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Part II—Aroma Profile of Twenty White Grapevine Varieties: A Chemotaxonomic Marker Approach

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Abstract: The importance of white wines in the northwest of the Iberian Peninsula has led to study on the aromatic profiles of 20 white grapevine varieties along three consecutive vintages. Volatile compounds were evaluated through solid-phase microextraction and gas chromatography-mass spectrometry methodology (SPME-GC-MS). The main aims of this study were as follow: test if their aromatic profile could be used as a potential chemotaxonomic tool to differentiate among grapevine varieties, evaluate if there is any relationship among the aromatic characteristics and the genetic origin of the varieties studied and try to bring out aromatically interesting varieties that could trigger diversified wine production in the study area. Free and bound volatile profiles were tentatively identified in each variety, grouping all identified compounds into thirteen volatile families to allow for a better discussion of the results obtained. Significant differences were found for terpenes among varieties in the free fraction and for acids, aldehydes, terpenes and norisoprenoids in the glicosidically bound fraction. Good differentiation among varieties was achieved by free and glicosidically bound profiles in the principal component analysis (PCA), as well as some clustering of varieties belonging to the same genetic reconstructed populations (RPP). Varietal aromatic profiling could be expected to be a good chemotaxonomic tool.

Keywords: *Vitis vinifera* L; white grapevine varieties; aromatic characterization; chemotaxonomic markers; varietal differentiation tools

1. Introduction

The northwest of the Iberian Peninsula, including Galicia in Spain and the northern territories of Portugal, are specialized in the production of white wines [1–4], recognized by their high quality and standing out for their freshness and good alcohol–acidity equilibrium, as well as for their aromaticity and fruity flavor [4]. Most of these wines are produced with autochthonous *Vitis vinifera* L. varieties, considering 'Albariño', 'Treixadura' and 'Godello', which represent the most important varieties for the Galician economy [2]. Moreover, some Portuguese regions are well known for their white wines from varieties, such as 'Alvarinho' (syn. 'Albariño'), 'Loureiro' (syn. 'Loureira'), 'Batoca' or 'Trajadura' (syn. 'Treixadura') in *Vinhos Verdes* regions or 'Fernão Pires' as the main white variety employed in the Bairrada Apellation, despite being also generally used in other Portuguese Apellations [5], making these white wines play an important economic role, with 'Alvarinho' and 'Loureiro' wines being mainly monovarietal ones [6].

In Galicia, the vineyard sector is of great economic importance, with a vineyard area of 24,000 ha and more than 194,000 wineries, with a total production of more than 800,000 hL, of which 300,000 hL is white wines [7]. Nowadays, the Galician wine sector is the most important one among the agro-industrial sectors, with around 20% of its total production

exported worldwide. Moreover, there has been a steady growth in average export costs from 2010 to 2019 [8].

Nowadays, global warming triggers earlier grape sugar ripening, generally unbalanced with aroma and flavor maturity, losing their potential acidity while the harvest is delayed for its aroma development [9]. Added to the fact that there is a highly competitive wine market, it is necessary to diversify products. The use of traditional varieties could help to preserve the genetic resources and to produce high-quality wines [10] elaborated in a sustainable agriculture [11].

Even though it is known that more than 6000 grapevine varieties exist over the world [12], only few of them cover the total world vineyard surface [13]. A deeper study of this varietal diversity could help to increase the varietal range of some appellations of origin regulations, which do not usually include minor varieties [14], despite some of them being able to offer high oenological potential. Varieties, such as 'Godello', recently included in different Galician wine appellations of origin, have already achieved a high impact in worldwide wine evaluations [15]. New consumption requirements [14], as well as more extreme and demanding environmental conditions arising from climate change [16,17], and multiple requests for the viticulture industry, such as globalization or economic factors [18], explain the necessity to study different grapevine varieties aiming to maintain a broad natural diversity and retain the wine regional typicity, as a way to respond to these risks to viticulture as we know it today.

Aroma is one of the main factors that contribute to the quality and character of a wine [19], being the variety used highly related to that quality [20]. The aroma specific to the grape variety will become the varietal or primary aroma of the resulting wines, and despite only those varieties with a high terpene composition, such as Muscat ones, will produce odoriferous floral musts, most varieties that are non-muscat varieties or neutral ones could equally produce different wines with specific aroma characteristics linked to the specific variety [6].

The constitutive levels of volatile organic compounds (VOCs) of a vegetal species are highly dependent of genetics [21] and more specifically, at least in fruit, dependent on the cultivars [22], which is the reason why aroma could be a way to differentiate and characterize varieties. Nevertheless, other factors, such as supplementary irrigation [23], as well as the training system or the vineyard soil management [24,25], could also affect the volatile composition.

Despite the difficulty of using volatile organic compounds as a chemotaxonomic tool is well-known, as they have complex biosynthesis and transformation mechanisms behind this, it is an interesting and plausible hypothesis thinking about a relationship between the volatile composition of different genetically related varieties, as it has recently been tested by Ŝikuten et al. [26]. Along the same lines, other studies have been recently carried out, in this case with the VOC profiling of different apple cultivars [22,27] and with pomegranate varieties [28].

Traditionally, grapevine varieties have been classified into different genetic groups using molecular markers [29,30]. Furthermore, some statistical tools for structural analysis have made it possible to create genetic–geographical relationships between certain grapevine varieties [29]. This have been developed by Díaz-Losada et al. [31–35] with varieties included in the grapevine germplasm bank established in EVEGA. This classification has been used in previous research works [36–39], in which a detailed phytochemical description was carried out. The phenolic, anthocyanin and non-anthocyanin profiles and the aromatic profile of red grapevine varieties were tested to be used as chemotaxonomic tools to discriminate among varieties. It was also analyzed whether those VOC profiles could cluster varieties by their genetic–geographic group, given the promising results obtained with grapevines by Ŝikuten et al. [26] or with pomegranate by Beghè et al. [28].

This study is therefore part of a broader characterization research, focusing in this case on the white grapevine varieties grown in the germplasm bank. The aromatic profile, including free, as well as glycosidically bound compounds, were evaluated, given that

grape's aromas and flavors could not only be attributed to their direct volatile compounds, but also to the effect of their aromatic precursor molecules, which will play different roles in translation into the varietal aroma of the wine, depending on the elaboration procedures, to become odorous compounds [40].

In this paper, the study of the volatile compounds present in twenty white grape varieties was performed in order to achieve the following objectives: (1) to assess if their aromatic profile allows a clear differentiation among varieties, (2) to evaluate if the aromatic profile could be used as a chemotaxonomic tool, testing how it groups the varieties in accordance to their aromatic similarities and if those clusters maintain any relationship with their genetic–geographic grouping and (3) try to bring out new varietal aromatic profiles from traditional varieties that trigger the diversification of winemaking in the study region in support of the grapevine biodiversity in this geographical area.

2. Materials and Methods

2.1. Vegetal Material

Grapes from 20 white genotypes of *Vitis vinifera* L. from the EVEGA germplasm bank were analyzed in three consecutive vintages, 2015, 2016 and 2017: 'Agudelo'—VIVC code 2527 [41] 'Albilla do Avia'—VIVC code 24392, 'Batoca'—VIVC code 1037, 'Blanca de Monterrei'—VIVC code 2124, 'Branco Lexítimo'—VIVC code 22838, 'Dona Branca'—VIVC code 2742, 'Fernão Pires'—VIVC code 4100, 'Garrido Fino'—VIVC code 4470, 'Godello'—VIVC code 12953, 'Italia'—VIVC code 5582, 'Jarrosuelto'—VIVC code 24138, 'Lado'—VIVC code 23156, 'Moscatel de Bago Miudo'—VIVC code 8193, 'Palomino'—VIVC code 8888, 'Pirixileira'—VIVC code 2476, 'Planta Fina'—VIVC code 9542, 'Ratiño'—VIVC code 24127, 'Silveiriña'—VIVC code 4178, 'Torrontés'—VIVC code 715 and 'Treixadura'—VIVC code 12629. All varieties are wine grape varieties, except for 'Italia', which is a table grape [42] and therefore not suitable for wine making; it is included in the study because it belongs to the EVEGA germplasm bank, and it could be important to complete the characterization to check how the aromatic grouping behaves with respect to the genetic–geographic one.

From all the varieties analyzed, 'Agudelo', 'Blanca de Monterrei', 'Branco Lexítimo', 'Dona Branca', 'Godello', 'Lado', 'Palomino', 'Torrontés' and 'Treixadura' are included within the different Protected Designations of Origin for wine in the community. 'Fernão Pires' is a white variety broadly used in Portugal, which was firstly recovered in the germplasm bank known as 'Torrontés II' and lately identified through molecular techniques. 'Albilla do Avia' and 'Ratiño' have been recently included in the commercial grapevine register (10/2021). The experimental field was in Ourense (42°21'34.5'' N, 8°07'08.2'' W, elevation 87 m above sea level (a.s.l.)), Galicia (Spain).

Vines are around 30 years-old, grafted on 196-17C rootstock and trained into a vertical trellis, with a planting frame of 1.8 m between rows and 1.2 m within the row and facing east–west. Varieties are in duplicate plots, from 6 to 11 plants [36,37].

Grape maturity was monitored weekly from veraison to harvest. At the optimum industrial ripening stage (19–23 °Brix), 500 berries were hand-picked from each variety from the top, bottom, and center of the bunch to obtain a representative sample. Subsequently, from the total of each sample collected, 100 berries, in duplicate, were frozen at −20 °C until the evaluation of aromatic compounds. Another two aliquots of 100 berries were saved to obtain the corresponding must to evaluate the physicochemical parameters.

2.2. Climatic Conditions

Maximum, minimum, and mean temperatures, as well as the accumulated rainfall, were registered by an automatic meteorological station (iMETOS, Pessl Instruments GmbH, Weiz, Austria) located in the same experimental field. There were also two heat summation indices calculated, the Heliothermal Index (HI) [43] and the Cool Night index (CI) [44].

2.3. Must Basic Chemical Composition

Must analysis was carried out as previously described by Díaz-Fernández et al. [36,37] to evaluate the oenological characteristics of the different varieties under study. Two samples (100 berries) from the 20 varieties were pressed and the following parameters were obtained: sugar content (g·L^{-1}), titratable acidity (g tartaric acid·L^{-1}) and pH were determined via Fourier transform infrared spectrometry (FTIR, OENOFOSS ™, FOSS, Hilleroed, Denmark). Malic and tartaric acid (g·L^{-1}) were determined using an autoanalyzer (LISA 2000, HYCEL DIAGNOSTICS, Massy, France). The tartaric:malic acid relationship, as well as two maturation indexes were estimated as follows: Cillis and Odifredi (MI-CO) and Baragiola & Scuppli (MI-BS) [45].

2.4. Volatile Composition

2.4.1. Chemicals

Referenced compounds and internal standards were from Sigma-Aldrich (Steinheim, Germany); absolute ethanol, dichloromethane and methanol were purchased from Merck (Darmstadt, Germany), and pure water was obtained from a Mili-Q purification system (Milipore, Bedford, MA, USA).

2.4.2. Varietal Volatile Compounds Based on SPME-GC-MS

Free and glicosidically bound volatile compounds were determined according to the methodology described by Díaz-Fernández et al. [36].

The free fraction was directly extracted from a 10 mL vial with 5 mL of clear juice with the addition of 1.5 g of NaCl and 20 µL of each internal standard, 4-methyl-2-pentanol (10 mg·L^{-1} in ethanol) and 3-octanol (1 g·L^{-1} in ethanol). Vials were equilibrated and shaken at 500 rpm in a 60 °C water bath for 2 min.

Then, 1 g C-18 cartridges (Hypersep Spe 1000 mg C-18), from Thermo Scientific (Waltham, MA, USA), were used to separate the volatile compounds from the glicosidically bound fraction, as it was described in previous studies [36,37]. Afterwards, those extracts containing the hydrolyzed bound compounds, using Enzyme AR2000 from Rapidase (DSM food specialties, Seclin, France) for hydrolyzation, were prepared equally as previously explained with the free-fraction samples.

The extraction and desorption of free and released glicosidically bound molecules were carried out via solid-phase microextraction (SPME), using a 2 cm 50/30 (DVB/CAR/PDMS) fiber from Supelco (Bellefonte, PA, USA). Desorption was carried out in the GC injector for 5 min at 250 °C in splitless mode.

Separation, identification and semi-quantification of volatile compounds were carried out on a GC 7820 A gas chromatograph (Agilent Technologies, Santa Clara, CA, USA) coupled with a 5975 Series MSD Agilent mass spectrometer detector. The column used was a ZBWax (Phenomenex; 60 m, 0.25 mm and 0.25 m film thickness). Chromatographic conditions used were reported in a previous paper [36,37].

