosynthesis by affecting isoprenoid metabolic pathways [20].

Therefore, the interannual differences observed in selected terpene and norisoprenoid profiles may partially reflect climatic modulation of grape secondary metabolism under Mediterranean conditions.

This trend is consistent with findings from studies on Mediterranean and Atlantic cultivars, where varietal identity significantly shapes volatile composition against a backdrop of environmental variability [36,43,44]. The ribbon chart serves as an informative visualization of how a curated set of structurally related compounds effectively distinguishes among grape varieties. When paired with methodologies such as headspace solid-phase microextraction combined with gas chromatography–time-of-flight mass spectrometry (HS-SPME/GC–TOFMS), these findings underscore the importance of terpenes and C_{13}-norisoprenoids as reliable markers for varietal identification, authenticity, and chemometric classification of young red wines [34].

4. Limitations

The present study should be interpreted within its context. First, all wines were produced from grapes grown in a single experimental vineyard (Mitra, Alentejo) and vinified at a single research facility under controlled, but location-specific, edaphoclimatic and management conditions. While this design allowed for a controlled assessment of varietal effects, it limits the ability to generalize the volatile patterns to other terroirs, vineyard systems, or commercial production contexts. As a result, the identified markers reflect varietal expression under the specific environmental conditions studied rather than representing universal signatures of Portuguese red wines. Second, the experimental design included five cultivars and two consecutive vintages (2021–2022), both characterized by warm and dry Mediterranean conditions. Although interannual consistency was observed for several compounds, the climatic similarity between the two years restricts conclusions regarding marker robustness under different weather conditions. Validation across additional vintages and macroclimatic scenarios would be desired. Third, wines were produced at pilot scale (50 L) using spontaneous alcoholic fermentation without technological corrections (e.g., yeast inoculation, sugar or acidity adjustment, oak aging). While this approach enhances the expression of intrinsic grape-derived variability, it does not encompass the diversity of commercial winemaking practices that can substantially influence volatile formation. Therefore, caution should be exercised when extending these findings to wines produced under different technologies. The semi-quantitative approach based on relative peak area percentages allows for consistent comparative chemometric profiling across samples. Direct quantitative comparisons with studies reporting absolute concentrations should be approached with caution.

5. Conclusions

This study provides an integrated assessment of VOC profiles in young monovarietal red wines from Alentejo, combining HS-SPME–GC–TOFMS with multivariate analysis to explore the influence of grape variety and vintage. The non-targeted semi-quantitative workflow captured clear varietal signatures and revealed that, under the conditions studied, variety exerted a stronger effect than vintage on the relative composition of esters, higher alcohols, terpenoids, and C_{13}-norisoprenoids.

Terpenoids and C_{13}-norisoprenoids, and particularly trans-β-ionone, emerged as stable and discriminant markers across the two vintages, supporting their use as varietal typicity and authenticity indicators in non-aromatic red wines. The 100% ribbon chart provided an intuitive visualization of these markers and their genotype-driven patterns, complementing the PCA-based separation of wines.

Overall, this work advances current knowledge of Portuguese red wine aroma by delivering a high-resolution HS-SPME–GC–TOFMS chemometric platform for varietal classification and by identifying terpenoids and C_{13}-norisoprenoids as key contributors to the aromatic identity of Alentejo monovarietal wines. Future research should couple targeted quantification and descriptive sensory analysis to directly link these markers to floral, fruity, and balsamic sensory attributes, and extend the approach to multiple sites and vintages to further evaluate its robustness for PDO valorization and regulatory authentication.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/beverages12040041/s1, Table S1: Mean and standard deviation values of the volatile compounds identified in the studied wines; Table S2: Characteristic aroma descriptors associated with the main terpenoids and C_{13}-norisoprenoids detected in the studied wines. Aroma descriptors were compiled from literature sources on wine volatile compounds, and Figure S1: Representative total ion chromatogram (TIC) of a young monovarietal Aragonez wine (2021 vintage) obtained by HS-SPME–GC–TOFMS. Figure S2: Representative total ion chromatogram (TIC) of a young monovarietal Aragonez wine (2022 vintage) obtained by HS-SPME–GC–TOFMS. Figure S3: Representative total ion chromatogram (TIC) of a young monovarietal Castelão wine (2021 vintage) obtained by HS-SPME–GC–TOFMS. Figure S4: Representative total ion chromatogram (TIC) of a young monovarietal Castelão wine (2022 vintage) obtained by HS-SPME–GC–TOFMS. Figure S5: Representative total ion chromatogram (TIC) of a young monovarietal Merlot wine (2021 vintage) obtained by HS-SPME–GC–TOFMS. Figure S6: Representative total ion chromatogram (TIC) of a young monovarietal Merlot wine (2022 vintage) obtained by HS-SPME–GC–TOFMS. Figure S7: Representative total ion chromatogram (TIC) of a young monovarietal Syrah wine (2021 vintage) obtained by HS-SPME–GC–TOFMS. Figure S8: Representative total ion chromatogram (TIC) of a young monovarietal Syrah wine (2022 vintage) obtained by HS-SPME–GC–TOFMS. Figure S9: Representative total ion chromatogram (TIC) of a young monovarietal Trincadeira wine (2022 vintage) obtained by HS-SPME–GC–TOFMS. Figure S10: Representative total ion chromatogram (TIC) of a young monovarietal Trincadeira wine (2022 vintage) obtained by HS-SPME–GC–TOFMS.

Author Contributions: S.G.-M.: methodology, writing, and investigation; N.M.: resources and supervision; M.J.C. and R.G.: conceptualization, supervision, project administration, and writing—review. All authors have read and agreed to the published version of the manuscript.

Funding: This work was funded by national funds through FCT—Portuguese Foundation for Science and Technology, under the Consortium of Agricultural Science Schools (CECA), under the FCT Research Grant Regulations (RBI)—PRT/BD/154325/2022.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The data presented in this study are available on request from the corresponding author.

Acknowledgments: The authors acknowledge the Oenology Laboratory of the University of Évora for technical assistance. The authors acknowledge the R&D unit MED—Mediterranean Institute for Agriculture, Environment and Development (https://doi.org/10.54499/UID/05183/2025) and the Associate Laboratory CHANGE—Global Change and Sustainability Institute (https://doi.org/10.54499/LA/P/0121/2020).

Conflicts of Interest: The authors declare no conflicts of interest.

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