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Potential of Different Eighteen Grapevine Genotypes to Produce Wines in a Hot Region: First Insights into Volatile and Sensory Profiles

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Abstract: A major challenge for the viticulture and oenology sector is to understand the impact of climate change on grapevine agronomic performance and wine quality. Genetic variability offers a key tool for adaptation, as some grape varieties may better withstand changing conditions while maintaining wine quality. As part of the WineClimAdapt research project (PDR2020-101-031010), a study was conducted on the adaptability of 18 white grape varieties to hot and dry conditions in Portugal. These grape varieties from Herdade do Esporão (Alentejo, Portugal) were vinified in duplicate at the INIAV winery, the result being 36 wines. The wines underwent physicochemical and sensory analyses, including gas chromatography–mass spectrometry (GC-MS) and gas chromatography–flame ionization detection (GC-FID), to assess their composition and sensory profiles. Tasters evaluated the wines using a structured scale (0–10) and rated their overall quality (0–20). Results from analysis of variance (ANOVA) revealed significant differences in the physicochemical composition and sensory profiles of the wines. Notably, some white wines displayed high acidity, which is advantageous for hot regions. The study also highlighted clear varietal differentiation across physicochemical, volatile and sensory analyses. Among the tested varieties, "Cayetana Blanca" and "Fernão Pires" achieved the highest average quality ratings, indicating promising potential for future studies and adaptation to climate change.

Keywords: white wines; variety differentiation; climate change; sensory profile; volatile profiles

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1. Introduction

Climate change is an undeniable reality that affects many aspects of the world, including the delicate balance of wine production. As temperatures rise and weather patterns change, winegrowers and winemakers around the world are facing significant challenges [1–3], since it is widely recognized that biotic and abiotic factors play a crucial role in shaping grape composition, in particular influencing aroma compounds [4–7] and consequently the overall wine quality.

Indeed, it is well known that the wine aroma, which is mainly determined by several volatile compounds originating from the grapes, pre-fermentative reactions, fermentative metabolism and post-fermentative reactions [8], is a significant driver of consumer preferences [9]. In addition, aromas can also be correlated with positive or negative emotions measured in consumers [10].

Santos et al. [3] highlighted that climate change could lead to the emergence of new regions for wine production. However, it is expected that in regions usually suitable for wine production, such as Southern Europe, viticulture could face major challenges due to increasing temperatures and dryness.

According to van Leeuwen et al. [2] and Santos et al. [3], several adaptation strategies can be used to increase resistance to climate change. Among the various strategies, one option is to select grape varieties that are most resistant to extreme high temperatures and dry conditions. Keller [11] encourages further research into utilizing the inherent genetic diversity of grapevines to choose or develop adapted cultivars as an adaptation strategy.

Europe, and Portugal in particular, has a vast and unique genetic heritage, with approximately 230 grape varieties native to Portugal or the Iberian Peninsula. These are listed in ordinance no. 380/2012 of 22 November, which identifies the 343 grapevine varieties suitable for wine production in Portugal [12]. Therefore, the study of lesser-known varieties should be a priority for these historic wine-producing countries, not only to diversify their offer and meet the needs of emerging market [13], but also to identify the grape varieties best suited to withstand the abiotic stresses expected in the near future.

Thus, this work was carried out within the framework of the WineClimAdapt project (project code PDR2020-101-031010; https://wineclimadapt.pt/, Accessed on 1 January 2025), which intended to study the adaptability of grape varieties in the hottest and driest region of Portugal. In this context, and following the idea of exploring the genetic diversity, this work intends to present a first approach to the production of several monovarietal white wines from varieties previously selected for their superior adaptation to abiotic stresses. The grape varieties selected were based on a ranking according to their agronomic performance, including the following variables: water use efficiency, vigor, heat wave tolerance, yield, among others [14]. They were "Parellada", "Cayetana blanca", "Pardina" ("Albillo Mayor"), "Trajadura", "Fernão Pires", "Galego Dourado", "Cercial", "Lameiro", "Folha de Figueira" ("Dona Branca "), "Malvasia Rei" ("Palomino Fino"), "Roupeiro Branco", "Bastardo Branco", "Pedro Ximenez", "Alvadurão", "Castelão Branco", "Uva Salsa" ("Chasselas Cioutat"), "Larião" and "Molinha Macia".

This set encompasses varieties from different geographical origins, namely, "Chasselas Cioutat" from France; "Parellada", "Cayetana Blanca", "Trajadura", "Palomino Fino" "Albillo Mayor" and "Pedro Ximenez" from Spain; and the majority from Portugal. Among the varieties from Portugal, some are considered as minority varieties according to the list published by the Portuguese authorities [15], such as "Lameiro", "Folha de Figueira", "Roupeiro Branco", "Bastardo Branco", "Alvadurão", "Castelão Branco" and "Larião". This means that these varieties are registered for wine production, but there is no propagating material in the certification system of Portugal [16]. Thus, the conservation and knowledge about the minor varieties have increased in recent years in several countries, with the aim of diversifying and improving production to meet emerging market demands, while also serving as a potential strategy for identifying grape varieties that are well adapted to climate change [13,17–20].

Regarding the varieties studied in this work, some of them have been studied, although not in the severe climatic conditions of this study, in terms of sensory attributes and volatile composition, such as “Cayetana Blanca” [21–23], “Trajadura” [24,25], “Parellada” [26], “Fernão Pires” [25,27], “Galego Dourado” [28], “Pedro Ximenez” [29] “Palomino Fino” [30] and “Albillo Mayor” [31]. However, for the remaining varieties, there is a lack of knowledge about the sensory attributes and volatile composition of the wines.

Therefore, the aim of this study was to provide a first approach to the oenological potential of 18 different grapevine genotypes for wine production under very hot and arid conditions. This study investigated, for the first time, the winemaking potential of certain grapevine genotypes subjected to extreme heat and drought stress.

2. Materials and Methods

2.1. Vineyard and Wine Experiment

The vineyard, approximately 10 years old, was located in the Herdade do Esporão in Reguengos de Monsaraz, Évora, Alentejo, Portugal, one of the warmest wine regions in the country. During the 2021 growing season (from March to September), the average temperature was 19.9 °C, rainfall totaled 205 mm and reference evapotranspiration reached 931 mm. Throughout the same period, the average maximum and minimum temperatures were 27.7 °C and 12.6 °C, respectively.

The vines were established in Eutric Cambisol soil with an ApBw1Bw2C profile originating from granite, containing 75–80% sand, and were grafted onto 1103P rootstock. Each grape variety was arranged in individual rows with 110 of plants, aligned North–South, with 3 m spacing between rows and 1.5 m between the vines. The training system employed was vertical shoot positioning.

Irrigation was carried out weekly using surface drip irrigation, with one dripper per meter dispensing water at a rate of 2.4 L/h. The irrigation volume, approximately 100 mm from pea size to maturation, was calculated using the FAO-56 method, with crop coefficients derived from the vegetation index and a stress coefficient of 0.5 applied.

For the 2021 vintage, grapes from each variety (around 60 kg) were hand-harvested, then transported and processed at the INIAV experimental winery in Dois Portos. The grapes were crushed, destemmed and pressed. The resulting juice was treated with SO_2 and clarified at 4 °C for 24 to 48 h. The clarified must was then split into similar batches and placed into two 20 L glass fermentation containers. Selected yeasts (Lalvin QA23, Lallemand) were added to the musts at 25 g/hL, and the fermentation process was conducted at a controlled temperature of 18 °C. Daily measurements of density and temperature were taken.

When the musts achieved the desired density, they were racked into 20 L glass containers. The containers were stored at 4 °C, and in February 2022, the wines were sampled to control SO_2 levels and confirm the absence of malolactic fermentation. SO_2 was added to achieve a free sulfur dioxide level of 30 mg/L using Oxyless (PROENOL S.A., Canelas, Portugal). After three weeks, in March 2022, the wines were bottled in 0.75 L amber glass bottles sealed with natural cork stoppers, without additional clarification procedures. Three samples from each wine variety replicate were collected for physicochemical and sensory analysis.