2.4.3. Statistical Treatment

Statistical differences between the mean values of the three-vintages for each parameter analyzed were tested by performing one-way analysis of variance (ANOVA). Fisher's least significant difference method was applied for the significance of comparisons. All values lower than 0.005 µg·L^{-1}, represented as <LQ in the tables, were considered as 0 for their consequent statistical analysis following other authors' methodology [36,37,46–48], to the detriment of the high standard deviations obtained in some cases but in favour of avoiding establishing overvaluations by omitting those vintages with very low values. Multivariate statistical analysis using the principal component analysis (PCA) was performed to attempt the separation of the varieties according to their content in the free and glicosidically bound compound profiles. XLstat-Basic+ (Addinsoft, Paris, France) software was used.

3. Results & Discussion

3.1. Climatic Conditions

Monthly climatic data, maximum temperature (T Max), mean temperature (T Mn), minimum temperature (T Min) and accumulated rainfall, are shown in Supplementary Table S1. In terms of temperature, the high similarity among the different vintages can be appreciated, while 2016 was highlighted by its high rainfall accumulation with 1211.8 mm against 659.2 and 869 in 2015 and 2017, respectively.

HI values reflected a warm climate (HI + 2) for the three vintages under study, while cool nights (CI + 1) in 2015 and very cool nights (CI + 2) in 2016 and 2017 were characterized by the CI [36].

3.2. Must Basic Chemical Composition

Analytical maturity parameters are shown in Table 1. Varieties were collected in different data in terms of achieving their optimal maturation and sanitary state. Intended °Brix were among 20–23 °Brix, with 'Blanca de Monterrei', 'Jarrosuelto', 'Palomino', 'Pirixileira' and 'Silveiriña' having slightly lower values of accumulated sugar.

Every parameter studied showed ANOVA significant differences among varieties except for the pH and the Baragiola & Scuppli (MI-BS) maturation index, with this last one showing the relationship between total acidity and the tartaric one. However, total acidity, tartaric acid, the ratio among tartaric and malic acid and the De Cillis and Odifredi maturation index showed lower significant differences than the sugar content.

Tartaric acid was 8 $g \cdot L^{-1}$ in 'Branco Lexítimo', being the highest value, and 4.9 $g \cdot L^{-1}$ in 'Blanca de Monterrei'. 'Ratiño' was outstanding based on its high malic acid value, with 4.7 $g \cdot L^{-1}$ against that of 'Palomino', with 0.4 $g \cdot L^{-1}$. In agreement with the De Cillis and Odifredi maturation index, which considers a theoretical range of correct industrial maturity among three and five points, almost every variety fulfilled it, except for 'Palomino', 'Pirixileira' and 'Torrontés', which had slightly higher values, and 'Ratiño', with a slightly lower one.

3.3. Volatile Composition

Volatile compounds identified, in free and bound forms, were classified into thirteen chemical families: acids, alcohols, esters, thiols, phenols, C_6 compounds, ketones, aldehydes, terpenes, sesquiterpenes, C_{13}-norisoprenoids, lactones and polycyclic aromatic hydrocarbons (PAHs).

In terms of clearly appreciating the VOC profiles and compare them among the studied varieties, Figure 1 shows the contribution percentage of each volatile family to the aromatic profile of each variety. In this sense, it could be easily observed which families would play an important role in the different aromatic profiles, if those compounds are in free form and could directly contribute to the aroma or if they act as aromatic precursors and should be previously released in order to play an active aromatic role. Furthermore, it could be observed if there are evident similarities among varieties from an aromatic point of view. Terpenes, alcohols, phenols and C_6 compounds were, in general, those families with a higher contribution, with up to 80% in some varieties, followed by esters, acids and aldehydes, with up to 30%. The rest of the chemical families contribute only up to 6%.

In order to go deeper and with regard to the free-volatile-fraction compounds, Table 2 shows the mean concentration values of the parameters in the different vintages. C_6 compounds were identified as the highest-content family in almost every variety, except for 'Fernão Pires', 'Italia' and 'Moscatel de Bago Miúdo', in which the terpene family was the major one, and 'Torrontés', in which alcohols were the most abundant volatile family.

In relation to the glicosidically bound fraction compounds, Table 3 shows the mean concentration values of the vintages studied. In this fraction, the alcohol family was identified as the one with the highest content in 13 out of 20 varieties; 'Fernão Pires', 'Italia' and 'Moscatel de Bago Miúdo' also had terpenes as the major contributing family in this

fraction, while 'Branco Lexítimo', 'Jarrosuelto' and 'Ratiño' stood out by their phenol family and 'Garrido Fino' by their ester one.
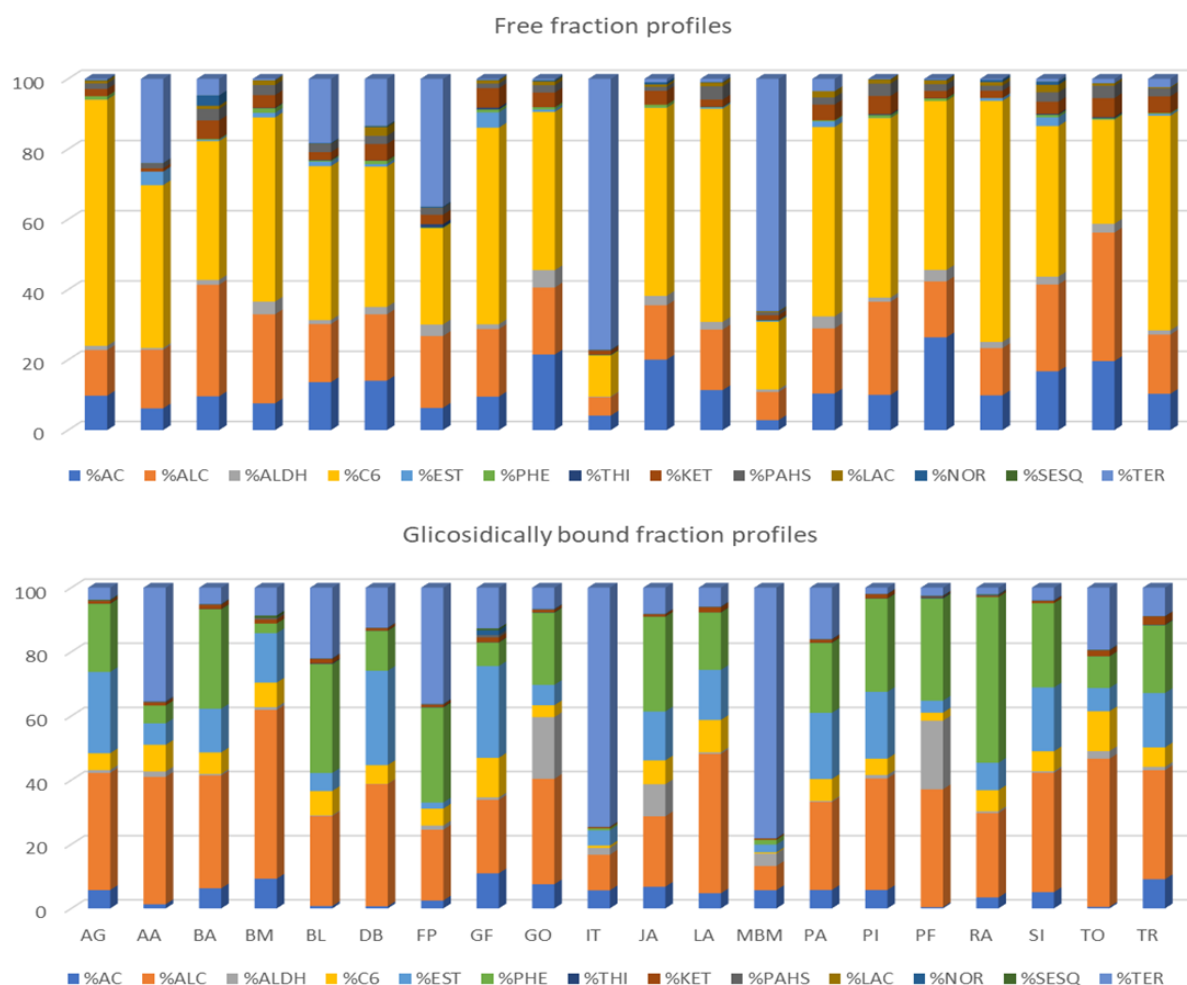

Figure 1. Aromatic family profile. % (AC: acids; ALC: alcohols; ALDH: aldehydes; EST: esters; PHE: phenols; THI: thiols; KET: ketones; LAC: lactones; NOR: norisoprenoids; SESQ: sesquiterpenes; TER: terpenes). Varieties (AG: 'Agudelo', AA: 'Albilla do Avia', BA: 'Batoca', BL: 'Branco Lexítimo', BM: 'Blanca de Monterrei', DB: 'Dona Blanca', FP: 'Fernão Pires', GF: 'Garrido Fino', GO: 'Godello', IT: 'Italia', JA: 'Jarrosuelto', LA: 'Lado', MBM: 'Moscatel de Bago Miúdo', PA: 'Palomino', PI: 'Pirixileira', PF: 'Planta Fina', RA: 'Ratiño', SI: 'Silveiriña', TO: 'Torrontés', TR: 'Treixadura').

The main volatile compounds identified in each chemical family can also be found in Supplementary Table S2.

Table 1. Chemical composition of the grapes in the three vintages studied (2015–2017).

Variety	Ab.	Sugar (g·L⁻¹)	Total Acidity (g·L⁻¹)	pH	Malic Acid (g·L⁻¹)	Tartaric Acid (g·L⁻¹)	MI:CO	MI:BS	T:M
'Agudelo'	**AG**	197.6 ± 19.1 abcde	5.4 ± 1.1 abcde	3.48 ± 0.24 abc	3.0 ± 0.6 cdef	5.1 ± 1.1 b	3.87 ± 0.86 abc	0.98 ± 0.36 ab	0.58 ± 0.10 efg
'Albilla do Avia'	**AA**	231.2 ± 10.6 hi	4.9 ± 0.8 abcd	3.45 ± 0.17 abc	1.0 ± 0.4 ab	6.5 ± 0.4 cdef	4.87 ± 1 bcd	1.37 ± 0.28 abc	0.15 ± 0.06 ab
'Batoca'	**BA**	199.5 ± 15.1 abcdef	6.2 ± 1.5 cdef	3.42 ± 0.17 abc	3.1 ± 1.7 defg	6.0 ± 1.1 abcde	3.47 ± 0.84 ab	1.03 ± 0.33 abc	0.54 ± 0.38 defg
'Branco Lexítimo'	**BL**	224.7 ± 17.5 hi	8.1 ± 2.2 ef	3.19 ± 0.30 a	3.1 ± 1.0 defg	8 ± 1.4 f	2.99 ± 1.12 ab	1.11 ± 0.21 abc	0.38 ± 0.06 abcdefg
'Blanca de Monterrei'	**BM**	176.6 ± 29.0 a	4.5 ± 0.7 abc	3.52 ± 0.15 abc	1.9 ± 0.9 abcde	4.9 ± 0.9 a	4.20 ± 0.86 abcd	1.13 ± 0.38 abc	0.40 ± 0.22 abcdefg
'Dona Branca'	**DB**	209.9 ± 13.4 bcdefghi	5.1 ± 1.1 abcd	3.58 ± 0.27 abc	2.9 ± 0.5 cdef	5.4 ± 1.3 abcd	4.33 ± 0.86 abcd	1.12 ± 0.48 abc	0.57 ± 0.19 defg
'Fernão Pires'	**FP**	205.0 ± 14.4 bcdefgh	6.2 ± 1.4 cdef	3.42 ± 0.22 abc	3.3 ± 0.9 efg	5.9 ± 0.7 abcde	3.45 ± 0.68 ab	0.98 ± 0.30 ab	0.58 ± 0.22 defg
'Garrido Fino'	**GF**	193.1 ± 3.2 abcde	4.9 ± 0.9 abcd	3.48 ± 0.21 abc	1.4 ± 0.3 abc	6.1 ± 0.8 abcde	4.23 ± 0.86 abcd	1.29 ± 0.40 abc	0.24 ± 0.08 abcd
'Godello'	**GO**	217.9 ± 14.6 efghi	5.6 ± 1.4 bcdef	3.50 ± 0.24 abc	2.3 ± 1.1 bcdef	6.6 ± 0.5 def	4.10 ± 1.17 abc	1.24 ± 0.39 abc	0.36 ± 0.19 abcdef
'Italia'	**IT**	201.7 ± 20.2 abcdefg	5.2 ± 1.70 abcd	3.62 ± 0.34 abc	3.1 ± 1.2 def	5.7 ± 1.1 abcde	4.29 ± 1.74 abcd	1.18 ± 0.59 abc	0.57 ± 0.32 defg
'Jarrosuelto'	**JA**	189.1 ± 0.8 abcd	6.5 ± 1.0 cdef	3.36 ± 0.04 abc	3.9 ± 0.9 fg	5.5 ± 1.0 abcde	3.07 ± 0.48 a	0.84 ± 0.02 a	0.71 ± 0.03 fg
'Lado'	**LA**	229.8 ± 9.6 hi	6.9 ± 1.7 def	3.30 ± 0.33 ab	3.1 ± 0.6 defg	6.7 ± 0.4 def	3.51 ± 0.96 ab	1.01 ± 0.22 abc	0.46 ± 0.10 bcdefg
'Moscatel de Bago Miúdo'	**MBM**	234.1 ± 4.9 i	5.7 ± 1.2 bcdef	3.48 ± 0.19 abc	2.9 ± 1.0 cdef	5.8 ± 0.5 abcde	4.24 ± 0.84 abcd	1.06 ± 0.26 abc	0.50 ± 0.21 cdefg
'Palomino'	**PA**	188.9 ± 14.4 abcd	3.7 ± 0.7 ab	3.62 ± 0.15 abc	0.4 ± 0.3 a	5.6 ± 0.7 abcde	5.41 ± 0.67 cd	1.57 ± 0.46 c	0.07 ± 0.01 a
'Pirixileira'	**PI**	184.4 ± 3.5 abc	3.4 ± 0.5 a	3.71 ± 0.10 c	0.8 ± 0.3 ab	5.2 ± 0.9 abc	5.77 ± 0.80 d	1.56 ± 0.40 bc	0.16 ± 0.07 abc
'Planta Fina'	**PF**	210.9 ± 17.3 cdefghi	5.1 ± 1.5 abcd	3.60 ± 0.35 abc	2.2 ± 0.0 bcde	7.0 ± 0.6 ef	4.38 ± 1.09 abcd	1.47 ± 0.54 abc	0.32 ± 0.03 abcde
'Ratiño'	**RA**	211.8 ± 17.8 defghi	7.7 ± 1.3 f	3.33 ± 0.37 abc	4.7 ± 2.0 g	6.8 ± 0.9 ef	2.82 ± 0.38 a	0.89 ± 0.10 a	0.72 ± 0.41 g
'Silveiriña'	**SI**	183.0 ± 23.5 ab	5.3 ± 0.3 abcd	3.46 ± 0.03 abc	1.9 ± 0.6 abcde	6.3 ± 0.6 bcde	3.65 ± 0.31 ab	1.20 ± 0.19 abc	0.31 ± 0.14 abcde
'Torrontés'	**TO**	226.8 ± 12.0 fghi	4.4 ± 0.7 abc	3.66 ± 0.26 abc	1.5 ± 0.3 abcd	6.3 ± 0.5 bcde	5.27 ± 0.80 cd	1.46 ± 0.33 abc	0.24 ± 0.04 abcde
'Treixadura'	**TR**	227.1 ± 12.6 ghi	4.8 ± 1.1 abcd	3.69 ± 0.25 bc	2.8 ± 0.7 cdef	5.3 ± 0.6 abcd	4.89 ± 1.07 bcd	1.16 ± 0.35 abc	0.54 ± 0.21 defg
Significance		***	*	ns	***	*	*	ns	*

