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# Preliminary Study of Enological Potential and Volatile Compounds of Tintilla de Rota Somatic Variant Grown in a Warm Climate

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**Abstract:** In an enological market notable for its use of universal varieties, the enological potential of the somatic variant Tintilla de Rota has been studied in a warm climate area from where it originates and has been compared with three universal cultivars and with a reference cultivar in Spain. It has been found that Tintilla de Rota is a grapevine cultivar that can adapt easily to the area where it has been grown traditionally, achieving adequate pH and acidity values while maintaining a moderate sugar content compared to the other varieties studied. Additionally, it has shown high anthocyanin content. In terms of aromatic composition, it has exhibited a content equal to or even higher than the other varieties analyzed in this study in most of the aromatic families studied, with a higher content of compounds such as 1-octanol, benzyl alcohol and citronellol, which can lead to wines with an interesting and distinguished aromatic profile. All these facts confirm the interest in the use of this cultivar and the importance of recovering and enhancing the value of autochthonous cultivars as opposed to other so-called universal varieties.

**Keywords:** Tintilla de Rota; somatic variants; autochthonous cultivars; aromatic precursors; aromatic compounds

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## 1. Introduction

Grapes are one of the world's most important fruit crops, with a production of 78 million tons approximately every year [1]. According to the Food and Agriculture Organization (FAO), 37% of the world's grape production was used for winemaking in 71 different countries, with more than 50% concentrated in just three countries (Spain, Italy and France) [2]. Thus, the wine industry can be considered a major contributor to the economy and reputation of many countries in the world [3].

However, viticulture today faces major challenges as one of the most important crop adaptations to climate change. All forecasts and simulations predict that climate change will be one of the major challenges for wine production in the near future, as vine quality and its yield depend on the complex interaction between temperature, water availability, plant material and viticultural techniques [4]. Thus, the characteristics imposed by climate change reduce flexibility in vine cultivation and require the search for strategies to adapt to the new growing conditions [5]. The main factors related to climate change with a direct influence on vine cultivation are the increase in temperature and carbon dioxide concentrations and the reduction in rainfall [6].

One strategy for adapting to climate change can be the use of varieties that are used in warm regions and are available in genetic resource collections around the world. In this way, appropriate combinations of rootstocks and clones can buffer the effects of high temperatures and drought that are currently occurring in wine-growing regions [7]. However, in order to maintain wine typicality, it is necessary to use other strategies, such as the use of autochthonous varieties and the exploration of intra-cultivar genetic variability to select late-ripening cultivars [8–10], selecting clones of those grapevine varieties that can reach ripening stages several weeks late [11].

The conservation of this plant material is not a recent development, since the work of prospection, collection and conservation of different vine varieties has been the subject of numerous scientific studies over the years [12–14]. More specifically, in an area with a warm climate such as South West (SW) Andalusia (Spain), the germplasm bank at Rancho de la Merced preserves different somatic variants of the main grapevine variety in Andalusia, Palomino Fino [15]. The geographical framework of the Jerez production area is located in the north-western quadrant of the province of Cádiz (SW Andalusia, Spain) and its production area is composed of different municipalities. Although it is characterized by the production of the typical sherry wine (a fortified wine) made from the Palomino Fino grape cultivar (which occupies more than 95% of the area's cultivated surface), there are historical references that define the region as an area with a great capacity for red wine production [16,17].

Traditionally, there has been an idea of the difficulty of producing quality red wines in warm climate areas due, among other factors, to the consequences that the climatic conditions can have on red grape production with more pronounced and faster ripening cycles than in any other areas, as well as the problems that can arise in different stages of winemaking and in adequate wine stabilization [18]. However, this is a handicap that is being surpassed in many areas by selecting the most suitable grape varieties in each case and carrying out the cultivation techniques and winemaking processes that will provide the desired product [18]. Additionally, grapevine variety and aromatic compounds and their precursors' concentrations present in grapes can also be affected by the conditions in which the grapes are grown [19–21].

The aromatic compounds present in grapes are responsible for the primary or varietal aroma of wines. These compounds can be found in free form and perceived by the sense of smell as they are in the grape. Furthermore, they can be found in a combined form as aroma precursors, normally in greater proportion, but are odorless and can be transformed into free aromas during winemaking by simply breaking a chemical bond, giving the wine its characteristic aromas [22,23]. From these will emerge the so-called pre-fermentative aroma, the result of the modifications that the grape undergoes during the pre-fermentative treatments and by reactions in grape musts.

Given the above, it is of particular interest to study and compare the presence and/or absence of volatile compounds and their precursors in red grape varieties grown in warm climates in order to improve and optimize the enological technology associated with obtaining wines from them. To this end, this research presents for the first time the preliminary study of the aromatic compounds (free and glycosylated) of a somatic variant of a grapevine widely used in a warm climate region such as Tintilla de Rota. For this purpose, the behavior of this cultivar has been compared at the physicochemical and aromatic levels with national and the most cultivated red grapevine varieties in Spain (Tempranillo) and universal varieties (Cabernet Sauvignon, Syrah and Merlot).

## 2. Materials and Methods

### 2.1. *Raw Material and Experimental Design*

The vineyard where the study was conducted is located in the Vinos de la Tierra de Cádiz appellation region, in Andalusia (SW Spain) (Latitude 36°42′3.89″ N, Longitude 6°11′25.52″ W), an area with limestone soil, which is rich in calcium carbonate, clay and silica, classified as Winkler Region IV. The vineyard density was 3500 plants/ha, with a plant framework of 2.40 m × 1.2 m, with a total of 3500 plants of Tintilla de Rota and Cabernet Sauvignon, 7000 plants of Merlot and 8200 plants of Tempranillo, with no fertilizer supplementation and non-irrigated managed. Average temperatures from July to September remained around 25 ± 1 °C, and there were no extremes in minimum or maximum temperatures, except for occasional days when maximum temperatures reached 38–39 °C.