Table 1 shows the list of grape varieties analyzed, along with their corresponding VIVC codes, wine codes, variety prime names and variety numbers from the Vitis International Variety Catalogue (VIVC) database. Throughout this manuscript, the prime name of the variety is used.

Table 1. List of grape varieties selected to be vinified and the corresponding wine codes and also the prime name of the variety and the corresponding variety number [32].

Prime Name of the Variety	Variety Number VIVC	Grape Variety Name in the Vineyard	Wine Code
Alvadurao	20806	Alvadurão	Al
Bastardo Branco	1026	Bastardo Branco	BB
Castelao Branco	2321	Castelão Branco	CB
Cayetana Blanca	5648	Cayetana	Ca
Cercial	16437	Cercial	Ce
Folha de Figueira	14142	Dona Branca	DB
Fernão Pires	4100	Fernão Pires	FP
Galego Dourado	4325	Galego Dourado	GD
Lameiro	6706	Lameiro	Lam
Lariao	6757	Larião	La
Palomino Fino	8888	Malvasia Rei	MR
Molinha Macia	40728	Molinha Macia	MoM
Albillo Mayor	12581	Pardina	Pa
Parellada	8938	Parellada	Par
Pedro Ximenez	9080	Pedro Ximenez	PX
Roupeiro Branco	17716	Roupeiro Branco	RB
Trajadura	12629	Trajadura	Tj
Chasselas Cioutat	2476	Uva Salsa	US

2.2. Reagents

The reagents used for volatile analyses included anhydrous sodium sulfate and ethanol, both obtained from Merck (Darmstadt, Germany), and dichloromethane purchased from Honeywell Riedel-de Haën (Steinheim, Germany). The GC-FID and GC–MS standards comprised various volatile compounds: 2-methyl-1-propanol, 2-methyl-1-butanol, 3-methyl-1-butanol, ethyl hexanoate, 1-pentanol, hexyl acetate, ethyl lactate, 1-hexanol, *trans*-3-hexen-1-ol, *cis*-3-hexen-1-ol, ethyl 3-hydroxybutanoate, butyrolactone, ethyl decanoate, butanoic acid, ethyl succinate, 3-methylbutanoic acid, phenethyl acetate, hexanoic acid, benzyl alcohol, 2-phenylethanol, octanoic acid and decanoic acid, all acquired from Fluka (Buchs, Switzerland). The following were purchased from Aldrich (Steinheim, Germany): 2-octanol (internal standard, IS), isoamyl acetate, acetoin, ethoxy-1-propanol, methional, methionol and monoethyl succinate. Malic acid diethyl ester was obtained from TCI (Zwijndrecht, Belgium). Ethyl butyrate, 1-propanol, 1-butanol and ethyl octanoate were bought from Merck (Darmstadt, Germany), while tyrosol was provided by Supelco (Steinheim, Germany).

2.3. Physicochemical General Analysis

For each wine sample, analytical determinations were performed following the official methods established by the International Organization of Vine and Wine [33]. These included density at 20 °C (ρ20), alcoholic strength (TAV), total acidity (TA), volatile acidity (VA), fixed acidity (FA), reducing substances and pH. Sulphur dioxide levels were measured using the OIV method, while malolactic fermentation was monitored via paper chromatography to ensure compliance with legal limits on sulfur dioxide and to confirm the absence of malolactic fermentation.

2.4. Analysis of Wine Volatile Compounds

The microextraction method of Vilanova et al. [34] was employed. In this procedure, 8 mL of wine, 400 μL of dichloromethane (solvent), 36 μL of 2-octanol (internal standard) and a magnetic stir bar were added to a test tube and gently stirred for 15 min. After stirring, the magnetic stir bar was removed, and the test tube was placed in a freezer at −20 °C for 10 min. The organic phase (lower layer in the test tube) was then extracted using a syringe and transferred to a labeled vial. All sample extracts were stored at −20 °C until being analyzed by GC-FID and GC-MS. Two duplicates of each wine replicate sample were prepared.

For GC-FID analysis of volatile compounds in the wine sample extracts, an Agilent Technologies 6890 N chromatograph was used under the following conditions: FID at 260 °C, injector at 250 °C in splitless mode (15 s closure time) and equipped with an HP INNOWax silica capillary column (J & W Scientific Technologies, Agilent, Palo Alto, CA, USA) measuring 30 m × 0.32 mm × 0.25 µm. The oven temperature program for chromatographic runs was as follows: an initial temperature of 35 °C, held for 6 min; a first ramp at 3.5 °C/min to 55 °C; a second ramp at 7.5 °C/min to 130 °C; and a third ramp at 5 °C/min to 210 °C, held for 30 min. Hydrogen was used as the carrier gas, and the injected sample volume was 1.4 µL. All injections were performed manually. Volatile compounds were quantified as 2-octanol equivalents, assuming a response factor of one between the internal standard (IS) and the analytes. Kovats Retention Indices (KIs) were calculated using alkane standards (C9–C26, C28, C30) under identical GC-FID conditions, following the procedure described by Philips [35].

For GC-MS identification, a Finnigan MAT Magnum (San Jose, CA, USA) was utilized. An aliquot of 0.4 µL was injected onto a fused silica capillary column coated with polyethylene glycol (HP-INNOWAX, dimensions: 30 m length × 0.25 mm inner diameter × 0.25 µm film thickness, Agilent J&W Technologies, Palo Alto, CA, USA). The operational parameters included injector and transfer line temperatures set to 250 °C, with helium (He ≥ 99.9992%) as the carrier gas at an inlet pressure of 12 psi and a split ratio of 1:60. The oven followed a temperature gradient program similar to that used in the GC-FID method. The mass spectrometer operated in electron impact mode at 70 eV, scanning in full scan mode within the m/z range of 40–340. Compound identification was systematically confirmed by comparing GC-MS chromatograms with data from the NIST and Wiley libraries by matching calculated Kovats Indices (KIs) with the literature references, and, where feasible, with the retention indices of pure standard compounds analyzed under the same conditions.

2.5. Wine Sensory Analysis

The samples underwent sensory evaluation at the sensory analysis laboratory of INIAV in Dois Portos, Portugal, utilizing the quantitative descriptive sensory method. The panel comprised eight trained members—six women and two men—aged between 30 and 61, all of whom were experienced tasters from the INIAV wine sensory taster group. Each taster performed their analysis in an individual booth equipped with proper lighting, a sink and white surfaces, following ISO 8589 guidelines [36]. Standard wine glasses [37] were used for the samples, with approximately 50 mL of wine poured into each glass. The tasters received training in accordance with international standards [38], which included the detection and identification of aromas and flavors, as well as the use of evaluation scales. The training involved assessing a range of flavor standards, such as apple, banana, strawberry, lemon, rock-rose, straw, nuts, raisin extracts, 1-hexanol, *cis*-3-hexenol, ethanol, ethyl acetate, ethyl butyrate, 2-phenylethanol, acetaldehyde, geraniol, isoamyl acetate, linalool, vanillin, glucose, fructose, tartaric acid, quinine sulfate, acetic acid, lactic acid, citric acid, malic acid and glycerol. During the sensory evaluation sessions, the panel also analyzed spiked wines containing known compounds to simulate common wine odor defects. Additionally, they evaluated commercial white wine samples to further hone their sensory skills and apply them to real-world samples.