Values are mean data of the studied vintages. MI-CO: maturation index De Cillis and Odifredi. MI-BS: maturation index Baragiola and Scuppli [45]. T:M: tartaric:malic acid relationship. *, *** and ns indicate significance at $p < 0.05$, $p < 0.0001$ and non-significant differences, respectively. The mean value, SD and different roman letters (a–i), showing significant differences according to Fisher's test ($p < 0.05$), are indicated in bold for each variety.

Table 2. Free volatile compounds (all values are expressed as $\mu g \cdot L^{-1}$ to allow for comparisons among families).

Variety	Alcohols	Acids	Aldehydes	C6	Thiols	Esters	Phenols
AG	712.11 ± 224.62 ab	514.08 ± 20.74 abc	59.00 ± 69.85 a	3845.38 ± 1121.59 cd	<LQ	4.03 ± 6.98 a	44.89 ± 27.79 f
AA	490.79 ± 172.21 a	134.53 ± 118.27 a	57.94 ± 100.35 a	3526.20 ± 5132.01 bcd	<LQ	45.66 ± 58.71 ab	<LQ
BA	717.24 ± 62.41 ab	233.11 ± 173.63 ab	35.87 ± 31.27 a	1029.51 ± 748.36 ab	<LQ	8.66 ± 7.61 a	8.18 ± 14.17 abc
BL	724.21 ± 144.84 ab	628.66 ± 245.70 abc	53.10 ± 50.97 a	1941.89 ± 418.93 abc	7.88 ± 8.11 ab	64.97 ± 67.60 ab	17.34 ± 17.79 abcd
BM	743.33 ± 248.92 ab	227.41 ± 165.33 ab	88.33 ± 152.99 a	1533.00 ± 344.98 abc	3.53 ± 6.11 ab	34.32 ± 28.18 ab	34.01 ± 30.19 bcd
DB	645.85 ± 279.15 ab	476.24 ± 189.32 abc	69.04 ± 35.35 a	1302.37 ± 424.77 ab	2.06 ± 3.57 ab	25.22 ± 21.99 a	32.55 ± 24.61 abcd
FP	854.36 ± 190.39 ab	223.35 ± 265.80 ab	121.13 ± 111.07 a	1513.36 ± 1586.81 abc	30.51 ± 43.14 c	<LQ	11.33 ± 8.43 abcd
GF	856.00 ± 378.88 ab	341.04 ± 215.42 abc	46.94 ± 39.34 a	2686.24 ± 2021.99 abcd	20.17 ± 0.65 bc	142.08 ± 163.96 b	40.81 ± 39.14 cd
GO	588.90 ± 68.03 ab	673.42 ± 400.69 abc	167.50 ± 244.82 a	1402.35 ± 508.50 abc	1.47 ± 2.55 ab	17.35 ± 30.04 a	22.70 ± 23.65 abcd
IT	487.24 ± 39.64 a	326.77 ± 250.60 abc	28.14 ± 39.80 a	894.69 ± 804.19 a	5.18 ± 7.32 ab	1.82 ± 2.58 a	13.08 ± 11.08 abcd
JA	518.45 ± 41.86 ab	674.24 ± 625.07 abc	89.77 ± 35.85 a	1793.43 ± 713.65 abc	<LQ	2.60 ± 3.67 a	26.74 ± 13.54 abcd
LA	612.69 ± 166.60 ab	414.61 ± 230.48 abc	84.26 ± 101.42 a	2109.31 ± 322.15 abcd	4.38 ± 4.77 ab	13.63 ± 23.61 a	5.08 ± 3.82 abc
MBM	750.87 ± 377.54 ab	318.11 ± 255.23 abc	70.46 ± 79.10 a	1787.66 ± 499.76 abc	15.87 ± 27.48 abc	17.26 ± 14.94 a	17.68 ± 30.61 abcd
PA	604.62 ± 39.64 ab	310.10 ± 234.20 ab	123.24 ± 117.61 a	1848.71 ± 821.92 abc	<LQ	47.17 ± 63.05 ab	8.41 ± 8.08 abc
PI	682.40 ± 177.29 ab	214.59 ± 218.64 ab	28.93 ± 43.76 a	1433.46 ± 877.61 abc	5.58 ± 7.64 ab	1.64 ± 2.83 a	17.38 ± 14.72 abcd
PF	575.94 ± 83.30 ab	926.98 ± 345.31 c	109.40 ± 131.85 a	1777.26 ± 907.47 abc	2.56 ± 3.62 ab	<LQ	23.86 ± 24.56 abcd
RA	933.52 ± 512.22 b	653.98 ± 320.71 abc	134.13 ± 164.55 a	4481.14 ± 879.52 d	1.60 ± 2.77 ab	54.75 ± 37.16 ab	2.12 ± 3.67 ab
SI	807.36 ± 103.22 ab	765.41 ± 901.79 bc	68.19 ± 19.34 a	1459.19 ± 401.07 abc	9.29 ± 3.95 ab	124.43 ± 176.97 b	30.20 ± 34.30 abcd
TO	875.23 ± 515.68 ab	517.17 ± 316.40 abc	67.69 ± 50.07 a	734.98 ± 518.34 a	5.86 ± 8.10 ab	1.30 ± 2.26 a	11.01 ± 13.40 abc
TR	621.25 ± 63.31 ab	438.54 ± 441.23 abc	50.57 ± 44.95 a	2314.78 ± 521.47 abcd	<LQ	24.53 ± 36.26 a	10.02 ± 3.78 abc
Significance	ns	ns	ns	ns	ns	ns	ns

Table 2. *Cont.*

Variety	Ketones	Lactones	Terpenes	Norisoprenoids	PAHs	Sesquiterpenes	Total
AG	105.75 ± 53.00 ab	40.53 ± 10.61 abc	17.61 ± 13.68 a	7.96 ± 9.45 a	82.84 ± 18.50 abc	1.76 ± 1.70 ab	5435.95 ± 1204.33 ab
AA	61.02 ± 75.92 a	7.95 ± 13.77 ab	1094.80 ± 1235.11 a	3.23 ± 5.37 a	76.59 ± 66.77 abc	<LQ	5498.69 ± 5481.40 ab
BA	114.19 ± 59.21 ab	18.95 ± 13.99 ab	103.65 ± 141.33 a	63.65 ± 92.05 b	65.39 ± 57.35 abc	0.39 ± 0.67 a	2398.78 ± 761.49 a
BL	100.49 ± 42.31 ab	5.15 ± 7.16 a	863.11 ± 568.20 a	9.91 ± 8.71 a	107.75 ± 56.19 bc	<LQ	4524.45 ± 1137.93 ab
BM	104.64 ± 14.85 ab	37.18 ± 11.49 abc	11.38 ± 4.90 a	<LQ	79.57 ± 27.75 abc	0.57 ± 0.98 a	2897.28 ± 391.11 a
DB	157.26 ± 11.47 ab	80.85 ± 75.28 c	472.50 ± 407.54 a	7.74 ± 8.46 a	76.25 ± 23.27 abc	5.75 ± 9.96 b	3353.68 ± 349.12 ab
FP	96.42 ± 104.41 ab	2.95 ± 4.17 a	1794.81 ± 1034.80 a	11.60 ± 0.85 ab	81.36 ± 26.75 abc	1.13 ± 0.08 ab	4742.31 ± 1893.58 ab
GF	193.63 ± 149.99 b	37.87 ± 8.84 abc	14.38 ± 9.94 a	5.26 ± 2.61 a	45.41 ± 41.34 ab	0.40 ± 0.56 a	4430.24 ± 1851.26 ab
GO	128.26 ± 17.35 ab	29.46 ± 30.03 abc	11.71 ± 8.33 a	10.22 ± 4.46 a	64.31 ± 6.02 abc	2.03 ± 2.37 ab	3119.68 ± 388.29 a
IT	107.26 ± 10.71 ab	2.80 ± 3.97 a	9169.02 ± 6950.02 c	7.58 ± 5.42 a	16.25 ± 22.98 a	<LQ	11,059.83 ± 5863.60 d
JA	132.56 ± 55.45 ab	21.48 ± 20.69 abc	31.94 ± 5.01 a	21.75 ± 12.85 ab	39.25 ± 11.90 ab	<LQ	3352.21 ± 11.15 ab
LA	77.30 ± 57.11 a	34.96 ± 20.32 abc	31.80 ± 12.07 a	6.02 ± 5.48 a	123.53 ± 47.06 c	<LQ	3517.58 ± 691.65 ab
MBM	115.92 ± 19.88 ab	34.72 ± 60.14 abc	6361.37 ± 2412.21 b	9.74 ± 8.63 a	64.38 ± 52.02 abc	<LQ	9564.03 ± 3476.58 cd
PA	143.73 ± 39.93 ab	54.91 ± 71.21 abc	113.44 ± 88.80 a	<LQ	65.59 ± 13.88 abc	<LQ	3319.93 ± 619.17 ab
PI	130.08 ± 11.83 ab	29.17 ± 2.92 abc	3.66 ± 3.73 a	<LQ	88.79 ± 9.07 bc	1.03 ± 1.14 a	2636.72 ± 868.91 a
PF	70.13 ± 81.77 a	40.57 ± 1.65 abc	8.31 ± 4.70 a	5.55 ± 3.13 a	68.95 ± 20.34 abc	<LQ	3609.51 ± 421.77 ab
RA	134.22 ± 25.34 ab	61.90 ± 21.30 abc	16.02 ± 0.97 a	44.68 ± 41.03 ab	96.74 ± 41.11 bc	<LQ	6614.81 ± 1750.04 bc
SI	126.87 ± 79.65 ab	66.00 ± 55.72 bc	23.19 ± 10.84 a	43.01 ± 63.36 ab	87.16 ± 26.05 bc	2.93 ± 2.82 ab	3613.24 ± 1720.19 ab
TO	134.79 ± 33.23 ab	17.23 ± 19.44 ab	31.51 ± 24.50 a	1.12 ± 1.93 a	91.40 ± 41.89 bc	0.65 ± 1.12 a	2489.95 ± 683.05 a
TR	190.39 ± 114.70 b	9.43 ± 12.56 ab	98.26 ± 74.99 a	<LQ	89.44 ± 40.23 bc	1.16 ± 1.60 ab	3848.38 ± 1054.21 ab
Significance	ns	ns	***	ns	ns	ns	**

PAHs: polycyclic aromatic hydrocarbons. LQ = 0.005. **, *** and ns indicate significance at $p \leq 0.01$, $p \leq 0.0001$ and non-significant differences, respectively. Internal: 4 methyl-2-pentanol for C_6 compounds, aldehydes, acids, esters, alcohols, thiols and phenols. 3-Octanol for terpenes, ketones, aromatic hydrocarbons, lactones, norisoprenoids and sesquiterpenes. Mean value, SD and different roman letters (a–d), showing significant differences according to Fisher's test ($p < 0.05$), are indicated in bold for each variety. See Table 1 for variety abbreviations.