Grape samples were collected once a week, from two weeks after veraison to technological ripening. In this sense, the relationship between sugar content and total acidity has been determined in each sample. This ratio increases gradually and at a constant rate during maturation. A slowdown in this ratio has been considered as the beginning of over-ripening and the optimal date to harvest. In order to compare the somatic variant Tintilla de Rota's behavior, grapes from Tempranillo (the most cultivated red grape in Spain) and three universal cultivars (Cabernet Sauvignon, Merlot and Syrah) were collected under equal conditions.

For each variety, 5% of the total grapevines regularly distributed throughout the vineyard were marked. From these grapevines, 2000 grapes were randomly collected at each sampling data point. The grapes were taken from different parts of the cluster and the plant and at different vineyard locations.

From each cultivar and sample, 1000 grapes were weighed and crushed in a stainless steel 5 L vertical press (2 atm). Grape musts obtained were used to determine general physicochemical parameters. Another 1000 grapes were used to extract both phenolic and volatile compounds. For this purpose, grape berries were crushed and pressed. The obtained musts were adjusted to an alcohol degree of 12% (by adding absolute ethanol), 3.5 pH (by adding tartaric acid if necessary) and 5 ghL$^{-1}$ of sulfur dioxide ($SO_2$) in order to simulate the typical conditions that occur in the industrial red winemaking process. Musts and skins were macerated for 12 h in an automatic agitator (Rotabit, P-Selecta, Barcelona, Spain) at a constant speed of 200 rpm at room temperature. After this time, grape musts were centrifuged (2500 g; 10 min) in a Biocen 22 R centrifuge (OrtoAlresa, Madrid, Spain).

### *2.2. Physicochemical Parameters Measurement*

Grape must sugar content (° Brix), pH and total acidity (tartaric acid g L$^{-1}$) were measured according to the methods of the International Organisation of Vine and Wine (OIV) in triplicate.

### *2.3. Phenolic Compounds Determination*

All the methods used in this section involve measuring absorbance at a certain wavelength in a spectrophotometer (Hitachi 200, Perkin-Elmer, Madrid, Spain) and are based on the methodologies proposed by Ribéreau-Gayon [24]. Total Polyphenols Index (TPI) involves direct measuring of absorbance at 280 nm. The total anthocyanin content is based on sample bleaching with sulfur dioxide, giving the results in mg L$^{-1}$ of anthocyanins, and the total tannin content is based on the development of a brown color after acidification and heating. The results are given in g L$^{-1}$ of tannins. All the measures were carried out in triplicate.

### *2.4. Colour Parameters Determination*

Grape must color was characterized by determining two variables: (i) the color intensity, i.e., the sum of absorbances at 420 nm (yellow), 520 nm (red) and 620 nm (blue) and (ii) wine hue, which is the ratio between the absorbances at 420 nm and 520 nm. All the measures were carried out in triplicate using a spectrophotometer (Hitachi 200, Perkin-Elmer, Madrid, Spain).

### *2.5. Volatile Compounds Determination*

In this case, the analytical method includes an extraction stage and an analysis stage. In the extraction stage, according to the methodology of Di Stefano [25], each sample (50 mL) was diluted four times with distilled water and 0.4 mL of internal standard (150 µg L$^{-1}$ of 1-heptanol in 40% ethanol/water) was added. The sample solution was passed through a 1 g DSC-18 cartridge (SUPELCO, Bellefonte, PA, USA) after washing with 3 mL of ethanol (HPLC Quality, Panreac, Barcelona, Spain). The retained compounds were eluted with

10 mL of dichloromethane (GC Quality, Panreac, Barcelona, Spain), dried over $Na_2SO_4$ anhydrous and concentrated to a final volume of 200 µL under a stream of nitrogen at room temperature. This procedure leads to the free aroma compounds being present in the concentrate but the glycosides are retained in the extraction cartridges.

Once the free compounds had been extracted, the glycosides were eluted with 5 mL of ethanol and dissolved in 25 mL of citrate-phosphate buffer (pH 5) with 1 mg of enzymatic preparation (Rapidase AR 2000, DSM Food Specialties, Herleen, The Netherlands). Samples were kept at 40 °C for 24 h in order to free the bound aroma compounds and afterward, the internal standard (150 µg/L of 1-heptanol in 40% ethanol/water) was added. The samples were then processed in the same way as previously described for the free form.

All samples were analyzed by gas chromatography-mass spectrometry (GC-MS) on a Voyager (Thermoquest, Italy) system equipped with a Supelcowax 10 column (60 m × 0.32 mm × 0.50 µm), with the detector and injector at 250 °C. The column temperature was programmed from 40 °C (5 min) to 200 °C (ramp 2 °C $min^{-1}$ increase) and a plateau of 200 °C (5 min). Samples (2 µL) were injected in splitless mode (40 s) with helium as the carrier gas at a flow rate of 1 mL $min^{-1}$. The detection was performed using electron impact mode at 70 eV in the mass spectrometer. The mass analyzer ranged from a ratio *m/z* of 45 to 400 at a scan rate of 1 scan/s.

Identification of volatile compounds was achieved using the GC-MS retention indexes (Authentic Chemicals Data Base) and by comparison of the mass spectra (Authentic Chemicals and Xcalibur Spectral Library Collection, Waltham, MA, USA). All of the volatile compound standards used for the identification and quantification were obtained from Sigma-Aldrich (Merck, Darmstadt, Germany).

### *2.6. Statistical Data Analysis*

The statistical study of the results was carried out by ANOVA analysis, using GraphPad Prism 6.01 software for Windows (GraphPad Software, San Diego, CA, USA), applying the Bonferroni Multiple Range (BSD) test with 95% confidence ($p < 0.05$). For the volatile compounds detailed analysis, a multivariate analysis was carried out using the Principal Components Analysis (PCA) method. The statistical software SPSS 24.0 (SPSS Inc., Chicago, IL, USA) was employed.

## 3. Results and Discussion

### *3.1. Physicochemical Characterisation of Grape Musts*

Table 1 shows the physicochemical composition in the grape must of Tintilla de Rota somatic variant and Tempranillo, Syrah, Merlot, and Cabernet Sauvignon grape musts at harvest.