The sensory sessions took place in the morning at 11 a.m., with the wine samples served at 14 °C ± 1 °C. Water was provided for tasters to rinse their mouths between samples. All tasters provided their informed consent, confirming their voluntary participation in the sensory sessions conducted in this study. The sensory evaluations were performed in accordance with the ethical guidelines approved by the INIAV executive board. The wine sensory assessment involved evaluating color, aroma and taste, with tasters instructed to rate the intensity of various attributes on a structured scale from 0 to 10. The evaluation form was based on previous work [39]. The score sheet included attributes related to color, aroma and flavor. Visual aspects such as color intensity (V1), green hue (V2), yellow hue (V3) and limpidity (V4) were evaluated. Aroma characteristics included global aroma intensity (A1) and the intensity of the following attributes: floral (A2), terpenic-muscat (A3), white fruit (A4), tropical fruit (A5), citrus fruit (A6), dried fruits (A7) and herbaceous/green (A8). Taste attributes included sweetness (F1), sourness (F2), bitterness (F3), body (F4) and harmonious persistency (F5). In addition, the tasters were also requested to rate the overall quality (OQ) of all the wine samples on a structured scale from 0 to 20. The panel assessed forty wine samples, which were randomly distributed across four sessions. This sample set consisted of 36 experimental wine samples, along with four repetitions comprised in each session to evaluate the reliability of the panel, as previously outlined [40]. Each session involved tasting ten wine samples, which were labeled with a unique 3-digit code and presented anonymously to the tasters. The wine samples were arranged in balanced sequences to minimize potential first-order carryover effects [41]. The sensory data were collected using Tastel software (ABT Informatique, Rouvroy-sur-Marne, France).

2.6. Statistical Analysis

A one-way Analysis of Variance (ANOVA) was conducted on the sensory and physicochemical wine results, with wine variety considered as the fixed factor. The Cochran test was used to assess variance homogeneity and, in cases where statistically significant effects were found ($p < 0.05$), mean comparisons were performed using the Bonferroni test.

Heatmap analysis was performed initially with the volatile compounds, followed by the sensory attributes that showed significant effects in ANOVA. The Pearson correlation coefficient (r value) was determined for both data groups and analyzed using clustering analysis. In the heatmaps, different colors represent positive and negative r values.

All the aforementioned analyses were conducted using Statistica version 7.0 software (StatSoft Inc., Tulsa, OK, USA) and Excel (version 16.63.1—2022, Microsoft, Redmond, Washington, DC, USA).

3. Results and Discussion

3.1. General Wine Composition

Table 2 presents the means obtained for each analytical determination, the outcomes of one-way ANOVA, as well as the results of the mean comparison test. The results displayed in Table 2 highlight a significant effect in all analyzed variables, as evidenced by the significance level, resulting in a clear differentiation between the wines based on the overall analysis.

It is worth noting that many of the wines had high total acidity levels (5.02–6.77 g/L), despite the fact that no acid correction was used in the vinification process. This is particularly true for the monovarietal wines “Cayetana Blanca”, “Albillo Mayor”, “Trajadura”, “Fernão Pires”, “Cercial”, “Lameiro”, “Bastardo Branco”, “Alvadurão” and “Castelão Branco”, which is an interesting oenological aspect, considering that white wines from warm regions typically have lower acidity levels, as other researchers have shown, with values ranging from 3.32 to 4.83 g/L for white wines from the Alentejo [42,43]. Similar results have been obtained with red grape varieties, such as Touriga Nacional and Aragonez in Alentejo, with total acidity values between 3.70 and 4.10 g/L [44]. In another region of Portugal, Vinhos Verdes, with fresher climatic conditions, 'Trajadura' wines tend to have higher acidity levels, ranging from 6.57 to 7.61 g/L [45].

Table 2. General physicochemical composition of varietal wines (average and standard deviation values of the two replicates of each variety) of different grapevine varieties from the 2021 vintage and the level of significance from ANOVA [*** ($p \leq 0.001$)].

Significance Level from ANOVA	***	***	***	***	***	***	***
Varietal Wine	**Density (g/cm^3)**	**Alcoholic Strength (% vol)**	**Total Acidity (g Tartaric Acid/L)**	**Volatile Acidity (g Acetic Acid/L)**	**Fixed Acidity (g Tartaric Acid/L)**	**Reducing Substances (g Glucose/L)**	**pH**
Parellada	0.9887 ^{ab} ±0.0002	12.8 ^{g} ±0.1	4.67 ^{cde} ±0.04	0.41 ^{de} ±0.02	4.16 ^{bcd} ±0.01	2.31 ^{abcd} ±1.90	3.61 ^{e} ±0.02
Cayetana Blanca	0.9901 ^{abcd} ±0.0000	11.6 ^{d} ±0.0	5.10 ^{fgh} ±0.09	0.28 ^{bc} ±0.03	4.76 ^{gh} ±0.05	1.38 ^{ab} ±0.01	3.68 ^{fg} ±0.00
Albillo Mayor	0.9902 ^{bcde} ±0.0000	11.7 ^{de} ±0.1	5.13 ^{fgh} ±0.172	0.27 ^{bc} ±0.01	4.80 ^{h} ±0.18	1.44 ^{ab} ±0.27	3.67 ^{efg} ±0.02
Trajadura	0.9882 ^{a} ±0.0001	15.5 ^{k} ±0.0	5.50 ^{h} ±0.05	0.55 ^{fg} ±0.03	4.81 ^{h} ±0.10	3.53 ^{abcd} ±0.18	3.78 ^{hi} ±0.02
Fernão Pires	0.9888 ^{ab} ±0.0003	14.9 ^{j} ±0.0	5.41 ^{fgh} ±0.01	0.61 ^{g} ±0.03	4.65 ^{fgh} ±0.03	5.16 ^{cd} ±1.12	3.63 ^{ef} ±0.02
Galego Dourado	0.9896 ^{abc} ±0.0002	13.9 ^{h} ±0.0	5.00 ^{def} ±0.02	0.52 ^{fg} ±0.02	4.35 ^{def} ±0.05	5.45 ^{d} ±0.86	3.66 ^{ef} ±0.01
Cercial	0.9889 ^{ab} ±0.0005	14.1 ^{hi} ±0.0	6.57 ^{i} ±0.00	0.52 ^{fg} ±0.02	5.92 ^{i} ±0.02	2.88 ^{abcd} ±0.68	3.36 ^{b} ±0.01
Lameiro	0.9895 ^{abc} ±0.0004	14.3 ^{i} ±0.0	5.44 ^{gh} ±0.02	0.31 ^{cd} ±0.01	5.05 ^{h} ±0.03	4.11 ^{abcd} ±0.23	3.72 ^{gh} ±0.03
Folha de Figueira	0.9900 ^{abcd} ±0.0007	14.6 ^{j} ±0.1	4.24 ^{ab} ±0.30	0.47 ^{ef} ±0.01	3.64 ^{a} ±0.28	4.49 ^{bcd} ±1.50	4.15 ^{j} ±0.02
Palomino Fino	0.9899 ^{abcd} ±0.0001	11.9 ^{de} ±0.1	4.58 ^{bcd} ±0.02	0.28 ^{bc} ±0.01	4.23 ^{cde} ±0.02	2.1 ^{abcd} ±0.21	3.62 ^{e} ±0.01
Roupeiro Branco	0.9896 ^{abc} ±0.0006	12.0 ^{e} ±0.2	4.10 ^{a} ±0.08	0.28 ^{bc} ±0.01	3.75 ^{ab} ±0.01	1.97 ^{abc} ±1.05	3.83 ^{ij} ±0.01
Bastardo Branco	0.9902 ^{bcde} ±0.0000	12.5 ^{f} ±0.0	6.77 ^{i} ±0.04	0.54 ^{fg} ±0.02	6.10 ^{i} ±0.06	2.65 ^{abcd} ±0.34	3.22 ^{a} ±0.00
Pedro Ximenez	0.9899 ^{abcd} ±0.0004	14.0 ^{h} ±0.1	4.33 ^{abc} ±0.01	0.37 ^{cde} ±0.01	3.86 ^{abc} ±0.03	5.53 ^{d} ±1.05	3.85 ^{i} ±0.01
Alvadurão	0.9908 ^{cde} ±0.0000	13.1 ^{g} ±0.1	6.60 ^{i} ±0.07	0.28 ^{bc} ±0.05	6.25 ^{i} ±0.13	2.15 ^{abcd} ±0.06	3.53 ^{d} ±0.01
Castelão Branco	0.9897 ^{abc} ±0.0000	12.0 ^{e} ±0.1	5.02 ^{efg} ±0.04	0.30 ^{bc} ±0.01	4.65 ^{fgh} ±0.05	1.91 ^{abc} ±0.12	3.44 ^{c} ±0.01