Table 3. Glicosidically bound fraction (all values are expressed as µg·L^{-1} to allow for comparisons among families).

Variety	Alcohols	Acids	Aldehydes	C6	Thiols	Esters	Phenols
AG	8441.40 ± 2843.02 a	1109.42 ± 1165.58 ab	226.71 ± 118.36 a	1308.83 ± 702.60 a	16.10 ± 27.89 a	6748.22 ± 6855.12 a	6128.44 ± 5124.32 ab
AA	10,749.58 ± 747.10 a	422.60 ± 731.96 ab	507.66 ± 786.89 a	1931.39 ± 1714.53 ab	<LQ	2072.10 ± 2141.97 a	1648.77 ± 805.69 a
BA	11,342.79 ± 8324.25 a	1235.82 ± 1658.38 abc	170.31 ± 158.45 a	2176.01 ± 1258.25 ab	16.59 ± 17.85 a	3241.36 ± 1802.72 a	10,634.04 ± 9394.24 abc
BL	8022.63 ± 3758.17 a	1059.24 ± 1834.66 ab	42.90 ± 37.24 a	1983.61 ± 614.25 ab	63.33 ± 94.70 ab	3774.35 ± 4643.14 a	40,810.41 ± 65026.25 c
BM	11,640.42 ± 9353.88 a	1620.68 ± 649.07 abc	207.97 ± 206.72 a	1864.39 ± 1842.87 ab	<LQ	4599.40 ± 5907.16 a	484.90 ± 82.02 a
DB	14,588.54 ± 2141.92 a	376.80 ± 456.55 ab	75.57 ± 130.89 a	2025.54 ± 462.22 ab	37.81 ± 45.05 ab	22,944.46 ± 35344.10 b	5601.72 ± 8421.99 a
FP	10,778.33 ± 5735.54 a	1786.87 ± 2097.68 abc	359.77 ± 340.73 a	2591.51 ± 1404.25 ab	25.94 ± 36.68 ab	887.49 ± 387.96 a	24,988.18 ± 33713.49 abc
GF	6624.45 ± 6762.63 a	1927.61 ± 876.97 abc	78.88 ± 46.34 a	4342.45 ± 5108.22 b	44.38 ± 62.77 ab	13,447.72 ± 18276.97 ab	1575.67 ± 1127.86 a
GO	8063.97 ± 2568.03 a	1833.42 ± 1981.54 abc	4338.09 ± 3760.32 c	943.84 ± 655.62 a	31.94 ± 38.81 ab	1346.81 ± 1009.48 a	6057.74 ± 6176.17 ab
IT	7073.49 ± 4672.08 a	3596.16 ± 2313.47 c	1141.63 ± 1420.57 ab	567.40 ± 65.59 a	<LQ	4107.10 ± 739.46 a	359.00 ± 365.25 a
JA	5840.73 ± 2531.64 a	2104.61 ± 2571.94 abc	3155.19 ± 4346.15 bc	1931.27 ± 1371.58 ab	18.73 ± 26.49 a	4802.03 ± 6495.65 a	7641.54 ± 5763.32 ab
LA	8879.90 ± 2452.78 a	952.01 ± 940.20 ab	92.70 ± 115.00 a	2067.17 ± 859.20 ab	12.52 ± 11.38 a	3740.61 ± 3451.63 a	4805.18 ± 5720.26 a
MBM	10,261.84 ± 3462.48 a	7340.23 ± 835.67 d	4951.25 ± 2925.32 c	524.54 ± 908.53 a	<LQ	2618.40 ± 4150.10 a	2079.51 ± 1537.89 a
PA	10,995.72 ± 7407.55 a	1601.96 ± 154.98 abc	155.76 ± 269.79 a	2793.34 ± 3349.62 ab	30.69 ± 27.11 ab	8293.59 ± 6317.18 ab	10,947.46 ± 17137.58 abc
PI	7509.08 ± 5062.42 a	1096.75 ± 652.84 ab	247.19 ± 156.24 a	1064.89 ± 773.05 a	22.43 ± 38.85 a	4263.72 ± 2694.46 a	7369.07 ± 6303.14 ab
PF	14,777.19 ± 1128.92 a	174.76 ± 76.28 ab	8929.35 ± 1206.32 d	1014.23 ± 40.47 a	105.60 ± 111.19 b	1778.93 ± 1485.39 a	15,443.26 ± 14250.90 abc
RA	13,988.46 ± 6347.67 a	2475.67 ± 2016.78 bc	352.71 ± 123.41 a	3054.76 ± 3122.61 ab	17.70 ± 30.66 a	4430.71 ± 1770.87 a	37,888.25 ± 31798.50 bc
SI	9876.00 ± 871.54 a	1270.35 ± 1454.49 abc	122.57 ± 83.60 a	1661.30 ± 107.67 ab	6.88 ± 11.91 a	5084.20 ± 3042.88 a	7392.87 ± 6484.80 ab
TO	11,178.70 ± 1489.17 a	146.29 ± 141.42 a	470.93 ± 554.09 a	2884.25 ± 416.57 ab	8.01 ± 13.88 a	1410.91 ± 1343.37 a	3515.08 ± 3987.11 a
TR	9837.80 ± 3088.79 a	2538.82 ± 1323.60 bc	287.90 ± 226.44 a	1762.90 ± 727.79 ab	43.77 ± 53.27 ab	4664.96 ± 3167.67 a	6134.49 ± 4369.58 ab
Significance	ns	***	***	ns	ns	ns	ns

Table 3. *Cont.*

Variety	Ketones	Lactones	Terpenes	Norisoprenoids	PAHs	Sesquiterpenes	Total
AG	190.80 ± 11.19 a	9.99 ± 17.30 b	851.14 ± 523.86 a	<LQ	25.32 ± 33.44 abc	41.84 ± 44.18 abc	25,098.20 ± 8941.37 a
AA	214.03 ± 109.88 a	<LQ	10,904.56 ± 6505.24 a	4.38 ± 7.59 ab	128.06 ± 59.77 d	<LQ	28,583.13 ± 8273.43 a
BA	311.59 ± 190.99 ab	<LQ	1472.98 ± 998.58 a	5.34 ± 9.25 ab	50.11 ± 50.22 abcd	16.92 ± 13.91 ab	30,673.85 ± 10,940.27 a
BL	352.98 ± 268.22 ab	<LQ	8110.17 ± 5514.22 a	<LQ	70.71 ± 61.40 abcd	0.83 ± 1.44 a	64,291.16 ± 70105.08 ab
BM	194.60 ± 19.30 a	<LQ	1856.28 ± 1457.11 a	<LQ	53.27 ± 62.62 abcd	105.98 ± 120.60 c	22,627.89 ± 19,109.58 a
DB	228.15 ± 32.74 a	<LQ	6776.31 ± 5167.83 a	1.77 ± 3.07 a	94.47 ± 19.65 bcd	5.16 ± 8.95 a	52,756.30 ± 36,559.87 a
FP	285.79 ± 31.78 ab	<LQ	15,127.22 ± 3121.87 a	<LQ	107.20 ± 21.19 cd	16.00 ± 6.92 ab	56,954.29 ± 46,100.87 ab
GF	128.93 ± 137.16 a	<LQ	1600.09 ± 188.93 a	139.81 ± 110.57 c	63.45 ± 78.46 abcd	47.88 ± 59.69 abc	30,021.32 ± 31,594.27 a
GO	190.70 ± 123.66 a	<LQ	1435.46 ± 770.39 a	22.63 ± 24.94 ab	39.84 ± 42.06 abc	8.68 ± 2.96 a	24,313.12 ± 5657.38 a
IT	173.57 ± 245.46 a	<LQ	98,597.04 ± 99149.28 b	<LQ	69.47 ± 98.24 abcd	42.55 ± 60.17 abc	115,727 ± 90,768 bc
JA	181.52 ± 38.40 a	2.33 ± 3.30 ab	2356.76 ± 1273.43 a	<LQ	55.02 ± 24.70 abcd	10.45 ± 5.73 ab	28,100.20 ± 5058.39 a
LA	296.82 ± 291.33 ab	0.98 ± 1.70 a	1105.64 ± 452.36 a	<LQ	54.27 ± 48.32 abcd	2.91 ± 5.04 a	22,010.72 ± 8129.79 a
MBM	493.99 ± 196.86 ab	<LQ	104834 ± 25440 b	<LQ	97.91 ± 90.39 cd	16.23 ± 28.12 ab	13,3218 ± 25158 c
PA	194.09 ± 148.05 a	<LQ	4132.31 ± 2525.20 a	6.68 ± 9.08 ab	64.11 ± 51.98 abcd	6.75 ± 6.46 a	39,222.46 ± 22,417.89 a
PI	234.56 ± 51.25 a	<LQ	355.10 ± 158.75 a	<LQ	37.80 ± 26.50 abc	1.44 ± 2.49 a	22,202.03 ± 9653.19 a
PF	134.13 ± 44.75 a	<LQ	873.30 ± 785.71 a	46.05 ± 65.12 ab	7.30 ± 10.32 ab	33.71 ± 42.18 abc	43,317.80 ± 14,879.73 a
RA	249.97 ± 128.68 a	<LQ	1081.71 ± 471.70 a	51.46 ± 59.29 b	98.04 ± 30.70 cd	81.18 ± 98.43 bc	63,770.63 ± 27,038.85 ab
SI	192.19 ± 64.02 a	<LQ	1052.09 ± 259.98 a	30.92 ± 35.27 ab	2.62 ± 4.54 a	8.75 ± 8.24 a	26,700.74 ± 2939.59 a
TO	379.17 ± 328.66 ab	<LQ	8603.15 ± 13,645.97 a	<LQ	57.88 ± 6.36 abcd	18.35 ± 16.62 ab	28,672.74 ± 17,653.21 a
TR	710.18 ± 762.90 b	<LQ	2544.15 ± 1016.31 a	<LQ	36.56 ± 35.14 abc	2.73 ± 2.37 a	28,564.26 ± 2535.54 a
Significance	ns	ns	***	**	ns	ns	**

PAHs.: polycyclic aromatic hydrocarbons. LQ = 0.005. **, *** and ns indicate significance at $p \leq 0.01$, $p \leq 0.0001$ and non-significant differences, respectively. Internal: 4 methyl-2-pentanol for C_6 compounds, aldehydes, acids, esters, alcohols, thiols and phenols. 3-Octanol for terpenes, ketones, aromatic hydrocarbons, lactones, norisoprenoids and sesquiterpenes. Mean value, SD and different roman letters (a–d), showing significant differences according to Fisher's test ($p < 0.05$), are indicated in bold for each variety. See Table 1 for variety abbreviations.

3.3.1. Free Volatile Fraction

Regarding the aromatic profile, which provides the contribution of each family to the global aromatic potential of each variety, it can be seen in Figure 1 that C_6, acids, ketones, PAHs and thiol compounds appeared mostly in higher percentages in the free fraction. Lactones and norisoprenoids were detected almost exclusively in the free form, with some exceptions, such as in 'Garrido Fino', with a higher percentage of norisoprenoids in its glycosidic fraction. It can also be noticed that there was almost no presence of phenols or esters in their free fraction, contrary to the glycosidic one.

Significant differences between varieties are shown in Table 2 for terpenes, with 'Pirixileira' showing the lowest mean value (3.66 µg·L^{-1}), while 'Italia' showed the highest one (9169 µg·L^{-1}). The mean content of total free volatiles also showed significant differences, with 'Italia' being the variety showing the highest mean value (11,059 µg·L^{-1}), while 'Batoca' showed the lowest one (2398 µg·L^{-1}). It is considered that the high deviation between the data obtained in the different years of the study is what has prevented us from obtaining greater statistical significance between families of compounds, except for terpenes, being, therefore in this fraction of aromatic compounds and in this specific study, the family that will allow us to obtain clear differentiation between varieties together with the mean total free volatile content.