**Table 1.** Grape must physicochemical composition of Tintilla de Rota somatic variant and Tempranillo, Syrah, Merlot, and Cabernet Sauvignon grape musts.

| | Tintilla de Rota | | | Tempranillo | | | Syrah | | | Merlot | | | Cabernet Sauvignon | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Berry weight (g) | 1.12 | ± | 0.13 $^{a}$ | 1.36 | ± | 0.09 $^{b}$ | 1.08 | ± | 0.15 $^{a,c}$ | 1.27 | ± | 0.06 $^{a,b}$ | 0.89 | ± | 0.07 $^{c}$ |
| pH | 3.49 | ± | 0.19 $^{a}$ | 3.67 | ± | 0.17 $^{a}$ | 3.68 | ± | 0.21 $^{a}$ | 3.54 | ± | 0.23 $^{a}$ | 3.48 | ± | 0.05 $^{a}$ |
| T.A. (g $L^{-1}$ $TH_2$) | 5.97 | ± | 0.05 $^{a,b}$ | 5.14 | ± | 0.12 $^{a}$ | 6.41 | ± | 0.11 $^{b}$ | 5.96 | ± | 0.13 $^{a,b}$ | 5.15 | ± | 0.06 $^{a}$ |
| ° Brix | 22.89 | ± | 0.53 $^{a}$ | 24.98 | ± | 0.56 $^{a,b}$ | 24.71 | ± | 0.16 $^{a,b}$ | 27.07 | ± | 0.22 $^{b}$ | 27.25 | ± | 0.33 $^{b}$ |
| TPI (AU) | 81.15 | ± | 7.21 $^{a}$ | 78.07 | ± | 6.54 $^{a,b}$ | 68.53 | ± | 3.96 $^{b}$ | 70.22 | ± | 8.41 $^{a,b}$ | 66.77 | ± | 5.98 $^{b}$ |
| Anthocyanins (mg $L^{-1}$) | 996.12 | ± | 54.13 $^{a}$ | 1077.87 | ± | 68.48 $^{a}$ | 968.03 | ± | 101.20 $^{a}$ | 628.40 | ± | 96.36 $^{b}$ | 693.12 | ± | 20.39 $^{b}$ |
| Tannins (g $L^{-1}$) | 2.66 | ± | 0.21 $^{a}$ | 2.86 | ± | 0.19 $^{a}$ | 2.55 | ± | 0.08 $^{a}$ | 2.88 | ± | 0.14 $^{a}$ | 2.84 | ± | 0.12 $^{a}$ |

**Table 1.** *Cont.*

| | Tintilla de Rota | | | Tempranillo | | | Syrah | | | Merlot | | | Cabernet Sauvignon | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| CI | 18.16 | ± | 0.98 [a] | 13.14 | ± | 0.47 [b] | 14.82 | ± | 1.12 [b] | 10.22 | ± | 1.02[c] | 10.38 | ± | 1.00 [c] |
| Hue | 0.46 | ± | 0.03 [a] | 0.54 | ± | 0.06 [a] | 0.56 | ± | 0.07 [a] | 0.69 | ± | 0.10 [b] | 0.67 | ± | 0.05 [b] |

T.A. (Total Acidity), TH2 (Tartaric acid), TPI (Total Polyphenol Index, AU: Absorbance Units), CI (Colour Intensity). Different superscript letters mean a significant difference between samples (ANOVA $p < 0.05$) determined by two-way ANOVA applying a Bonferroni's multiple range (BSD) test.

One of the most important parameters to deciding harvest date is sugar accumulation in grape berries as this will largely determine the wine's final alcohol content. The sugar content ranged between 22.89 ± 0.53 and 27.25 ± 0.33° Brix for Tintilla de Rota and Cabernet Sauvignon, respectively, showing significant differences (ANOVA $p < 0.05$) between the somatic variant and the rest of the universal varieties. Regarding sugar content, the total acidity shows an inverse behavior. While sugars accumulate in berries during ripening, the acid content decreases. For Tintilla de Rota, a value of 5.97 ± 0.05 g $L^{-1}$ of tartaric acid was observed, which is the third highest value of the whole series analyed with respect to the universal varieties studied. This drop in acidity implies an increase in grape must pH values. The values observed ranged between 3.48 ± 0.05 and 3.68 ± 0.21 for Cabernet Sauvignon and Syrah, respectively, with no significant differences between the data analyzed. Finally, grape berry average weight ranged between 0.89 ± 0.07 g for Cabernet Sauvignon and 1.36 ± 0.09 g for Tempranillo, with significant differences between the study cultivar Tintilla de Rota and the two abovementioned cultivars. This parameter fluctuates as ripening progresses, as do the previous three, increasing at the beginning and stabilizing or even decreasing at the end of the grape ripening phase. Combining these four physicochemical parameters, it can be seen that the autochthonous cultivar Tintilla de Rota has acidity values located in the middle of the range studied, while it shows a lower concentration of sugars. This could be beneficial in producing wines with a fresher sensory profile and lower alcohol content in line with current consumer and market trends [26]. On the other hand, the use of autochthonous grapevine varieties [10], or their somatic variants [27], has proven to be an efficient viticultural strategy to cope with the adverse effects linked to climate change in a warm climate zone.