Table 2. *Cont.*

Significance Level from ANOVA	***	***	***	***	***	***	***
Varietal Wine	**Density (g/cm^3)**	**Alcoholic Strength (% vol)**	**Total Acidity (g Tartaric Acid/L)**	**Volatile Acidity (g Acetic Acid/L)**	**Fixed Acidity (g Tartaric Acid/L)**	**Reducing Substances (g Glucose/L)**	**pH**
Chasselas Cioutat	0.9915 ^{def}	9.5 ^{b}	4.43 ^{abc}	0.20 ^{ab}	4.18 ^{bcd}	1.30 ^{ab}	3.34 ^{b}
	±0.0002	±0.1	±0.10	±0.0012	±0.03	±0.11	±0.03
Larião	0.9930 ^{f}	8.5 ^{a}	4.44 ^{abc}	0.14 ^{a}	4.26 ^{cde}	0.95 ^{a}	3.44 ^{c}
	±0.0012	±0.0	±0.01	±0.01	±0.00	±0.03	±0.01
Molinha Macia	0.9919 ^{ef}	9.9 ^{c}	4.56 ^{bc}	0.31 ^{bcd}	4.17 ^{bcd}	0.80 ^{a}	3.68 ^{efg}
	±0.0000	±0.0	±0.11	±0.00	±0.11	±0.00	±0.00

Means with different superscript letters within the same column are significantly different ($p < 0.05$).

It is worth noting that many of the wines had high total acidity levels (5.02–6.77 g/L), despite the fact that no acid correction was used in the vinification process. This is particularly true for the monovarietal wines "Cayetana Blanca", "Albillo Mayor", "Trajadura", "Fernão Pires", "Cercial", "Lameiro", "Bastardo Branco", "Alvadurão" and "Castelão Branco", which is an interesting oenological aspect, considering that white wines from warm regions typically have lower acidity levels, as other researchers have shown, with values ranging from 3.32 to 4.83 g/L for white wines from the Alentejo [42,43]. Similar results have been obtained with red grape varieties, such as Touriga Nacional and Aragonez in Alentejo, with total acidity values between 3.70 and 4.10 g/L [44]. In another region of Portugal, Vinhos Verdes, with fresher climatic conditions, 'Trajadura' wines tend to have higher acidity levels, ranging from 6.57 to 7.61 g/L [45].

On the other hand, several wines had very high alcohol content, as well as elevated levels of residual sugars (reducing substances ~5 g/L), including "Fernão Pires", "Galego Dourado" and "Pedro Ximenez". This highlights one of the challenges faced by producers in hot climates: the increase in grape sugar content, which leads to a corresponding rise in alcohol levels [44,46,47].

3.2. Volatile Composition Results

The chromatographic analysis (GC-FID and GC-MS) enabled the identification and quantification of 35 compounds. Figure 1 presents a chromatogram of the "Alvadurão" wine, showcasing these 35 identified compounds, which are representative of the other samples, as the chromatographic profiles were identical across the various wines analyzed. Additionally, three more compounds were identified: acetic acid (RT ≈ 18.7 min), 2,3-butanediol (RT ≈ 20.09 min) and dodecanoic acid (RT ≈ 35.7 min). Acetic acid was not quantified, as its analysis had already been included in the previously presented volatile acidity values, and the latter two compounds were not quantified due to their trace concentrations.

Table 3 presents the results of the ANOVA analysis concerning the contents of the different volatile compounds identified in the wines. It includes the identification of the compounds, odor descriptors, sensory threshold, retention indices and the significance level of the variety factor for each compound. The analysis revealed that the wine variety had a significant effect on the content of all the volatile compounds analyzed (Table S1).

In other words, wines from different varieties, despite having a similar profile, differ in the quantities of each compound. Additionally, Table 4 presents the results organized by the main physicochemical families detected and quantified.