Terpenes are the compounds of grape-origin that mainly determine the characteristic wine aroma and muscat wines, with linalool, geraniol, hotrienol, nerol and *α*-terpineol being some of the most extended terpenes in muscat and other aroma-related grapes. Their different compositions among varieties could be used for varietal differentiation [49]. Nevertheless, their chemical structure is highly variable reason why there is a broad terpene diversity, being monoterpenoids, sesquiterpenoids and diterpenoids only found in determined species [50]. In this study, they were detected in their free form in every variety studied but in very different contents, with the olfactory impact of these compounds being synergistic [51]. As it could be easily appreciated in Figure 1, those varieties with higher percentages were 'Albilla do Avia', 'Branco Lexítimo', 'Dona Branca', 'Fernão Pires', 'Italia' and 'Moscatel de Bago Miúdo', which also showed similar terpene percentage contributions in their glycosidic fraction. Of these, 'Italia' and 'Moscatel de Bago Miúdo' differed statistically from the rest in terms of their contents, with 9169 µg·L^{-1} and 6361 µg·L^{-1}, respectively, with linalool being the major terpene detected (Supplementary Table S2), which could contribute to citric, sweet, and floral aromas [52]. Compared to them, 'Pirixileira' and 'Planta Fina' were the varieties that showed the lowest contents (3.66 and 8.31 µg·L^{-1} respectively), with m-cymene, a geometric p-cymene isomer [53], and *β*-cyclocitral, with minty as descriptor [54], as the major terpenes detected.

Sesquiterpenes are C_{15} terpene compounds enriched in grapes' external layer wax [55]. More than 60 sesquiterpenes were identified in grapes [56] and could be used for varietal discrimination [57]. Their biological functions are thought to be involved in grape defense strategies against biotic and abiotic stress factors, as well as in grape quality [58]. In this study, they were detected in 11 of the 20 varieties studied in their free form, having a low percentage contribution to their aromatic profiles, as shown in Figure 1, involving up to 1% of the total profile. 'Dona Branca' showed the highest average value.

C_{13}-norisoprenoids are interesting compounds, as their odoriferous properties are well-known to contribute to the perception of wine's aroma, derived from the breakage of carotenoids [51]. In this research, they were detected almost exclusively in the free aromatic form of almost every variety studied (Figure 1), except in 'Blanca de Monterrei', 'Palomino', 'Pirixileira' and 'Treixadura'. 'Batoca', 'Ratiño' and 'Silveiriña' were those varieties with the highest content, with *β*-damascenone as the major content one in the last two varieties, suggested as a fruity aroma-enhancer in some studies [59]. Other studies identify it as a high-floral-intensity compound [60].

Organic acids together with other families, such as terpenoids, thiol precursors and esters, among others, are one of the most important families of aromatic compounds [61]. Figure 1 reflects this previous information, with acids appearing in both free and glycosidic

fractions in every variety studied, representing up to 25% of the free aromatic profile of the varieties studied, with 'Planta Fina' being the variety in which they have the greatest contribution in their free form, followed by 'Godello', 'Jarrosuelto' and 'Torrontés', with up to 20%. They are characterized by fatty and cheesy smells, and they are partly related to the generic vinous character of the wine [62]. As it was previously mentioned, 'Planta Fina' stood out based on its high content, with hexanoic acid as the major compound, such as for 'Agudelo', 'Albilla do Avia', 'Batoca', 'Branco Lexitimo', 'Fernão Pires', 'Garrido Fino', 'Godello', 'Lado', 'Pirixileira' and 'Ratiño'.

C_6 compounds are broadly described as having characteristic odors of green, grass and vegetation. C_6 alcohols derived from grapes were tested to serve as varietal markers [63]. Six carbon alcohols and aldehydes have been identified as the major compounds in the free fraction of several Spanish varieties [64], as has also been observed in this study, being detected as the main contributing family in the free aromatic profile of every variety studied, accounting for more than 40% of the free aromatic fraction, except for 'Fernão Pires', 'Italia', 'Moscatel de Bago Miúdo' and 'Torrontés' (Figure 1). Concentrations ranged from 4481 $\mu g \cdot L^{-1}$ in 'Ratiño' to 734 $\mu g \cdot L^{-1}$ in 'Torrontés'. Hexanol and 2-hexenal were two of the major compounds detected with descriptors, such as resin, flower, green and apple and green in the second compound [40,54], being also some of the major compounds found in other studies [26], being classified in that work as alcohols and aldehydes, resulting these families the major compounds detected in that study.

Alcohols were detected and semi-quantified in high contents as have already been found in other studies [65]. In this study, similar percentage contributions to the aromatic profile of each studied variety were detected in both free and glycosidic fractions, with a slightly higher percentage in the glycosidic fraction in every variety (Figure 1). 'Albilla do Avia' and 'Italia' could be highlighted for having the lowest amounts of alcohol compared to the rest of varieties studied, with 2-ethyl-1-hexanol, as the major compound in every variety, being also the major compound identified in the free fraction of varieties studied in [36,37], characterized by having fresh, floral, citrus, and sweet aroma descriptors [66].

Aldehydes are organic compounds that are very commonly found in food or flavoring agents and usually have a low odor threshold [67]. In this study, they were detected in lower quantities compared with C_6 compounds and alcohols, with 'Godello' showing the highest content (167 $\mu g \cdot L^{-1}$) and 'Italia' and 'Pirixileira' the lowest one (28 $\mu g \cdot L^{-1}$). They also had a low percentage contribution to the free, aromatic fraction, with a maximum of 5% in the 'Godello' free aromatic profile (Figure 1).

Esters are one of the main aromatic family compounds related to the fruity aroma of wines [62]. In this study, it could be seen that they were detected in low contents in almost every variety, also contributing at a very low percentage to their free aromatic profile (Figure 1), with 'Garrido Fino' and 'Silveiriña' being those varieties that showed the highest values (142 and 124 $\mu g \cdot L^{-1}$ respectively). 'Albilla do Avia', despite having a lower concentration value than those varieties previously mentioned, showed a similar contribution percentage to its free aromatic profile as the latter ones.

Phenols were detected in free form in every variety, except in 'Albilla do Avia', where they were not detected in any vintage. 'Agudelo' was the variety with the highest values with 44 $\mu g \cdot L^{-1}$, while 'Ratiño' was the one showing the lowest one, with 2 $\mu g \cdot L^{-1}$, and they were only detected in one vintage. However, they were found in low contents and contributed as a low percentage to their free aromatic profiles (Figure 1).

Ketones were detected in all varieties and years of study, mainly in the free aromatic fraction. 'Garrido Fino' and 'Treixadura' showed the highest values (193 and 190 $\mu g \cdot L^{-1}$ respectively) but contributed to a higher percentage in the free aromatic profile of 'Batoca', 'Pirixileira' and 'Torrontés' compared to that of 'Treixadura' (Figure 1). On the contrary, 'Albilla do Avia' showed the lowest value (61 $\mu g \cdot L^{-1}$). Methyl isobutyl ketone was the major compound detected in all the studied varieties, identified in other varieties and other kind of foods, such as lemon juice, papaya, or ginger [68].

Referring to PAHs, 'Lado' stood out for its highest value (123 µg·L^{-1}) and 'Italia' with the lowest one (16 µg·L^{-1}). However, they did not contribute, with more than 4%, to the free aromatic profile of any of the studied varieties (Figure 1). Hemimellitene and mesitylene were those major compounds in almost every variety, being isomers of trimethylbenzene, as has already been shown in previous studies [36,37].

Lactones were detected in every variety, mostly in their free form. 'Dona Branca' stood out for the highest content detected (80 µg·L^{-1}), with furaneol as the major compound, which is related to the strawberry-like aroma, generally found in higher amounts in some *Vitis lambrusca* or hybrid varieties [69].

The thiol content depends on the variety, and some of them are important providers of varietal wine aroma odors [70]. In this study, they were detected in their free form in 14 out of 20 varieties studied in low contents, not being detected in every year. Major compounds detected were 2-methyl- 2-undecanethiol and 3-(methylthio)-nonanal.

3.3.2. Glycosidically Bound Volatile Fraction

Regarding the glycosidically bound aromatic profile, which provides the contribution of each family to the global aromatic precursor potential, it can be seen in Figure 1 that terpenes, alcohols and sesquiterpenes contributed in higher percentages to the bound fraction than they did to the free one in most of the varieties. Phenols and esters contributed almost exclusively to the bound aromatic form. It could also be noticed that there was almost no presence or contribution of lactones in the bound fraction, contrary to what was observed in the free one. The alcohol family is the one with the highest percentage contribution, except for 'Fernão Pires', 'Italia' and 'Moscatel de Bago Miúdo', where terpenes were the main volatile family, 'Branco Lexítimo', 'Jarrosuelto' and 'Ratiño' in which phenols were the major family and 'Garrido Fino' in which esters were the major one. These previous families were considered to have a higher contribution to the bound aromatic potential of studied varieties.

Significant differences among varieties are shown in Table 3 for the content of acids, aldehydes, terpenes, norisoprenoids and the total glicosidically bound fraction content, with the mean value of the latter parameter ranging from 22,010 µg·L^{-1} for 'Lado' to 133,218 µg·L^{-1} for 'Moscatel de Bago Miúdo'.

As it was previously mentioned with the free fraction, terpenes were detected every year in every studied variety, showing higher contents than in their corresponding free fraction, despite having a similar percentage contribution in both fractions. It is also worth mentioning that 'Moscatel de Bago Miúdo', 'Italia', 'Fernão Pires' and 'Albilla do Avia' could be highlighted for their higher contents. The first two ones also differed statistically from the rest of varieties with mean values of 98,597 and 104,834 µg·L^{-1}. 'Pirixileira' and 'Planta Fina' together with 'Agudelo' are those varieties in which a lower glycosidic terpene content was detected, which also corresponded to the free fraction, with 355, 873 and 851 µg·L^{-1}, respectively, with dihycitronellol being the major compound (Supplementary Table S2). As it could also be seen in Supplementary Table S2, linalool in 'Italia' and cis geraniol in 'Moscatel de Bago Miúdo' were the major terpenes.

In contrast to the free fraction, sesquiterpenes were detected in their bound form in 19 out of the 20 varieties studied, except for 'Albilla do Avia', with trans-Z-β-bisabolene epoxide and patchoulane as two of the major compounds detected. Patchoulane was detected in species, such as *Eugenia klotzschiana* O. Berg, which is considered to have important nutritional value [71], or in the essential oil of *Pogostemon cablin* (Blanco) Benth, being referred as an important anti-inflammatory activity compound [72]. Trans-Z-β-bisabolene epoxide, already identified in previous studies [36,37], was also identified in 'Merlot' wines [73]. 'Blanca de Monterrei' and 'Garrido Fino' were those varieties that showed a higher percentage contribution to the bound aromatic profile, involving no more than 1% (Figure 1).

C$_{13}$-norisoprenoids showed significant differences among the different varieties studied in their bound fraction, being detected only in 9 out of 20 varieties studied: 'Albilla do Avia', 'Batoca', 'Dona Branca', 'Garrido Fino', 'Godello', 'Palomino', 'Ratiño' and

'Silveiriña', with dihydro-β-ionol, with a woody–flowery and camphoraceous odor [74] and β-damascenone with fruity, balsamic and tobacco descriptors [75], as major compounds (Supplementary Table S2).

Significant differences were found in the bound fraction of organic acids among varieties, with a range of mean values of 7340 µg·L^{-1} in 'Moscatel de Bago Miúdo' and 146 µg·L^{-1} in 'Torrontés', with nonanoic acid being the major compound in almost every variety. In general, they have a more important contribution to the free aromatic fraction, except in 'Blanca de Monterrei', 'Garrido Fino', 'Italia' and 'Moscatel de Bago Miúdo', in which the bound fraction percentage contribution was higher (Figure 1).

C$_6$ compounds were detected in every variety, with 'Garrido Fino' showing the highest content (4342 µg·L^{-1}) and the highest percentage contribution to its bound aromatic profile, together with 'Torrontés', with around 12%. 'Italia' and 'Moscatel de Bago Miúdo' showed the lowest concentrations (567 and 524 µg·L^{-1} respectively) contributing to these last ones, in less than 1%, to their bound aromatic profile (Figure 1).

The alcohol family was the most contributing one to the bound aromatic profile for most of the studied varieties, with almost 40% (Figure 1). Concentration values ranged from 6624 µg·L^{-1} in 'Garrido Fino' up to 14,777 µg·L^{-1} in 'Planta Fina'. Benzyl alcohol and phenylethyl alcohol were the major alcohols detected, as shown in previous studies [36,37], with sweet and fruity descriptors for the first [76] and honey and rose ones for the second compound [77].

Aldehydes showed significant differences among varieties, with three varieties showing much higher values than the rest, 'Planta Fina' with 8929 µg·L^{-1}, 'Moscatel de Bago Miúdo' with 4951 µg·L^{-1} and 'Godello' with 4338 µg·L^{-1} in front of 'Branco Lexítimo', in which only 42 µg·L^{-1} was detected. Regarding the bound aromatic profile, 'Godello' and 'Planta Fina' must be highlighted for their higher percentage contributions (Figure 1). Benzaldehyde was found to be one of the major compounds in most varieties studied, characterized for providing a bitter almond taste in wine [51].