Along with the physicochemical parameters discussed above, the content of polyphenolic compounds must also be studied, given the importance of these compounds from a sensory point of view in wines. If the overall phenolic content is evaluated, the most direct parameter is the Total Polyphenol Index (TPI). TPI values ranged between 81.15 ± 7.21 AU for Tintilla de Rota and 68.53 ± 3.96 AU for Syrah, showing significant differences between some universal cultivars and Tintilla de Rota. Contrary to expectations, no direct correlation was found between berry weight and the result in the TPI analysis, proving that the accumulation of colored compounds is determined by the nature of the grapevine variety, beyond the weight and size of its berries. Given the complexity of TPI analysis, as it encompasses a large number of polyphenolic compounds, it was decided to analyze the anthocyanin content due to its direct relationship with wine color and the tannins due to their sensory involvement and the role they can play in wine color stabilization processes through the co-pigmentation phenomena [28]. The results of the anthocyanin analysis show how, on one hand, Tintilla de Rota, Tempranillo and Syrah are grouped together, and on the other hand, Merlot and Cabernet Sauvignon are grouped together. Furthermore, significant differences between these two groups can be appreciated (ANOVA $p < 0.05$). Anthocyanins are families of compounds that increase in concentration during the grape ripening process, reaching a maximum and then decreasing in the case of grape over-ripening [18]. As for the tannin content, the results show similar values between the different varieties studied, with no significant differences being observed in any of the cases, with values between 2.55 ± 0.08 and 2.88 ± 0.14 for Syrah and Merlot respectively. The tannin content fluctuates greatly in concentration during the grape ripening process, so its analysis at the time of maximum ripening is not of relevant interest, for example, to determine the appropriate date of harvest or to discriminate between varieties, as other polyphenolic compounds could be [9].

In addition to phenolic compound contents, grape must chromatic characteristics are also very important. For this reason, the Colour Intensity (CI) and hue of each of the samples studied were determined. In terms of color intensity, a parallelism was obtained with that observed for anthocyanin content, with the distribution being repeated in two significantly different groups, with values ranging between $10.22 \pm 1.02$ and $18.16 \pm 0.98$ for Merlot and Tintilla de Rota, respectively. This parallelism denotes the direct relationship between these two magnitudes. In turn, if the color and hue values of the different cultivars are analyzed, the previous grouping is repeated. The values ranged between $0.46 \pm 0.03$ and $0.69 \pm 0.10$ for Tintilla de Rota and Merlot, with the results showing the inverse behavior to those observed for CI. From a technological point of view, lower hue values in a grape variety will be beneficial since brownish colors will be avoided, i.e., those undesirable when making red wine [29]. According to its own name (in Spanish), Tintilla de Rota presented a grape must with higher color content, and is the must with the highest CI values. This is consistent with the higher values regarding TPI analysis compared to the results presented by the universal varieties. Furthermore, in this sense, it could be considered potentially a grape must capable of producing wines with a stronger stable color intensity compared to the other grape musts.

### *3.2. Evolution of Volatile Compounds during Ripening*

A total of 26 compounds were identified among the four universal varieties (Tempranillo, Syrah, Merlot, Cabernet Sauvignon) and the somatic variant (Tintilla de Rota) studied, grouped into five families: C6 alcohols, alcohols and aldehydes, aromatic alcohols, terpenes and terpenic derivatives, and norisoprenoids. In order to simplify the study, the obtained results in each of the aromatic families have been clustered in order to show the evolution profile of free and glycosylated aromas for all the universal varieties and the somatic variant Tintilla de Rota organized by compound families and during the ripening period. Figure 1 shows the evolution of free (Figure 1a) and glycosylated (Figure 1b) C6 compound contents during the period studied.

As can be seen, both glycosylated and free C6 compounds showed a decreasing tendency during the ripening period for all the cultivars studied. In all cases, the lowest values were shown by the glycosylated C6 compounds, highlighting Syrah as having the lowest values in both cases. Tintilla de Rota somatic variant samples showed intermediate values for both free and glycosylated C6 alcohols, which may be of interest since the final wines will not present the high concentrations of herbaceous and green aromas typical of these compound groups [30]. As was expected, a progressive decrease in the sum of all these compounds was observed in all cultivars with a slower decrease, as was observed for Syrah and Tintilla de Rota and more markedly in Tempranillo. This decrease allows the herbaceous aromas of grape must to disappear during ripening, which, if they appear in wines in a significant amount, can become unpleasant.

Figure 2 shows the evolution of free (Figure 2a) and glycosylated (Figure 2b) alcohols and aldehydes during the ripening period. For these compounds, an increase was generally observed during ripening, with some ups and downs in certain varieties. Once again, as expected, the lowest values corresponded to the lower proportion of glycosylated compounds. Nevertheless, it should be noted that significantly higher values (ANOVA $p < 0.05$) were obtained at the end of the ripening period for Tintilla de Rota samples, closely followed by Cabernet Sauvignon. This is mainly due to the contribution of 1-octanol, which is present in both grape cultivars at a higher concentration than in the rest of the varieties analyzed, and can be associated with floral, jasmine aromas, or may form octyl acetate and/or ethanoate during fermentation, providing also fruity aromas such as orange [30,31]. However, this increase was not observed for Tempranillo and Merlot, where small decreases were observed in the last sampling points. The results corresponding to the glycosylated compounds showed significantly lower levels than the free ones. However, it should be pointed out that once again Tintilla de Rota showed the highest results during the entire ripening period compared to the universal varieties. This means that, once again, this somatic variant offers a very attractive aromatic potential for the production of red wines in a warm climate zone.