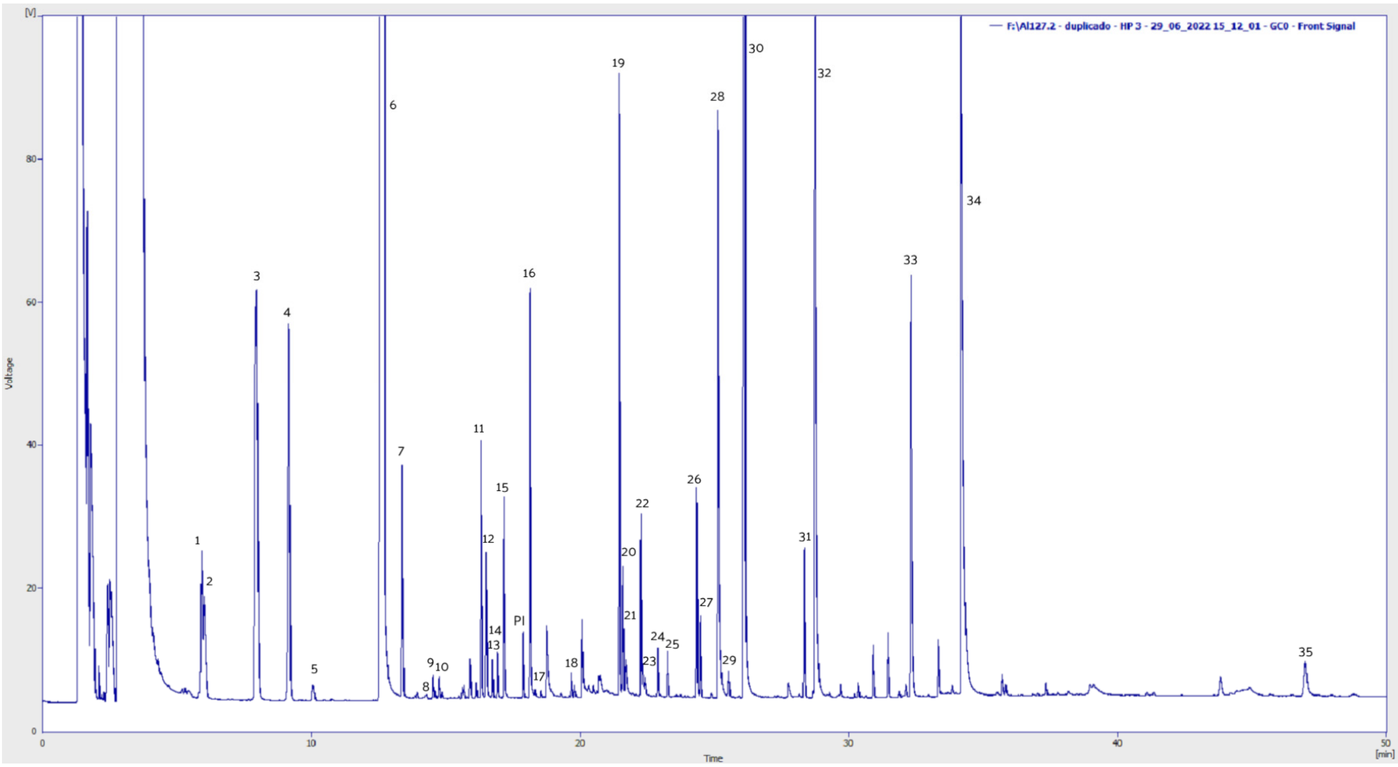


Figure 1. Example of a chromatogram from a wine ("Alvadurão") produced in the experiment (identification of compounds at Table 3) [1-Ethyl butanoate (Butanoic acid ethyl ester), 2-Propan-1-ol (1-propanol), 3-2-Methyl-1-propanol (Isobutanol), 4-3-Methylbutyl ethanoate (Isoamyl acetate), 5-Butan-1-ol (1-Butanol), 6-2-Methyl-1-butanol+3-methyl-1-butanol (Isoamylic alcohols), 7-Ethyl hexanoate (Hexanoic acid ethyl ester), 8-Pentan-1-ol (1-Pentanol), 9-Hexyl acetate (Hexyl ethanoate), 10-3-Hydroxybutan-2-one (Acetoin), 11-Ethyl 2-hydroxypropanoate (Ethyl lactate), 12-Hexan-1-ol (1-Hexanol), 13-(E)- 3-Hexen-1-ol (*trans*-3-Hexenol), 14-3-Ethoxypropan-1-ol (3-Ethoxy-1-propanol), 15-(Z)-3-Hexen-1-ol (*cis*-3-Hexen-1-ol), 16-Ethyl octanoate (Ethyl caprylate), 17-3-(Methylsulfanyl) propanal (Methional), 18-Ethyl 3-hydroxybutanoate, 19-Butyrolactone (γ-Butyrolactone), 20-Ethyl decanoate (Decanoic acid ethyl ester), 21-Butanoic acid (Butyric acid), 22-Diethyl butanedioate (Diethyl succinate), 23-3-Methylbutanoic acid (isovaleric acid), 24-Ethyl 9-decenoate (9-Decenoic acid ethyl ester), 25-3-(Methylsulfanyl)-1-propanol (Methionol), 26-Ethyl 4-hydroxybutanoate, 27-2-Phenylethyl acetate (Phenylethyl acetate), 28-Hexanoic acid (Caproic acid), 29-Phenylmethanol (Benzyl alcohol), 30-2-Phenylethanol, 31-Diethyl hydroxybutanoate (Diethyl malate), 32-Octanoic acid (Caprylic acid), 33-Decanoic acid (Capric acid), 34-Monoethyl butanedioate (Ethyl monosuccinate), 35-4-Hydroxy-benzeneethanol (Tyrosol)].

The identified and analyzed volatile compounds are largely generated during the fermentation process. Alcohols are the primary group in terms of quantity (Table 4), as confirmed by other research groups [34]. Concentrations around 300 mg/L enhance the complexity of the wine, while levels exceeding 400 mg/L tend to have a detrimental impact on wine quality [48]. Among the alcohols detected, isoamyl alcohols were the most prevalent in all white wines, with their concentration being highest in "Cayetana Blanca" wines. These alcohols are often associated with malt and burnt descriptors in the literature (Table 3).

Among the C6 alcohols, 1-hexanol and *cis*- and *trans*-3-hexenol were detected. Several authors consider these compounds to be varietal markers [45], as they are produced through the enzymatic breakdown of linoleic and linolenic acids, primarily through the pre-fermentation phase. However, they contribute to green aromas (Table 3). The highest levels were found in "Cayetana Blanca" and "Larião" wines, while the lowest levels were observed in "Folha de Figueira" wines (Table 4).

Esters are mainly formed during fermentation and contribute to the fruity, sweet and floral aromas (Table 3). However, yeast metabolism, which is responsible for their formation, can be influenced by grape composition [49]. These compounds represent the second most abundant group (Table 4), as verified by other researchers [23–28]. In monovarietal white wines, the average concentrations of esters ranged from 8.09 to 32.30 mg/L (Table 4). The highest amounts were found in "Fernão Pires" wines, followed by "Cercial", "Trajadura" and "Alvadurão" wines. Conversely, the lowest values were observed in "Larião" wines.

Fatty acids are the third most abundant group of volatile compounds (Table 4). These compounds are associated with unpleasant aromas (Table 3), but Etievant [48] reported that they could enhance the freshness of wines and contribute to balancing their fruity aromas. The concentrations found in the white wines ranged from 6.84 mg/L in "Parellada" wines to 19.96 mg/L in "Alvadurão" wines.

Regarding other volatile compounds, acetoin, another compound resulting from yeast metabolism [50], is related to a butter aroma (Table 3). Its concentrations in wines ranged from 0.04 mg/L in "Roupeiro Branco" wines to 0.22 mg/L in "Larião" wines. Butyrolactone, also produced during fermentation [51], is often associated with caramel and sweet descriptors (Table 3), and its levels ranged from 0.51 mg/L in "Larião" wines to 6.31 mg/L in "Fernão Pires" wines.

Tyrosol, a phenolic compound derived from tyrosine by yeast during fermentation, has also been identified in white wines by other researchers [52]. This compound is believed to play a significant role in the mouthfeel of white wine [53], and its concentrations ranged from 0.25 mg/L in Larião wines to 1.12 mg/L in "Pedro Ximenez" wines. Methional, which could result from methionine oxidation, is an odorant compound related to cooked potato aroma notes (Table 3). Other researchers have also detected it in white wines [54]. Its concentrations ranged from 13.79 µg/L in "Parellada" wines to 33.23 µg/L in "Alvadurão" wines.

Table 3. Results of ANOVA variety effect for each identified and quantified compound, along with additional information about odor descriptors from the scientific literature and calculated retention index [** ($p \leq 0.01$), *** ($p \leq 0.001$)].

Identified Compound (Compound Code Figure 2)	Odor Descriptors $^{\alpha}$	Retention Index	Significance Level from ANOVA
Ethyl butanoate (C1)	apple	1047	***
1-Propanol (C2)	ripe fruit, alcohol	1050	***
Isobutanol (C3)	wine, solvent, bitter	1105	***
Isoamyl acetate (C4)	banana	1133	***
1-Butanol (C5)	medicinal	1155	***
Isoamylic alcohols (C6)	malt, burnt	1223	***
Ethyl hexanoate (C7)	apple peel, fruit	1245	**
1-Pentanol (C8)	green, wax	1263	**
Hexyl acetate (C9)	fruit, herb	1282	***
Acetoin (C10)	butter/cream	1289	***
Ethyl lactate (C11)	fruity	1354	***
1-Hexanol (C12)	resin, flower, green	1362	***
trans-3-Hexenol (C13)	moss, fresh	1373	***
3-Ethoxy-1-propanol (C14)	ripe pear [55]	1381	***

Table 3. *Cont.*

Identified Compound (Compound Code Figure 2)	Odor Descriptors $^{\alpha}$	Retention Index	Significance Level from ANOVA
cis-3-hexen-1-ol (C15)	grass	1391	***
Ethyl octanoate (C16)	fruity, fat	1441	**
Methional (C17)	cooked potato	1417	***
Ethyl 3-hydroxybutanoate (C18)	fruity [56]	1525	***
Butyrolactone (C19)	caramel, sweet [57]	1633	***
Ethyl decanoate (C20)	grape	1642	**
Butanoic acid (C21)	rancid, cheese, sweat	1649	***
Diethyl succinate (C22)	wine, fruit	1684	***
Isovaleric acid (C23)	sweat, acid, rancid	1687	***
Ethyl 9-decenoate (C24)	rose [58]	1695	***
Methionol (C25)	sweet, potato	1723	***
Ethyl 4-hydroxybutanoate (C26)	fruity [59]	1813	***
2-Phenylethyl acetate (C27)	rose, honey, tobacco	1821	***
Hexanoic acid (C28)	fat, cheese, barnyard	1861	***
Benzyl alcohol (C29)	fruity, walnut, bitter almond [54]	1884	***
2-Phenylethanol (C30)	honey, spice, rose, lilac	1918	***
Diethyl malate (C31)	brown sugar, sweet	2050	***
Octanoic acid (C32)	sweat, cheese	2074	***
Decanoic acid (C33)	rancid, fat	2288	**
Ethyl monosuccinate (C34)	-	2404	***
Tyrosol (C35)	-	3025	***

α—descriptors are mainly taken from the database Flavornet [60].

In previous studies, terpenic compounds were also detected in monovarietal wines from certain grape varieties, such as “Fernão Pires” [27,61], “Trajadura” [24] and “Palomino Fino” [30], which were also evaluated in this work. However, in this study, these compounds were not detected in the monovarietal wines. It is hypothesized that abiotic stress conditions have influenced the loss of these volatile compounds, reducing the wines’ potential in terms of varietal aroma. Despite the very heterogeneous results, it is recognized that abiotic factors influence the composition of grape aroma compounds, especially terpenic compounds, as highlighted by Rienth et al. [6].

Table 4. Free volatile compounds to allow for comparisons among physicochemical families (values are expressed as mg/L, with the exception of methional, which is expressed as μg/L).