Esters showed much higher contents and percentage contributions in their bound form than in the free one (Figure 1), as was already the case in previous studies with red varieties [37]. No significant differences were observed among varieties. Major compounds; for most of the varieties, were nonanoic acid, methyl ester and salicylic acid, with methyl ester having coconut and wine-like odor descriptors for the first compound [74] and green and minty flavor nuance descriptors for the second one [78].

Phenols, as was observed with the esters, notably increased their content and profile contribution in the bound fraction. It was the aromatic family that contributed most to the bound profile for 'Branco Lexítimo', 'Jarrosuelto' and 'Ratiño', with almost 34, 29 and 52% of their bound profile, respectively (Figure 1). Phenol, 2,4-bis(1,1-dimethylethyl)- was the major compound for almost every variety studied. It is a kind of m-cresol, for which its appearance in wines has been related, in some studies, to the fire–smoke exposition of grapes [79].

Ketones were identified in every studied variety, as shown with the free form, with benzophenone being the major ketone among 11 out of the 20 varieties studied, with descriptors, such as rose, balsam, metallic or powdery geranium [80].

PAHs were also detected in every studied variety with a range of values of 2.2 µg·L^{-1} in 'Silveiriña' up to 128 µg·L^{-1} in 'Albilla do Avia'. As in the free fraction, hemimellitene, mesitylene and psi-cumene were the major PAHs detected.

Lactones were only detected in three varieties, 'Agudelo' in 2016, 'Jarrosuelto' and 'Lado' in 2017, with δ dodecalactone as the major one in these last two varieties, with a coconut descriptor and previously detected in wines elaborated with varieties, such as 'Airén', 'Albillo Dorado' or 'Montonera del Casar' [81].

Finally, thiols were detected in every variety in the bound form, except in 'Albilla do Avia', 'Blanca de Monterrei', 'Italia' and 'Moscatel de Bago Miúdo', not being detected in every studied year. As was shown for the free form, 2-methyl- 2-undecanethiol was the

major compound in almost every variety studied and only contributed to the aromatic profile in very small percentages, less than 0.5 % (Figure 1).

3.3.3. Variety Classification: Aromatic Relationship PCAs

Two principal component analyses (PCAs) were carried out, with the free and glycosidic bound aromatic profile, as we did in previous studies [36,37], in terms of testing their capacity to differentiate among varieties, to see how they place varieties according to their aromatic similarities and to check if they maintain any relationship with their corresponding genetic–geographic groups. Percentages were used instead of concentrations, since according to different studies, such as those of Dimitrovska et al., Mattivi et al., Pomar et al. and Yang et al. [82–85], they observed quantitative variations in studied compounds due to non-genetic factors, whereas the qualitative composition remained consistent.

Mean data of the studied vintages were used, since our main objective was to establish the potential relationships between varieties without considering the influence of the year factor on their distribution.

Figure 2 shows the free volatile aromatic profile PCA.
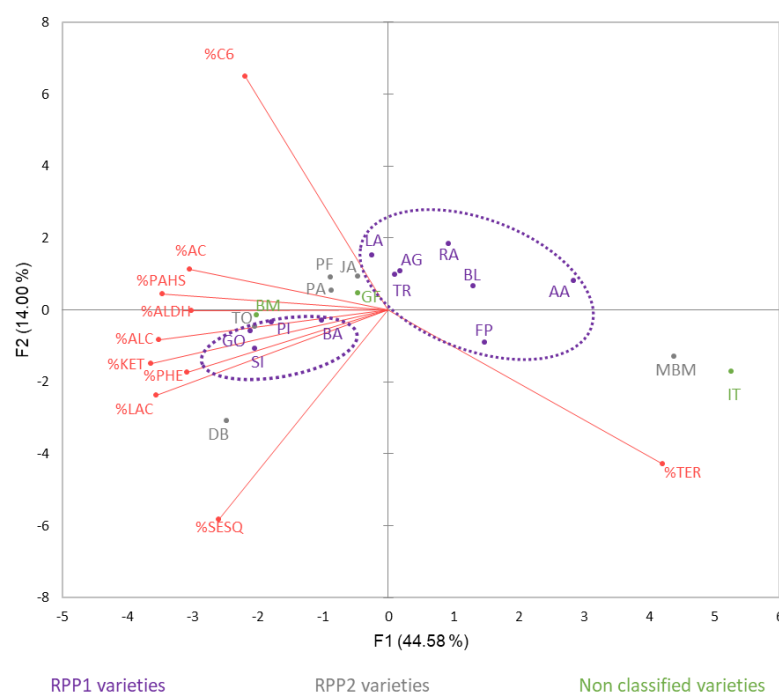
Figure 2. Principal component analysis of the free-aromatic-fraction profile (percentage) of grapes. AC: acids, KET: ketones, PAHs: polycyclic aromatic hydrocarbons, ALC: alcohols, ALDH: aldehydes, C6: C_6 compounds, SESQ: sesquiterpenes, PHE: phenols, TER: terpenes, LAC: lactones. AG: 'Agudelo', AA: 'Albilla do Avia', BA: 'Batoca', BL: 'Branco Lexítimo', BM: 'Blanca de Monterrei', DB: 'Dona Blanca', FP: 'Fernão Pires', GF: 'Garrido Fino', GO: 'Godello', IT: 'Italia', JA: 'Jarrosuelto', LA: 'Lado', MBM: 'Moscatel de Bago Miudo', PA: 'Palomino', PI: 'Pirixileira', PF: 'Planta Fina', RA: 'Ratiño', SI: 'Silveiriña', TO: 'Torrontés', TR: 'Treixadura'. Varieties in purple correspond to the Reconstructed Population (RPP) RPP1; varieties in grey correspond to RPP2, and varieties in green correspond to non-classified varieties in the genetic–geographical structure stablished by Díaz-Losada et al. [34].

The first two principal components (Figure 2) accounted for 58.6% of the total variance (14% and 44.6%), showing good varietal differentiation. Thiols, esters and C_{13}-norisoprenoids were left out of the analysis as they contributed at very low percentages to explaining the variance. The aromatic clustering coincided to some extent with the genetic–geographic grouping, with varieties included in the RPP1 [34] aromatically clustered in two main groups: one embracing 'Batoca', 'Pirixileira', 'Godello' and 'Silveiriña', mainly character-

ized by their content of ketones, alcohols, phenols and lactones, and a second one, which included 'Agudelo', 'Albilla do Avia', 'Branco Lexítimo', 'Fernão Pires', 'Lado', 'Ratiño' and 'Treixadura'. From those varieties included in the RPP1, considered as northwestern Iberian Peninsula varieties, 'Albilla do Avia' and 'Fernão Pires' could stand out aromatically, for having a higher terpene content and being the closest to 'Italy' and 'Moscatel de Bago Miúdo', two varieties noted for being highly terpenic and therefore serving as a reference for aromaticity.

Regarding RPP2 varieties, 'Jarrosuelto', 'Palomino' and 'Planta Fina' were aromatically grouped fairly close in the first quadrant, and all RPP2 varieties, together with 'Torrontés' and 'Dona Branca', except for 'Moscatel de Bago Miudo', were classified in the negative side of F1. It seems that the aromatic profile clustering coincided to some degree with the genetic–geographic one.

Figure 3 shows the PCA based on the glicosidically bound volatile aromatic profile.
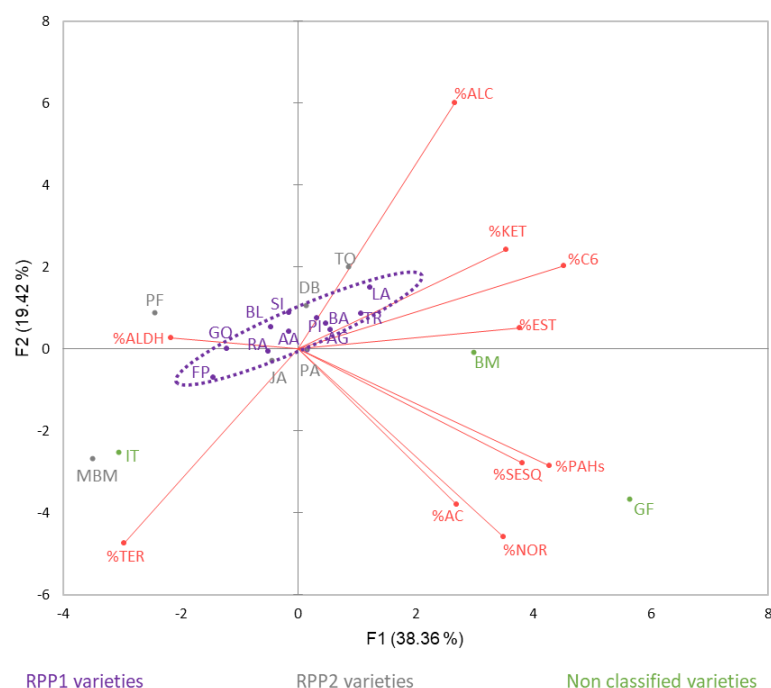
Figure 3. Principal component analysis of glicosidically bound aromatic fraction profile (percentage) of grapes. NOR: C_{13}-norisoprenoids, AC: acids, EST: esters, KET: ketones, PAHs: polycyclic aromatic hydrocarbons, ALC: alcohols, ALDH: aldehydes, C6: C_6 compounds, SESQ: sesquiterpenes, TER: terpenes, AG: 'Agudelo', AA: 'Albilla do Avia', BA: 'Batoca', BL: 'Branco Lexítimo', BM: 'Blanca de Monterrei', DB: 'Dona Blanca', FP: 'Fernão Pires', GF: 'Garrido Fino', GO: 'Godello', IT: 'Italia', JA: 'Jarrosuelto', LA: 'Lado', MBM: 'Moscatel de Bago Miudo', PA: 'Palomino', PI: 'Pirixileira', PF: 'Planta Fina', RA: 'Ratiño', SI: 'Silveiriña', TO: 'Torrontés', TR: 'Treixadura'. Varieties in purple correspond to the Reconstructed Population (RPP) RPP1; varieties in grey correspond to RPP2, and varieties in green correspond to non-classified varieties in the genetic–geographical structure stablished by Díaz-Losada et al. [34].

This second PCA (Figure 3) also achieved good varietal differentiation, although in this case, varieties were closer than in the previous one (Figure 2). The first two principal components accounted for 57.8% of the total variance (19.4% and 38.4%). Thiols, lactones, and phenols were left out of the analysis as they contributed at very low percentages to explaining the variance. Based on their aromatic profile, RPP1 varieties were all grouped together but with three RPP2 varieties, 'Dona Branca', 'Jarrosuelto' and 'Palomino', quite near and hardly distinguishable exclusively from an aromatic point of view. From those varieties included in the RPP1, as was the case for the free fraction, 'Fernão Pires' appeared

closer to the muscat reference varieties, being probably the most aromatic variety among studied ones from the northwestern Iberian Peninsula.

Regarding RPP2 varieties, two pairs, 'Torrontés' with 'Dona Branca' and 'Palomino' with 'Jarrosuelto', seemed to be more similar to each other appearing closer in the PCA, while 'Planta Fina' and 'Moscatel de Bago Miúdo' appeared further among them and from the rest.

Based on the results obtained, the aromatic profile could be used to discriminate between grapevine varieties. At least in this study, the free aromatic fraction profile better distinguished among varieties than the glycosidic one. The aromatic profile seems to have a certain chemotaxonomic approach since it places some varieties that are grouped in the same genetic–geographic group closer on the basis of their molecular characterization, something already seen in our previous studies [36,37] and which was similarly tested by Ŝikuten et al. [26], in which they were able to differentiate genetic–geographically grouped varieties based on their volatile profiles.

4. Conclusions

This study, which is part of a wider aromatic and phenolic study, shows the aromatic profile of 20 white grapevine varieties from the EVEGA germplasm bank. Firstly, the aromatic profile allowed us to differentiate among varieties, being better achieved with the free profile than with the glycosidic one.

Secondly, both aromatic profiles placed some varieties that belong to the same genetic–geographic group or reconstructed population (RPP) closer based on their molecular characterization [34], which could be a promising chemotaxonomic approach prospective.

To conclude, the results obtained confirmed that there are some minor varieties, in some cases included in few community wine PODs, such as 'Branco Lexítimo' and 'Albilla do Avia', or even varieties not included in the community register, such as 'Fernão Pires', broadly used for the elaboration of quality white wines in Portugal, which could provide great aromatic potential, richness and diversity to the geographic area of study, even more than some other varieties already included in the community's PODs. This has already been observed in previous studies with some red varieties.

Despite the promising prospects of the grape aromatic characterization, first for being used as a chemotaxonomic tool and secondly to reveal the aromatic potential of those minor varieties, further research is desirable to firmly correlate genetic–geographic and volatile metabolism clustering and confirm the varietal aromatic potential.