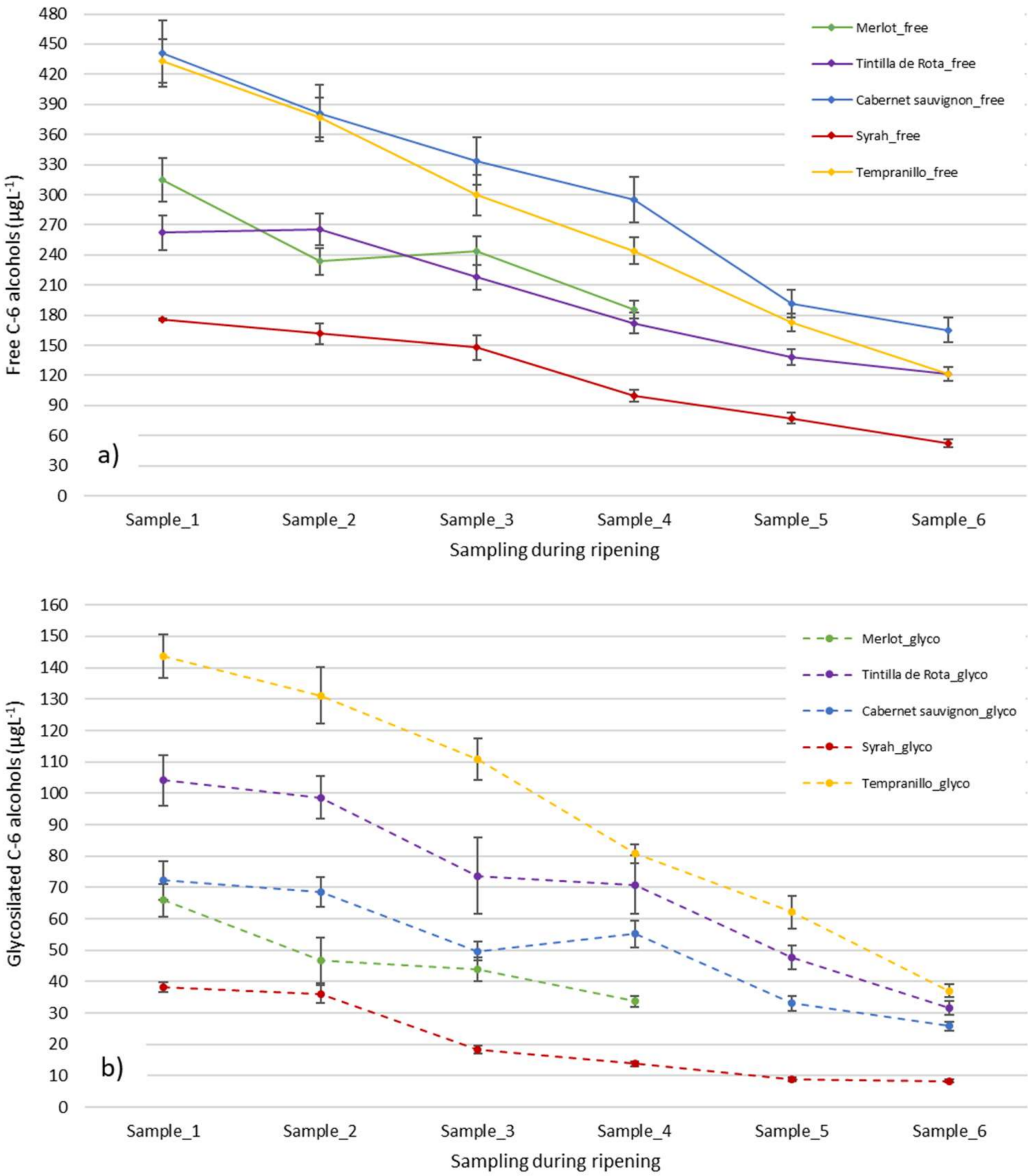


**Figure 1.** Evolution of the content of free (**a**) and glycosylated (**b**) C6 compounds during the period studied.

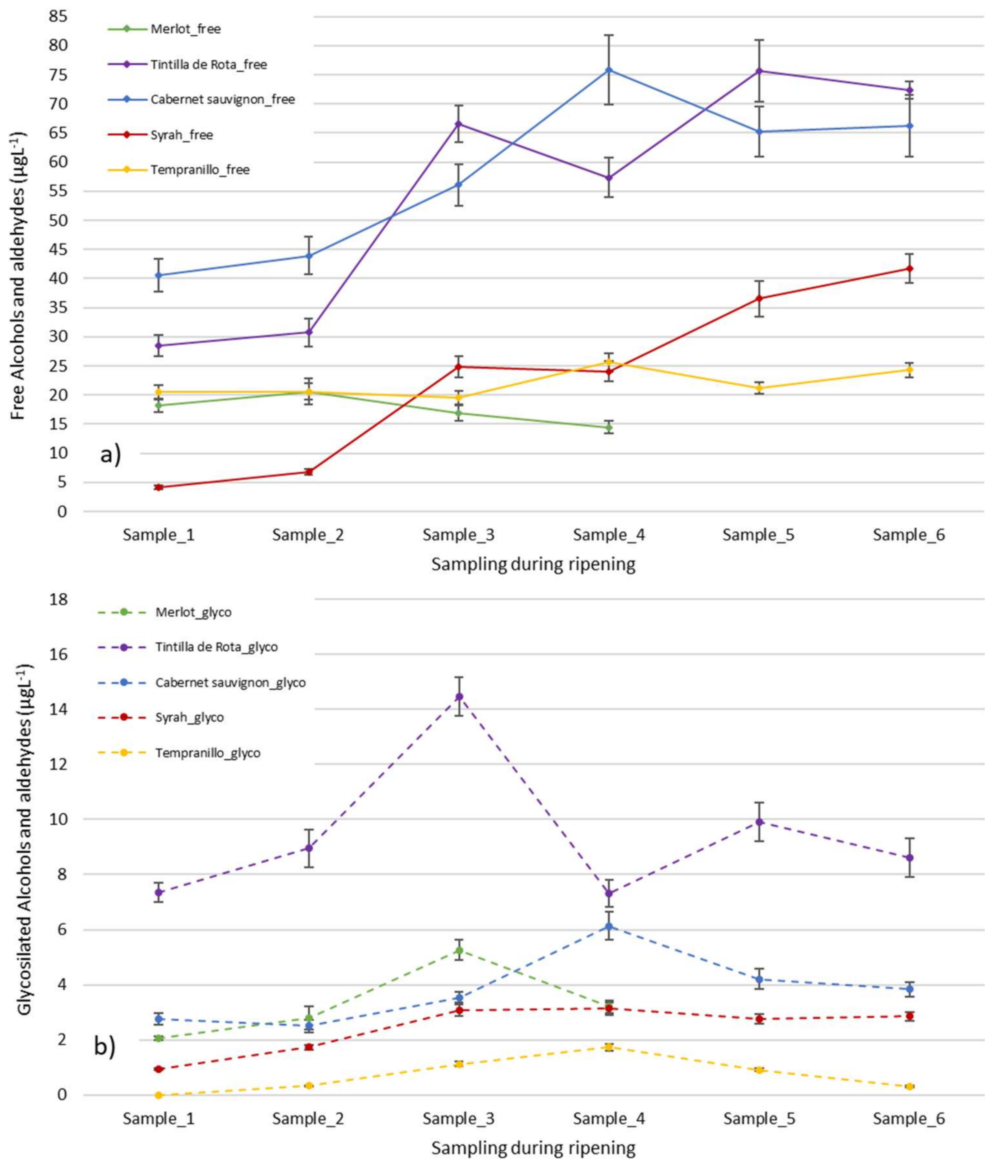


**Figure 2.** Evolution of the content of free (**a**) and glycosylated (**b**) alcohols and aldehydes during the period studied.