Varietal White Wine	Esters	Alcohols	C6 Alcohols	Acids	Cetones	Lactones	Others	
					Acetoin	Butyrolactone	Methional	Tyrosol
Alvadurão	24.42 bcd	222.16 abc	1.99 bcd	19.96 c	0.17 abcd	2.99 de	33.23 d	0.68 abcde
Bastardo Branco	16.70 abc	200.45 ab	1.31 abc	8.16 ab	0.09 abcd	2.92 de	13.79 a	0.54 abc
Castelão Branco	14.35 ab	197.04 ab	1.88 abcd	14.45 abc	0.05 ab	1.83 abcd	16.46 ab	0.48 ab
Cayetana blanca (Sarigo)	17.41 abcd	360.43 c	2.42 d	17.63 bc	0.07 ab	2.62 bcde	28.62 bcd	0.82 bcde
Cercial	26.76 cd	302.97 bc	1.61 abcd	14.09 abc	0.16 abcd	5.36 fg	32.21 cd	0.99 cde

Table 4. *Cont.*

Varietal White Wine	Esters	Alcohols	C6 Alcohols	Acids	Cetones	Lactones	Others	
					Acetoin	Butyrolactone	Methional	Tyrosol
Folha de Figueira (Dona Branca)	13.83 [ab]	187.18 ab	0.83 [a]	10.68 abc	0.06 [ab]	2.04 [abcd]	23.05 [abcd]	0.46 [ab]
Fernão Pires	28.58 [d]	306.29 bc	1.74 abcd	12.12 abc	0.17 [bcd]	6.31 [g]	27.71 [abcd]	0.84 bcde
Galego Dourado	15.53 abc	207.58 ab	1.13 [abc]	11.97 abc	0.09 [abc]	2.79 [cde]	20.45 [abcd]	0.63 abcd
Lameiro	19.43 abcd	255.61 abc	1.02 [abc]	11.09 abc	0.10 abcd	3.19 [de]	18.92 [abc]	1.00 [de]
Larião	8.09 [a]	132.50 [a]	2.08 cd	14.40 abc	0.22 [d]	0.51 [a]	27.06 [abcd]	0.25 [a]
Palomino fino (Malvasia Rei)	12.44 [a]	246.98 abc	1.72 abcd	8.57 [ab]	0.07 [ab]	1.78 [abcd]	20.72 [abcd]	0.61 abcd
Molinha Macia	9.82 [a]	184.30 ab	1.87 abcd	15.02 abc	0.04 [a]	0.94 [ab]	20.89 [abcd]	0.39 [ab]
Albillo Mayor (Pardina)	11.11 [a]	232.77 abc	1.87 abcd	9.65 [ab]	0.08 [abc]	1.67 [abcd]	25.83 [abcd]	0.61 abcd
Parellada	9.74 [a]	172.69 ab	1.40 abcd	6.84 [a]	0.04 [a]	1.52 [abcd]	14.24 [a]	0.48 [ab]
Pedro Ximenez	18.78 abcd	284.75 bc	1.18 [abc]	13.44 abc	0.07 [abc]	2.78 [cde]	21.49 [abcd]	1.12 [e]
Roupeiro Branco	9.83 [a]	188.61 ab	1.14 [abc]	11.80 abc	0.04 [a]	1.16 [abc]	21.37 [abcd]	0.50 [ab]
Trajadura	25.26 bcd	223.05 abc	0.95 [ab]	12.49 abc	0.14 abcd	4.17 [ef]	21.70 [abcd]	0.61 abcd
Chasselas Cioutat (Uva Salsa)	11.99 [a]	211.91 ab	1.18 [abc]	19.36 [c]	0.20 [cd]	0.84 [a]	30.32 [bcd]	0.58 abcd

Means with different superscripts letters within the same column are significantly different ($p < 0.05$).

Considering the effect of the variety factor on all volatile compounds, a multidimensional analysis was chosen to better understand the differentiation of the wines based on the volatile compounds analyzed. The heatmaps, created using the average composition of the volatile compounds across all wine samples, are presented in Figure 2.

The wines exhibit different correlations with the analyzed volatile compounds, forming a few clusters. One cluster includes the wines Tj, Gd and DB, which are positively correlated with C1 (Ethyl butanoate), C2 (1-Propanol), C14 (3-Ethoxy-1-propanol), C4 (Isoamyl acetate), C18 (Ethyl-3-hydroxybutanoate) and C20 (Ethyl decanoate), all of which also form a cluster of variables related to fruity notes (Table 3). This wine cluster is negatively correlated with C12 (1-Hexanol), related to green notes.

A second cluster appears, formed by Par, Pa, MR and Ca wines, which are negatively correlated with the levels of C1, C2, C14, C18 and C20, as these compounds are present at lower levels in these wines.

A third cluster includes La, MoM and CB wines, which are negatively correlated with the levels of C18 (Ethyl 3-hydroxybutanoate), C20 (Ethyl decanoate), C3 (Isobutanol), C5 (1-butanol), C19 (butyrolactone), C34 (Ethyl monosuccinate), C6 (Isoamylic alcohols), C21 (Butanoic acid), C23 (Isovaleric acid), C30 (2-Phenylethanol), C35 (Tyrosol) and C26 (Ethyl 4-hydroxybutanoate), suggesting that these wines are characterized by low levels of most of the volatile compounds analyzed.

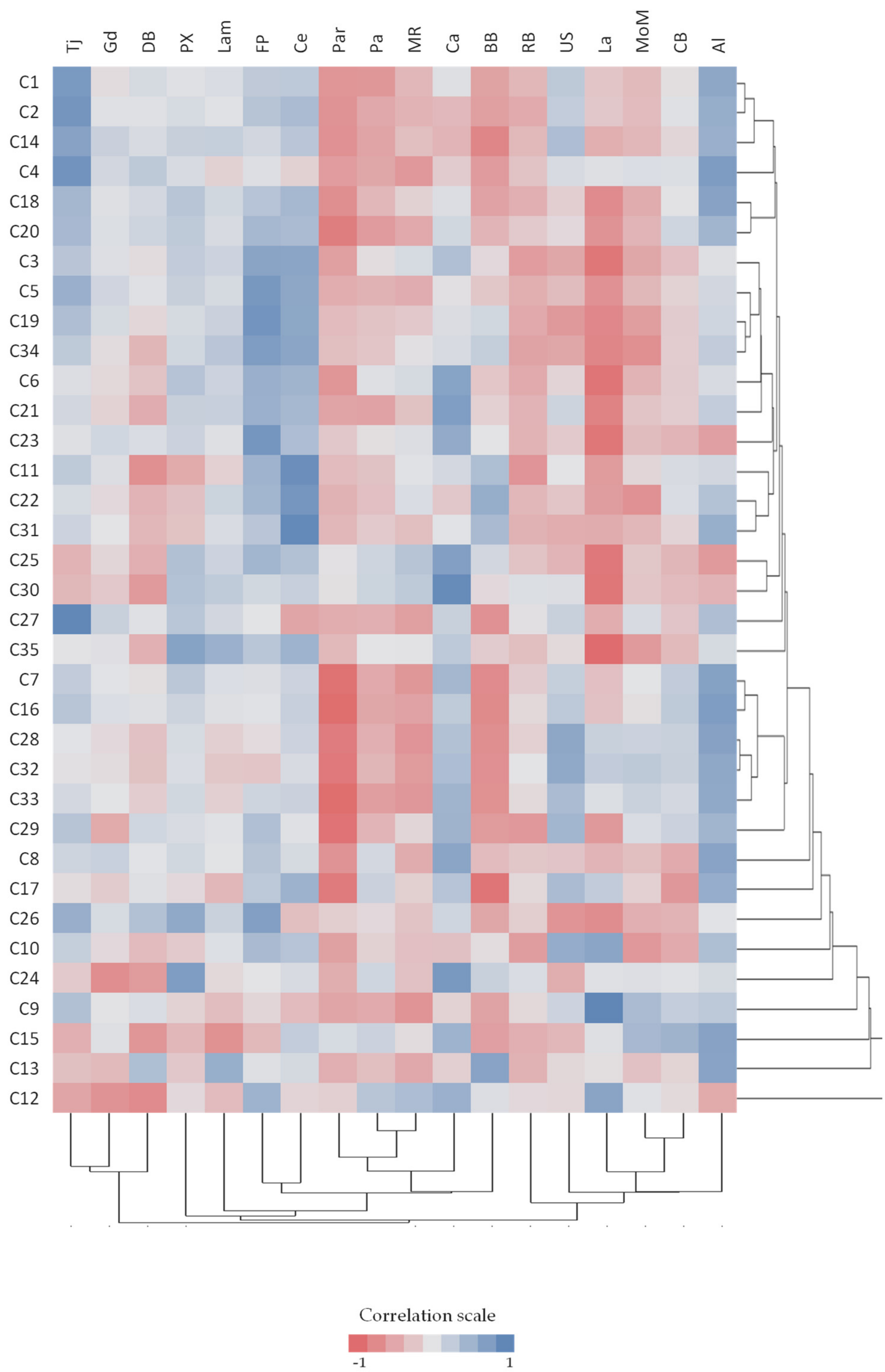


Figure 2. Heatmap of volatile compounds of varietal white wines (codes of volatile compounds from C1 until C35 from Table 3 and the varietal wines from Table 1).