References

1. Resco, P.; Iglesias, A.; Bardaji, I.; Sotés, V. Exploring adaptation choices for grapevine regions in Spain. *Reg. Environ. Chang.* **2016**, *16*, 979–993. [CrossRef]
2. Vilanova, M.; Genisheva, Z.; Masa, A.; Oliveira, J.M. Correlation between volatile composition and sensory properties in Spanish Albariño wines. *Microchem. J.* **2010**, *95*, 240–246. [CrossRef]
3. Vilanova, M.; Campo, E.; Escudero, A.; Graña, M.; Masa, A.; Cacho, J. Volatile composition and sensory properties of *Vitis vinifera* red cultivars from Northwest Spain: Correlation between sensory and instrumental analysis. *Anal. Chim. Acta* **2012**, *720*, 104–111. [CrossRef] [PubMed]
4. Vilanova, M.; Oliveira, J.M.; Rivas, R.; Alonso, J.C.; Martínez-Zapater, J.M.; Ibáñez, J.; Cacho, J. Primera parte: El sector vitivinícola gallego en el siglo XXI. El triunfo de las variedades de cultivo tradicional. In *El Potencial Aromático de las Variedades de Vid Cultivadas en Galicia*; Xunta de Galicia-Consellería de Medio Rural: Santiago de Compostela, Spain, 2017; Available online: https://libraria.xunta.gal/sites/default/files/downloads/publicacion/libro_vid_i_castellano.pdf (accessed on 10 December 2022).
5. Rocha, S.M.; Coutinho, P.; Coelho, E.; Barros, A.S.; Delgadillo, I.; Coimbra, M.A. Relationships between the varietal volatile composition of the musts and white wine aroma quality. A four-year feasibility study. *LWT -Food Sci. Technol.* **2010**, *43*, 1508–1516. [CrossRef]
6. Genisheva, Z.; Oliveira, J.M. Monoterpenic characterization of white cultivars from Vinhos Verdes appellation of origin (North Portugal). *J. Inst. Brew.* **2009**, *115*, 308–317. [CrossRef]
7. Vineyard Enquises. Ministerio de Agricultura, Pesca y Alimentación. *(MAPA).* Available online: https://www.mapa.gob.es/es/estadistica/temas/estadisticas-agrarias/agricultura/encuestas-de-vinedo/ (accessed on 2 November 2022).
8. Estratexia de Dinamización Económica, Territorial e Turística das Comarcas Vitivinícolas de Galicia. Available online: https://mediorural.xunta.gal/sites/default/files/publicacions/portada/ESTRATEXIA-DINAMIZACION_V9_1_sep.pdf (accessed on 2 November 2022).
9. Vierra, G. Pretenders at the Table—Are table wines no longer food friendly? *Wine Bus. Mon.* **2004**, *11*, 17–21.
10. Antolín, M.C.; Toledo, M.; Pascual, I.; Irigoyen, J.J.; Goicoechea, N. The Exploitation of Local Vitis vinifera L. Biodiversity as a Valuable Tool to Cope with Climate Change Maintaining Berry Quality. *Plants* **2021**, *10*, 71. [CrossRef]
11. Carbonell-Bejerano, P.; de Carvalho, L.C.; Eiras Dias, J.E.; Martinez-Zapater, J.M.; Amancio, S. Chapter 15: Exploiting *Vitis* genetic diversity to manage with stress. In *Grapevine in a Changing Environment: A Molecular and Ecophysiological Perspective*; Gerós, H., Chaves, M.M., Gil, H.M., Delrot, S., Eds.; John Wiley & Sons, Ltd.: Hoboken, NJ, USA, 2015; pp. 347–380. [CrossRef]
12. Lacombe, T. Contribution á L'étude de L'histoire Evolutive de la Vigne Cultivée (Vitis vinifera L.) par L'analyse de la Diversité Génétique Neuter et de Genes D'intérêt, Centre International d'Etudes Supérieures en Sciences Agronomiques, Montpellier SupAgro, 18 December 2012. Available online: https://www.supagro.fr/theses/extranet/12-0040_Lacombe.pdf (accessed on 1 November 2022).
13. Wolkovich, E.M.; de Cortázar-Atauri, I.G.; Morales-Castilla, I.; Nicholas, K.A.; Lacombe, T. From Pinot to Xinomavro in the world's future wine-growing regions. *Nat. Clim. Chang.* **2018**, *8*, 29–37. [CrossRef]
14. García-Muñoz, S.; Muñoz-Organero, G.; Fernández-Fernández, E.; Cabello, F. Sensory characterisation and factors influencing quality of wines made from 18 minor varieties (*Vitis vinifera* L.). *Food Qual. Prefer.* **2014**, *32*, 241–252. [CrossRef]
15. Álvarez, M.G.; González-Barreiro, C.; Cancho-Grande, B.; Simal-Gándara, J. Relationships between Godello white wine sensory properties and its aromatic fingerprinting obtained by GC–MS. *Food Chem.* **2011**, *129*, 890–898. [CrossRef]
16. Duchêne, E.; Huard, F.; Dumas, V.; Scheneider, C.; Merdinoglu, D. The challenge of adapting grapevine varieties to climate change. *Clim. Res.* **2010**, *41*, 193–204. [CrossRef]
17. Duchêne, E.; Butterlin, G.; Dumas, V.; Merdinoglu, D. Towards the adaptation of grapevine varieties to climate change: QTLs and candidate genes for developmental stages. *Theor. Appl. Genet.* **2012**, *124*, 623–635. [CrossRef] [PubMed]
18. Belliveau, S.; Smit, B.; Bradshaw, B. Multiple exposures, and dynamic vulnerability: Evidence from the grape industry in the Okanagan Valley, Canada. *Glob. Environ. Chang.* **2006**, *16*, 364–378. [CrossRef]
19. Petronilho, S.; Lopez, R.; Ferreira, V.; Coimbra, M.A.; Rocha, S.M. Revealing the Usefulness of Aroma Networks to Explain Wine Aroma Properties: A Case Study of Portuguese Wines. *Molecules* **2020**, *25*, 272. [CrossRef]
20. Vilanova, M.; Escudero, A.; Graña, M.; Cacho, J. Volatile composition and sensory properties of North West Spain white wines. *Food Res. Int.* **2013**, *54*, 562–568. [CrossRef]
21. Wason, E.; Hunter, M. Genetic variation in plant volatile emission does not result in differential attraction of natural enemies in the field. *Oecologia* **2014**, *174*, 479–491. [CrossRef]
22. Yang, S.; Meng, Z.; Fan, J.; Yan, L.; Yang, Y.; Zhao, Z. Evaluation of the volatile profiles in pulp of 85 apple cultivars (*Malus domestica*) by HS–SPME combined with GC–MS. *J. Food Meas. Charact.* **2021**, *15*, 4215–4225. [CrossRef]

23. Bouzas-Cid, Y.; Trigo-Córdoba, E.; Falqué, E.; Orriols, I.; Mirás-Avalos, J.M. Influence of supplementary irrigation on the amino acid and volatile composition of Godello wines from the Ribeiro Designation of Origin. *Food Res. Int.* **2018**, *111*, 715–723. [CrossRef]

24. Alem, H.; Rigou, P.; Schneider, R.; Ojeda, H.; Torregrosa, L. Impact of agronomic practices on grape aroma composition: A review. *J. Sci. Food Agric.* **2019**, *99*, 975–985. [CrossRef]

25. Coletta, A.; Toci, A.T.; Pati, S.; Ferrara, G.; Grieco, F.; Tufariello, M.; Crupi, P. Effect of Soil Management and Training System on Negroamaro Wine Aroma. *Foods* **2021**, *10*, 454. [CrossRef]

26. Šikuten, I.; Štambuk, P.; Tomaz, I.; Marchal, C.; Kontić, J.K.; Lacombe, T.; Maletić, E.; Preiner, D. Discrimination of genetic and geographical groups of grape varieties (*Vitis vinifera* L.) based on their volatile organic compounds. *Front. Plant Sci.* **2022**, *13*, 942148. [CrossRef] [PubMed]

27. Chitarrini, G.; Lazazzara, V.; Lubes, G.; Agnolet, S.; Valls, J.; von Lutz, H.; Brunner, K.; Lozano, L.; Guerra, W.; Ciesa, F.; et al. Volatile profiles of 47 monovarietal cloudy apple juices from commercial, old, red-fleshed and scab-resistant apple cultivars. *Eur. Food Res. Technol.* **2021**, *247*, 2739–2749. [CrossRef]

28. Beghè, D.; Cirlini, M.; Beneventi, E.; Miroslav, Č.; Tatjana, P.; Ganino, T.; Petruccelli, R.; Dall'Asta, C. Volatile profile of Italian and Montenegrine pomegranate juices for geographical origin classification. *Eur. Food Res. Technol.* **2021**, *247*, 211–220. [CrossRef]

29. Laucou, V.; Launay, A.; Bacilieri, R.; Lacombe, T.; Adam-Blondon, A.-F.; Berard, A.; Chauveau, A.; de Andrés, M.T.; Hausmann, L.; Ibáñez, J.; et al. Extended diversity analysis of cultivated grapevine *Vitis vinifera* with 10 K genome-wide SNPs. *PLoS ONE* **2018**, *13*, e0192540. [CrossRef] [PubMed]

30. Cunha, J.; Ibáñez, J.; Teixeira-Santos, M.; Brazão, J.; Fevereiro, P.; Martínez-Zapater, J.M.; Eiras-Dias, J.E. Genetic Relationships Among Portuguese Cultivated and Wild *Vitis vinifera* L. *Germplasm. Front. Plant Sci.* **2020**, *11*, 127. [CrossRef]

31. Díaz-Losada, E.; Salgado, A.T.; Ramos-Cabrer, A.M.; Río Segade, S.; Diéguez, S.C.; Pereira-Lorenzo, S. Twenty microsatellites (SSRs) reveal two main origins of variability in grapevine cultivars from Northwestern Spain. *Vitis* **2010**, *49*, 55–62. [CrossRef]

32. Díaz-Losada, E.; Salgado, A.T.; Ramos-Cabrer, A.M.; Pereira-Lorenzo, S. Determination of genetic relationships of Albariño and Loureira cultivars with the Caíño group by microsatellites. *Am. J. Enol. Viticult.* **2011**, *62*, 3. [CrossRef]

33. Díaz-Losada, E.; Fernández, I.O.; Martínez, F.R.; Salgado, A.T.; Cabrer, A.R.; Lorenzo, S.P. A Colección de Vides da Estación de Viticultura e Enoloxía de Galicia; Xunta de Galicia-Consellería do Medio Rural: Santiago de Compostela, Spain. 2011. Available online: https://libraria.xunta.gal/sites/default/files/documents/11-0247_0.pdf (accessed on 15 December 2022).

34. Díaz-Losada, E.; Salgado, A.T.; Ramos-Cabrer, A.M.; Díaz-Hernández, B.; Pereira-Lorenzo, S. Genetic and geographical structure in grapevines from northwestern Spain. *Ann. Appl. Biol.* **2012**, *161*, 24–35. [CrossRef]

35. Díaz-Losada, E.; Cortés-Diéguez, S.; Rodríguez-Torres, I.; Mirás-Avalos, J.M.; Orriols-Fernández, I.; Pereira-Lorenzo, S. Characterization of the nearly extinct 'Albilla' cultivar from Galicia and its relationships with other Spanish 'Albillos'. *J. Int. Sci. Vigne Vin.* **2013**, *47*, 261–268. [CrossRef]

36. Díaz-Fernández, Á.; Díaz-Losada, E.; Cortés-Diéguez, S. Approach to the Chemotaxonomic Characterization of Traditional Cultivation Grape Varieties through Their Varietal Aroma Profile. *Foods* **2022**, *11*, 1427. [CrossRef]

37. Díaz-Fernández, Á.; Díaz-Losada, E.; Cortés-Diéguez, S. Diversity among Traditional Minority Red Grape Varieties According to Their Aromatic Profile. *Agronomy* **2022**, *12*, 1799. [CrossRef]

38. Díaz-Fernández, Á.; Díaz-Losada, E.; Moreno, D.; Valdés Sánchez, M.E. Anthocyanin profile of Galician endangered varieties. A tool for varietal selection. *Food Res. Int.* **2022**, *154*, 110983. [CrossRef] [PubMed]

39. Díaz-Fernández, Á.; Díaz-Losada, E.; Vázquez-Arias, A.; Pujol, A.P.; Cardona, D.M.; Valdés-Sánchez, M.E. Non-Anthocyanin Compounds in Minority Red Grapevine Varieties Traditionally Cultivated in Galicia (Northwest Iberian Peninsula), Analysis of Flavanols, Flavonols, and Phenolic Acids. *Plants* **2023**, *12*, 4. [CrossRef]

40. Ferreira, V.; Lopez, R. The Actual and Potential Aroma of Winemaking Grapes. *Biomolecules* **2019**, *9*, 818. [CrossRef] [PubMed]

41. Vitis International Variety Catalogue VIVC. Available online: https://www.vivc.de/ (accessed on 5 November 2022).

42. Torres, R.; Ferrara, G.; Soto, F.; López, J.A.; Sanchez, F.; Mazzeo, A.; Pérez-Pastor, A.; Domingo, R. Effects of soil and climate in a table grape vineyard with cover crops. Irrigation management using sensors networks. *Ciênc. Téc. Vitiviníc.* **2017**, *32*, 72–81. [CrossRef]

43. Huglin, P. Nouveau mode d'évaluation des possibilités héliothermiques d'en milieu viticole. *Comptes. Rendus. Acad. Agric. Fr.* **1978**, *64*, 1117–1126.