Figure 3 shows the progression of the aromatic alcohol's free (Figure 3a) and glycosylated (Figure 3b) content during ripening. It is important to note that, in comparison with the other families, results for the aromatic alcohols families reached values ten times higher than the results obtained for alcohols, aldehydes and terpenes and terpenic derivatives. All the universal varieties showed fluctuations during the different sampling points, unlike the somatic variant Tintilla de Rota, which at all times showed increasing values for this group of compounds. Regarding free compounds, at the final sampling point, both Cabernet Sauvignon and Tintilla de Rota showed significantly higher values compared to the other varieties. However, observing the glycosylated compounds, Tintilla de Rota showed significantly higher values (ANOVA $p < 0.05$) at all times compared to the universal varieties. This is mainly due to the high benzyl alcohol content, which is associated with floral (rose) and sweet aromas [32], which during fermentation can be partly transformed into benzyl acetate, which also contributes to floral notes [33]. Once again, Tintilla de Rota's aromatic potential is higher than the rest of the cultivars studied, and these levels remain high even at the date closest to harvest.

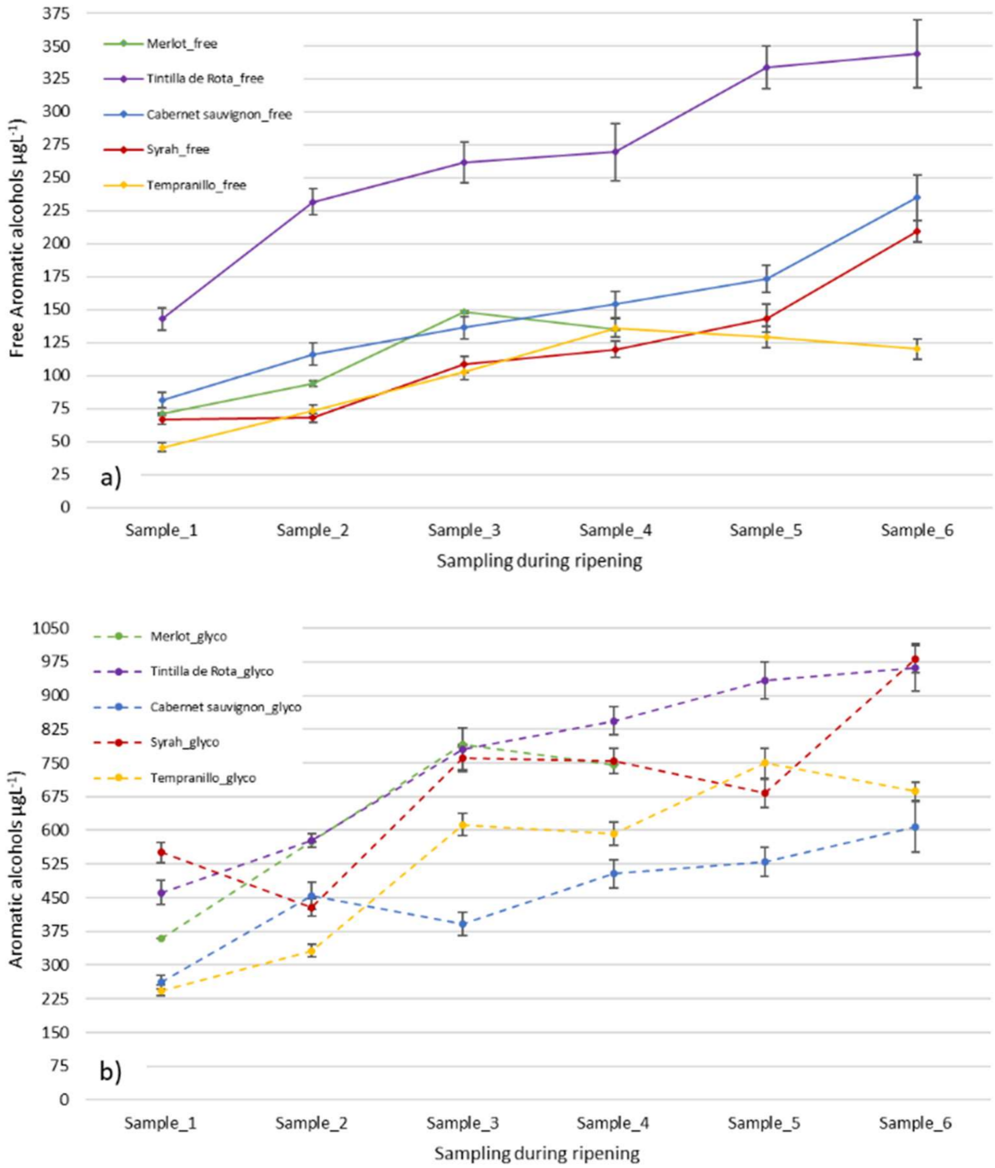


**Figure 3.** Evolution of the content of free (**a**) and glycosylated (**b**) aromatic alcohols during the period studied.