The other monovarietal wines, "Pedro Ximenez" (Px), "Lameiro" (Lam), "Fernão Pires" (FP), "Cercial" (Ce), "Bastardo Branco" (BB), "Roupeiro Branco" (RB), "Chasselas Cioutat" (US) and "Alvadurão" (Al), appear isolated and separated from the others. The Al wine is positively correlated with the first cluster of variables (C1, C2, C14, C4, C18 and C20), which are associated with fruity notes, as well as with another cluster of variables [C16 (Ethyl octanoate), C28 (Hexanoic acid), C32 (Octanoic acid), C33 (Decanoic acid) and C29 (Benzyl alcohol)] related to different odor notes, such as fruity and sweat. It is also associated with high levels of C8 (1-Pentanol), C17 (Methional), C15 (*cis*-3-Hexen-1-ol) and C13 (*trans*-3-Hexenol), which are more related to green and earthy notes.

The "Fernão Pires" (FP) wine is positively associated with the levels of C3, C5, C19, C34, C23 and C36, while the nearby "Cercial" wine is also correlated with C3, C5, C19 and C34, in addition to showing correlations with C11, C22 and C31. The "Pedro Ximenez" (Px) and "Lameiro" (Lam) wines are associated with intermediate levels of most volatile compounds.

The wines BB, RB and US are also presented separately in the heatmap (Figure 2). BB shows a positive correlation with the levels of C11 (Ethyl lactate), C22 (Diethyl succinate), C31 (Diethyl malate) and C13 (trans-3-Hexenol) and a negative correlation with a group of variables, including C7 (Ethyl hexanoate), C16 (Ethyl octanoate), C28 (Hexanoic acid), C32 (Octanoic acid), C33 (Decanoic acid), as well as C14 (3-Ethoxy-1-propanol) and C17 (Methional). RB wine shows negative correlations with most volatile compounds, reflecting its low levels of the majority of the analyzed compounds. US wines, also presented separately, show positive correlations with the levels of C28 (Hexanoic acid), C32 (Octanoic acid), C33 (Decanoic acid), C29 (Benzyl alcohol) and C10 (1-Hexanol).

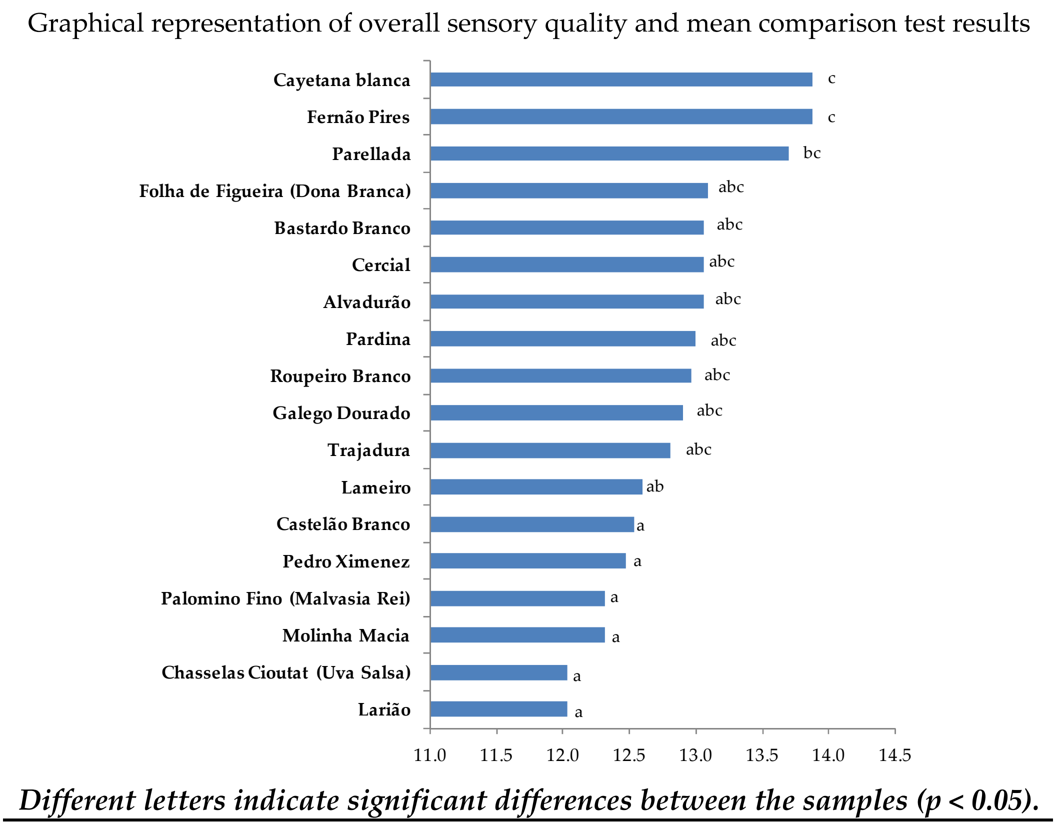
3.3. Sensory Results

Based on the average values from the eight tasters (Table S2), a one-way ANOVA was performed on all the descriptors present in the tasting form, with grape variety as the sole factor. A significant effect was detected for the following 11 descriptors (Table 5): color intensity, yellow hue, green hue, limpidity, tropical fruit, aroma positive intensity, sweetness, acidity, bitterness, body, harmonious persistence and overall quality. Puig and Pujol [22] also found similar results in a study of minority varieties, detecting significant effects of grape variety on sensory descriptors such as floral, fruity, tertiary aromas, post-fermentative aromas, sourness and astringency.

Table 5. Summary of one-way ANOVA for various sensory analysis descriptors and overall quality of the wines [ns ($p > 0.05$), * ($p \leq 0.05$), ** ($p \leq 0.01$), *** ($p \leq 0.001$)].

Descriptor	Effect of Variety Factor (Significance Level)
Color intensity (V1)	**
Green hue (V2)	*
Yellow hue (V3)	***
Limpidity (V4)	*
Aroma positive intensity (A1)	**
Floral (A2)	ns
Terpenic/muscat (A3)	ns
White fruit (A4)	ns
Tropical fruit (A5)	*
Citrus fruit (A6)	ns
Dried fruits (A7)	ns
Herbaceous/green (A8)	ns
Sweetness (F1)	***
Sourness (F2)	*
Bitterness (F3)	ns
Body (F4)	*
Harmonious persistency (F5)	**
Overall quality (OQ)	*

The heatmap generated from the sensory analysis (Figure 3) similarly highlights the formation of only a few wine clusters. These clusters do not correspond to those found in the analysis of volatile compounds, suggesting that, in addition to volatile composition, other aspects of the wine's composition also influence its sensory profile.