44. Tonietto, J.; Carbonneau, A. A multicriteria climatic classification system for grape growing regions worldwide. *Agric. For. Meteolo.* **2004**, *124*, 81–97. [CrossRef]

45. Togores, J.H. Chapter 3: Vendimia. Recepción de Uva en la Bodega. In *Tomo I: Tratado de Enología*, 2nd ed.; Mundi-Prensa: Madrid, Spain, 2010.

46. Bazán, D.L.; del Río, P.G.; Domínguez, J.M.; Cortés-Diéguez, S.; Mejuto, J.C.; Pérez-Guerra, N. The Chemical, Microbiological and Volatile Composition of Kefir-Like Beverages Produced from Red Table Grape Juice in Repeated 24-h Fed-Batch Subcultures. *Foods* **2022**, *11*, 3117. [CrossRef]

47. Masa, A.; Vilanova, M. Flavonoid and aromatic characterisation of cv. Albarín blanco (*Vitis vinifera* L.). *Food Chem.* **2008**, *107*, 273–281. [CrossRef]

48. Pedroza, M.A.; Zalacain, A.; Lara, J.F.; Salinas, M.R. Global grape aroma potential and its individual analysis by SBSE-GC-MS. *Food Res. Int.* **2010**, *43*, 1003–1008. [CrossRef]

49. Marais, J. Terpenes in the Aroma of Grapes and Wines: A Review. *S. Afr. J. Enol. Vitic.* **1983**, *4*, 49–60. [CrossRef]
50. Yu, F.; Utsumi, R. Diversity, regulation, and genetic manipulation of plant mono- and sesquiterpenoid biosynthesis. *Cell Mol. Life Sci.* **2009**, *66*, 3043–3052. [CrossRef] [PubMed]
51. Conde, C.; Silva, P.; Fontes, N.; Dias, A.; Tavares, R.; Sousa, M.; Agasse, A.; Delrot, S.; Gerós, H. Biochemical changes throughout Grape Berry development and fruit and wine quality. *Food* **2007**, *1*, 1–22. Available online: https://www.researchgate.net/publication/228555202_Biochemical_changes_throughout_Grape_Berry_development_and_fruit_and_wine_quality (accessed on 5 December 2022).
52. Zellner, B.D.A.; Casilli, A.; Dugo, P.; Dugo, G.; Mondello, L. Odour fingerprint acquisition by means of comprehensive two-dimensional gas chromatography-olfactometry and comprehensive two-dimensional gas chromatography/mass spectrometry. *J. Chromatogr. A* **2007**, *1141*, 279–286. [CrossRef] [PubMed]
53. Marchese, A.; Arciola, C.R.; Barbieri, R.; Silva, A.S.; Nabavi, S.F.; Sokeng, A.J.T.; Izadi, M.; Jafari, N.J.; Suntar, I.; Daglia, M.; et al. Update on monoterpenes as antimicrobial agents: A particular focus on p-Cymene. *Materials* **2017**, *10*, 947. [CrossRef] [PubMed]
54. Flavornet. Available online: https://www.flavornet.org/flavornet.html (accessed on 12 December 2022).
55. May, B.; Wust, M. Sesquiterpene profiles of different grape varieties. In *Advances and Challenges in Flavor Chemistry & Biology*; Hofmann, T., Meyerhof, W., Schieberle, P., Eds.; Deutsche Forschungsanstalt für Lebensmittelchemie: Freising, Germany, 2011; pp. 323–327.
56. Petronilho, S.; Coimbra, M.A.; Rocha, S.M. A critical review on extraction techniques and gas chromatography-based determination of grapevine derived sesquiterpene. *Anal. Chim. Acta* **2014**, *846*, 8–35. [CrossRef]
57. Kalua, C.M.; Boss, P.K. Comparison of major volatile compounds from Riesling and Cabernet Sauvignon grapes (*Vitis vinifera* L.) from fruitset to harvest. *Aust. J. Grape Wine Res.* **2010**, *16*, 337–348. [CrossRef]
58. Todorova, I.T.; Batovska, D.I.; Parushev, S.P.; Djakova, G.J.; Popov, S.S. Surface metabolic composition of berries collected from 13 Bulgarian grapevine plants. *Nat. Prod. Res.* **2010**, *24*, 935–941. [CrossRef]
59. Pineau, B.; Barbe, J.C.; Van Leeuwen, C.; Dubordieu, D. Which impact for β-damascenone on red wines aroma? *J. Agric. Food Chem.* **2007**, *55*, 4103–4108. [CrossRef]
60. Tomasino, E.; Bolman, S. The potential effect of β-Ionone and β-Damascenone on sensory perception of Pinot Noir wine aroma. *Molecules* **2021**, *26*, 1288. [CrossRef]
61. González-Barreiro, C.; Rial-Otero, R.; Cancho-Grande, B.; Simal-Gándara, J. Wine aroma compounds in grapes: A critical review. *Crit. Rev. Food Sci. Nutr.* **2015**, *55*, 202–218. [CrossRef]
62. Slaghenaufi, D.; Peruch, E.; De Cosmi, M.; Nouvelet, L.; Ugliano, M. Volatile and phenolic composition of monovarietal red wines of Valpolicella appellations. *OENO One* **2021**, *55*, 279–294. [CrossRef]
63. Oliveira, J.M.; Faria, M.; Sá, F.; Barros, F.; Araújo, I.M. C6-alcohols as varietal markers for assessment of wine origin. *Anal. Chim. Acta* **2006**, *563*, 300–309. [CrossRef]
64. López-Tamames, E.; Carro-Mariño, N.; Gunata, Y.Z.; Sapis, C.; Baumes, R.; Bayonove, C. Potential aroma in several varieties of Spanish grapes. *J. Agric. Food Chem.* **1997**, *45*, 1729–1735. [CrossRef]
65. Canosa, P.; Oliveira, J.M.; Masa, A.; Vilanova, M. Study of the Volatile and Glycosidically Bound Compounds of Minority *Vitis vinifera* Red Cultivars from NW Spain. *J. Inst. Brew.* **2011**, *117*, 462–471. [CrossRef]
66. The Good Scent Company. Available online: https://www.thegoodscentcompany.com (accessed on 5 January 2023).
67. Yu, Q.; Xie, B.J.; Zhang, Y.; Zhou, H.Y.; Pan, S.Y. Study on Aroma Components in Fruit from Three Different Satsuma Mandarin Varieties. *Agric. Sci. China* **2007**, *6*, 1487–1493. [CrossRef]
68. IARC Working Group of the Evaluation of Carcinogenic Risks to Humans. *Some Chemicals Present in Industrial and Consumer Products, Food and Drinking-Water*; International Agency for Research on Cancer: Lyon, France, 2013. Available online: https://www.ncbi.nlm.nih.gov/books/NBK373195/ (accessed on 10 January 2023).
69. Sasaki, K.; Takase, H.; Tanzawa, F.; Kobayashi, H.; Saito, H.; Matsuo, H.; Takata, R. Identification of Furaneol Glucopyranoside, the Precursor of Strawberry-like Aroma, Furaneol, in Muscat Bailey A. *Am. J. Enol. Vitic.* **2014**, *66*, 91–94. [CrossRef]
70. Cerreti, M.; Esti, M.; Benucci, I.; Liburdi, K.; de Simone, C.; Ferranti, P. Thiol precursors in grape. *Aust. J. Grape Wine Res.* **2015**, *21*, 411–416. [CrossRef]
71. Mariano, A.P.; Ramos, A.L.; de Oliveira Júnior, A.H.; García, Y.M.; de Paula, A.C.; Silva, M.R.; Augusti, R.; de Araújo, R.L.; Melo, J.O. Optimization of Extraction Conditions and Characterization of Volatile Organic Compounds of *Eugenia klotzschiana* O. Berg Fruit Pulp. *Molecules* **2022**, *27*, 935. [CrossRef]
72. Chen, X.Y.; Dou, Y.X.; Luo, D.D.; Zhang, Z.B.; Li, C.L.; Zeng, H.F.; Su, Z.R.; Xie, J.H.; Lai, X.P.; Li, Y.C. β-Patchoulene from patchouli oil protects against LPS-induced acute lung injury via suppressing NF-κB and activating Nrf2 pathways. *Int. Immunopharmacol.* **2017**, *50*, 270–278. [CrossRef]
73. Welke, J.E.; Manfroi, V.; Zanus, M.; Lazarotto, M.; Zini, C.A. Characterization of the volatile profile of Brazilian Merlot wines through comprehensive two-dimensional gas chromatography time-of-flight mass spectrometric detection. *J. Chromatogr. A* **2012**, *1226*, 124–139. [CrossRef]
74. Burdock, G.A. *Fenaroli's Handbook of Flavour Ingredients*, 6th ed.; CRC Press: Boca Raton, FL, USA; Taylor & Francis Group: Boca Raton, FL, USA; London, UK; New York, NY, USA, 2010.
75. Weyerstahl, P.; Meisel, T.; Mewes, K.; Negahdari, S. Struktur und Geruch, XIII. Synthese und olfaktorische Eigenschaften von Megastigmatrienon-Analoga. *Liebigs Ann. Der Chem.* **1991**, *1*, 19–25. [CrossRef]

76. García-Carpintero, E.G.; Sánchez-Palomo, E.; Gallego, M.A.G.; González-Viñas, M.A. Volatile and sensory characterization of red wines from cv. *Moravia Agria minority grape variety cultivated in La Mancha region over five consecutive vintages*. *Food Res. Int.* **2011**, *44*, 1549–1560. [CrossRef]

77. Peinado, R.A.; Moreno, J.; Bueno, J.E.; Moreno, J.A.; Mauricio, J.C. Comparative study of aromatic compounds in two young white wines subjected to prefermentative cryomaceration. *Food Chem.* **2004**, *84*, 585–590. [CrossRef]

78. Poitou, X.; Redon, P.; Pons, A.; Bruez, E.; Delière, L.; Marchal, A.; Cholet, C.; Geny-Denis, L.; Darriet, P. Methyl salicylate, a grape and wine chemical marker and sensory contributor in wines elaborated from grapes affected or not by cryptogrammic diseases. *Food Chem.* **2021**, *360*, 130120. [CrossRef] [PubMed]

79. Culbert, J.A.; Jiang, W.; Ristic, R.; Puglisi, C.J.; Nixon, E.C.; Shi, H.; Wilkinson, K.L. Glycosylation of Volatile Phenols in Grapes following Pre-Harvest (On-Vine) vs. Post-Harvest (Off-Vine) Exposure to Smoke. *Molecules* **2021**, *26*, 5277. [CrossRef] [PubMed]

80. Vararu, F.; Moreno-Garcia, J.; Cotea, V.V.; Moreno, J. Grape musts differentiation based on selected aroma compounds using SBSE-GC-MS and statistical analysis. *Vitis* **2015**, *54*, 97–105. [CrossRef]

81. Pérez-Navarro, J.; Izquierdo-Cañas, P.M.; Mena-Morales, A.; Chacón-Vozediano, J.L.; Martínez-Gascueña, J.; García-Romero, E.; Hermosín-Gutiérrez, I.; Gómez-Alonso, S. Comprehensive Chemical and Sensory Assessment of Wines Made from White Grapes of *Vitis vinifera* Cultivars Albillo Dorado and Montonera del Casar: A Comparative Study with Airén. *Foods* **2020**, *9*, 1282. [CrossRef]

82. Dimitrovska, M.; Bocevska, M.; Dimitrovski, D.; Murkovic, M. Anthocyanin composition of Vranec, Cabernet Sauvignon, Merlot and Pinot Noir grapes as indicator of their varietal differentiation. *Eur. Food Res. Technol.* **2011**, *232*, 591–600. [CrossRef]

83. Mattivi, F.; Guzzon, F.; Vrhowsek, U.; Stefanini, M.; Velasco, R. Metabolite profiling of grape: Flavonols and anthocyanins. *J. Agric. Food Chem.* **2006**, *54*, 7692–7702. [CrossRef]

84. Pomar, F.; Novo, M.; Masa, A. Varietal differences among the anthocyanin profiles of 50 red table grapes cultivars studied by high performance liquid chromatography. *J. Chromatogr. A* **2005**, *1094*, 34–41. [CrossRef]

85. Yang, C.; Wang, Y.; Liang, Z.; Fan, P.; Wu, B.; Yang, L.; Wang, Y.; Li, S. Volatiles of grape berries evaluated at the germplasm level by headspace-SPME with GC–MS. *Food Chem.* **2009**, *114*, 1106–1114. [CrossRef]