Figure 4 presents the variation in terpenes and their free (Figure 4a) and glycosylated (Figure 4b) derivatives during ripening. For this family of compounds, it was observed that the glycosylated compounds showed higher values than free compounds. However, it is a common phenomenon in many grapevine varieties [34]. These compounds, which contribute to varietal aromas, will be released naturally during winemaking, or by applying certain technologies (i.e., enzyme addition or pre-fermentative cold maceration), which makes it very interesting to enhance their extraction and their subsequent release. Considering the behavior of the glycosylated compounds corresponding to this family, in all cases, there was a more or less constant increase during the first weeks until reaching a stabilizing or even a decreasing point. Among these, the highest results were obtained for Syrah, followed by Tintilla de Rota, the latter being notable for its citronellol concentration which contributes to fresh fruit aromas. The behavior shown by Tempranillo should be highlighted, as the results showed a decrease after a short period of stabilization, possibly coinciding with the beginning of the so-called overripening period. In general, a slightly increasing trend was observed for free compounds, except Tintilla de Rota, which, besides presenting low values for this compound group, showed a small decrease in levels during the last two weeks of sampling.

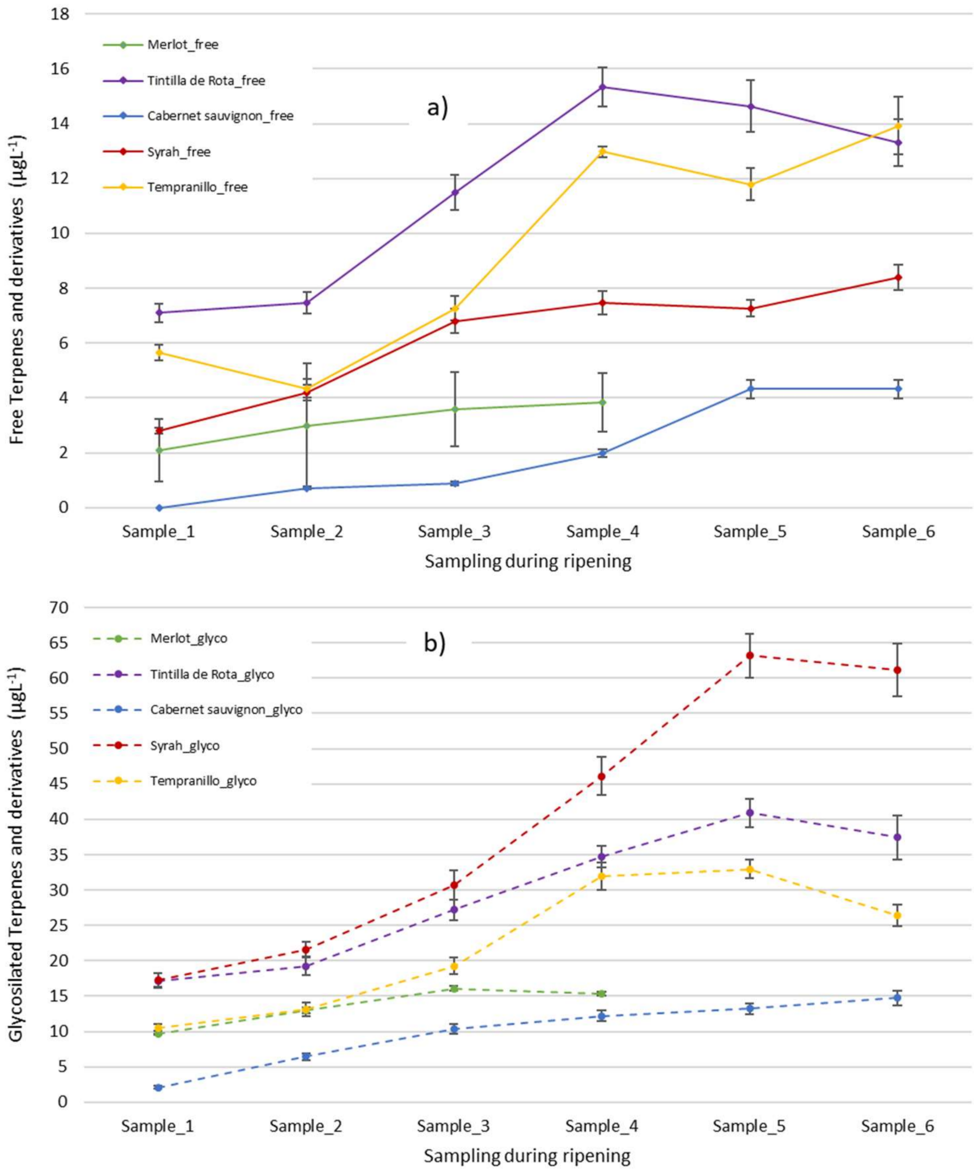


**Figure 4.** Evolution of the content of terpenes and derivatives free (**a**) and glycosylated (**b**), during the period studied.

Figure 5 showed the development of the free (Figure 4a) and glycosylated (Figure 4b) norisoprenoids content during the period studied. A similar phenomenon happens for these compounds, as was observed for terpenes and their derivatives, with the exception of Syrah, where results continue increasing during the last sampling point. Furthermore, greater results are obtained for glycosylated compounds than its free form, so that it may again be interesting to promote their release. Furthermore, it should be remarked that the amount of this type of compound found in Syrah was much higher than in the rest of the universal varieties and the somatic variant Tintilla de Rota, which is a varietal trait [35].