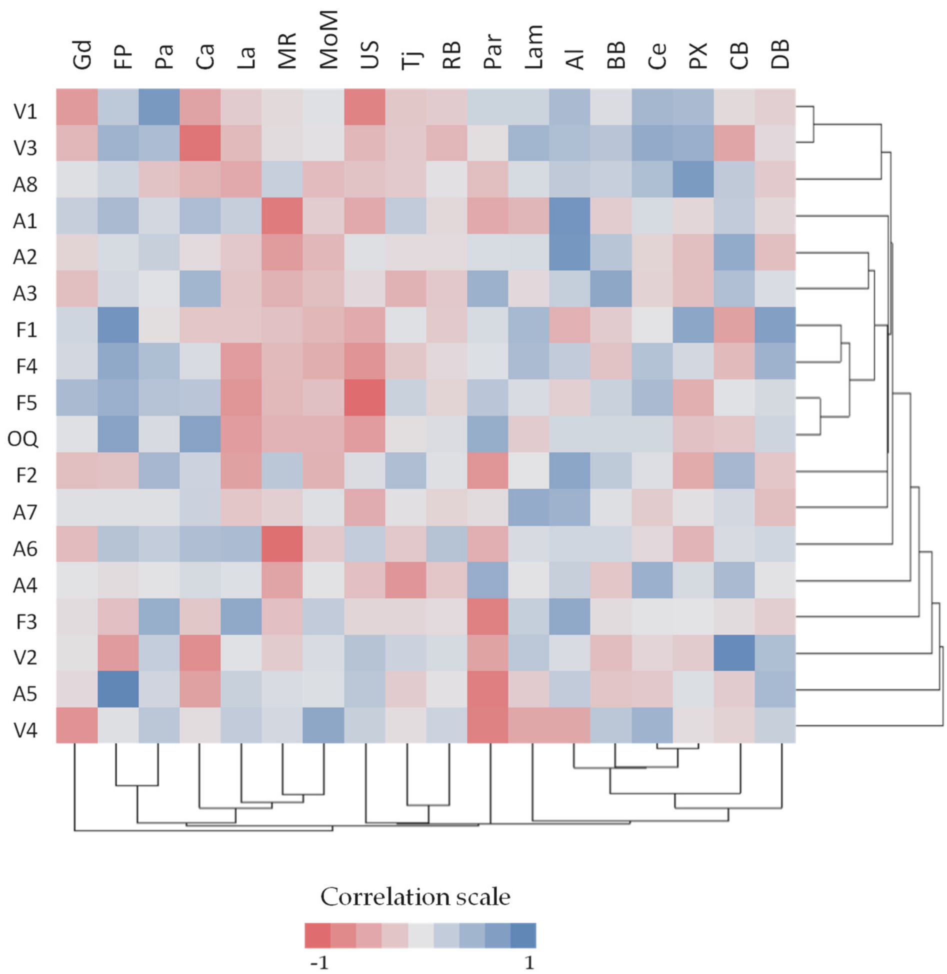


Figure 3. Heatmap of sensory attributes of varietal white wines (codes of sensory attributes in Table 5 and varietal wines codes in Table 1).

However, it is recognized that, beyond volatile compounds, the non-volatile composition significantly influences sensory characteristics and consumer preferences [62]. In the heatmap, overall quality appears in the same cluster as F1 (sweetness), F4 (body), F5 (harmonious persistence) and two aroma descriptors: A2 (floral) and A3 (terpenic).

The FP and Pa wines appear in the same cluster and show a stronger positive correlation with visual descriptors such as V1 and V3, aroma descriptors such as A5, and flavor descriptors, such as F1, F4 and F5, as well as with overall quality (OQ). In fact, these wines exhibited significantly high overall quality (Table 5).

A wine cluster consisting of La, Mr and MoM is negatively correlated with the descriptors F1, F4, F5, F2 and OQ, corresponding to wines with significantly low overall quality assessments.

Another cluster includes samples of Al, BB, Ce, PX and CB. Within this group, the CB wine appears slightly separate from the others. The variables most correlated with this cluster are V1, V3 and A8. This group exhibits intermediate overall quality, except for CB, which has the lowest quality.

The remaining wines—Gd, Ca, US, Tj, RB, Par, Lam and DB—appear separately, each displaying distinct characteristics. The other group of wines with generally high-quality assessments (Table 5) includes DB, BB, Ce, AL, Pa, RB, Gd and Tj. "Trajadura" (Tj) is a variety that has shown promising results in the Vinho Verde region [45], while "Cercial" (Ce) has also demonstrated positive outcomes in other Portuguese regions such as Bairrada [63]. "Galego Dourado" (Gd), traditionally used in a small region for fortified wines, was considered a minority variety until recently. However, it is no longer classified as such due to the promising results obtained from monovarietal white wines over the years [28]. "Albillo Mayor" ("Pardina") is a variety used in Spain [31].

Regarding the remaining wines in this group—specifically DB, BB, AL and RB—these are minority Portuguese varieties that warrant further study due to the limited information available on their potential.

4. Conclusions

The results obtained represent an initial approach to compare these 18 grape varieties for white wine production in a very hot region. However, it is important to recognize the limitation that these findings are based on data from a single harvest, while it is well-known that significant variability can occur across multiple harvests [27,28]. Despite this limitation, for several varieties—namely, "Alvadurão", "Bastardo Branco", "Castelão Branco", "Folha de Figueira", "Lameiro", "Larião", "Molinha Macia", "Roupeiro Branco" and "Chasselas Cioutat"—this study displays, for the first time, results regarding the physicochemical and sensory composition of the respective wines.

The sensory and volatile compound compositions do not reveal a direct correspondence. However, it is recognized that, beyond volatile compounds, the non-volatile composition significantly influences sensory characteristics and consumer preferences.

Considering the results obtained, particularly in terms of overall wine quality, the findings confirm "Fernão Pires" as an excellent variety for white wine production. This supports its status as one of the most cultivated varieties in Portugal and various other regions. Similarly, the "Cayetana Blanca" variety produced wines of high overall quality. In Portugal, this variety is known as "Sarigo", while in Spain, it is considered a minority variety that has recently attracted the attention of researchers [22,23]. Until a few years ago, it had remained relatively underexplored in Portugal. The monovarietal wines of "Fernão Pires" and "Cayetana Blanca" exhibited high levels of several groups of volatile compounds.

Additionally, "Parellada" produced wines with similarly high overall quality. A third group of wines, while exhibiting slightly lower overall quality, still showed promising characteristics. This group included the varieties “Folha de Figueira”, “Bastardo Branco”, “Cercial”, “Alvadurão”, “Albillo Mayor”, “Roupeiro Branco”, “Galego Dourado” and “Trajadura”. While “Cercial”, “Galego Dourado”, “Trajadura” and “Albillo Mayor” have previously been studied and demonstrated oenological potential, the other varieties are minority varieties, and these findings represent the first published data on the characteristics of their wines. Among these grape varieties, the high natural acidity of certain wines (>6 g/L) is particularly noteworthy in a hot region, especially for “Alvadurão”, “Cercial” and “Bastardo Branco”.

Based on these promising results obtained in a deficit irrigated vineyard, further research is needed to validate and build on the findings presented here, as well as to gain more knowledge about the behavior of these varieties under different conditions of water availability.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/beverages11030068/s1, Table S1: Average values of volatile compounds (mg/L) determined in varietal white wines; Table S2: Average values of sensory attributes evaluated in varietal white wines.

Author Contributions: Conceptualization, J.S.; Methodology, M.D., J.d.D., S.L., R.R. and I.C.; Software, R.R., I.C. and O.A.; Formal analysis, R.R., S.L., O.A. and I.C.; Investigation, R.R., I.C., M.D. and J.S.; Resources, J.S.; Data curation, J.S., I.C., M.D. and O.A.; Writing—original draft, I.C. and J.d.D.; Writing—review and editing, I.C., M.D., R.R., S.L., O.A., J.d.D. and J.S.; Supervision, I.C., J.S. and O.A.; Project administration, J.S.; Funding acquisition, I.C. and J.S. All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement: This study was conducted in accordance with the Declaration of Helsinki and approved by the CD-INIAV (protocol code CEC-INIAV-Ed. 2-2018 and date of approval: 7 January 2019).

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: The original contributions presented in the study are included in the article/Supplementary Materials; additional information could be requested from the corresponding author.

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