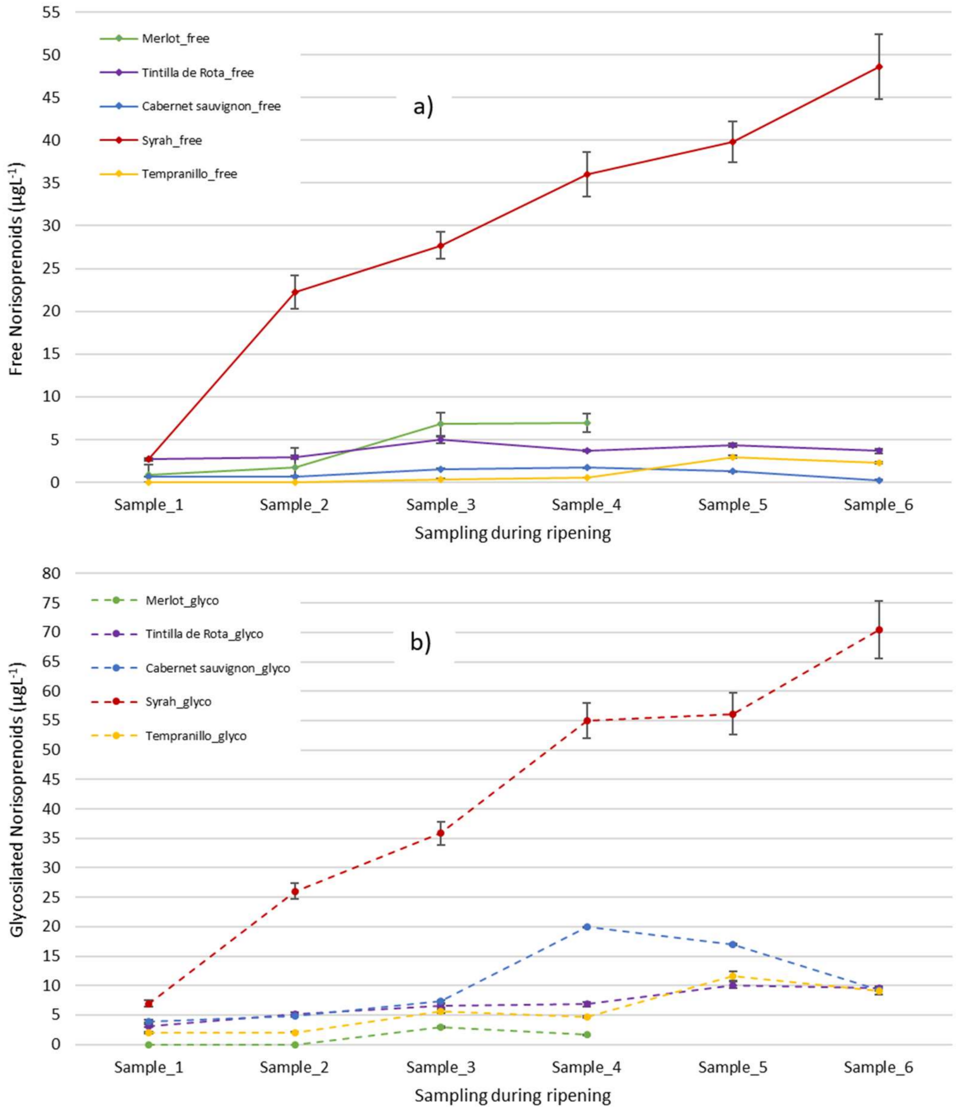


**Figure 5.** Evolution of the content of free (**a**) and glycosylated (**b**) norisoprenoids during the period studied.

### 3.3. Evolution of Aromas Families

A comparison of the behavior of each compound's family, both free and glycosylated, for each of the universal varieties and the somatic variant Tintilla de Rota, was carried out.

For Tempranillo, free aromatic compounds mainly showed a decrease in C6 alcohols as the sampling date approached the harvest date. However, when glycosylated compounds were studied, most of them belonged to the terpene families and were mainly phenols, as well as a slight increase in the norisoprenoid content. These compounds are generally desirable in grape musts and wines from an aromatic point of view. Therefore, the potential of releasing them can be of interest in aromatic young wine production with a marked varietal character.

It was observed an increase at the beginning of the sampling period, but then these values began to decrease, especially terpenes and norisoprenoids, and were reduced by more than a half when the grapes remained on the vine too long before harvest.

Compared to Tempranillo, a very different aromatic profile was observed in free aromatic compounds for Syrah, with significantly fewer C6 compounds and a considerably increased content of norisoprenoids. In addition, this profile becomes more interesting as the ripening process carries on, with an increase in these compounds' content. Analyzing the glycosylated fraction revealed levels of terpenic compounds and phenolic derivatives not found in their free form. In addition to the increasing flavor intensity, the release of glycosylated compounds in this case could also modulate and enrich the aromatic profile by increasing the fruity and floral notes, as well as the sensory properties typical of these compounds. Furthermore, it should be noted that in these compounds' evolution, as in the case of free compounds, increasing values were observed even at the last sampling point. This would suggest that a delay in its harvest could lead to an increase in the concentration of these families of compounds in grape must.

A different aromatic profile was observed for Merlot, which showed a limited content in the four most desirable aromatic compounds in free form. No significant differences appeared between the different sampling points, except for the norisoprenoid compounds, which showed a considerable increase in the last two weeks of the period studied. Nevertheless, regarding the glycosylated fraction, an interesting content in terpenes and, above all, in its phenolic derivatives, was observed. This fact would suggest that an improvement in the aromatic profile and the aroma intensity of wines made with this grapevine cultivar could be possible if the proper techniques were applied for its release.

In comparison with the other varieties and the somatic variant Tintilla de Rota, the results for Cabernet Sauvignon showed a less balanced profile in relation to the free-form compounds. Higher values in alcohols and aldehydes families are remarkable, mainly due to a high content of 1-octanol. However, observing the glycosylated compounds, in addition to finding a large amount of phenolic derivatives, the results showed interesting levels of norisoprenoids and terpenes, those levels not being observed in the free form of this family of compounds.

Regarding Tintilla de Rota, as with Syrah, it initially showed, in general, higher content of volatile compounds. In contrast to Syrah, Tintilla de Rota showed a low content norisoprenoids. In a similar way to Cabernet Sauvignon, Tintilla de Rota showed a large number of aromas included in the alcohols and aldehydes families, with 1-octanol once again standing out, and not a very high T-2-heptenal content, which differentiates it from the other varieties. The high content in aromatic alcohols in the free form is also remarkable, highlighting the content in benzyl alcohol, which is higher than in the rest of the varieties. In terms of terpenes, the presence of citronellol, which is not present in the free form in any of the other varieties studied, also presents a higher content of this compound in the glycosylated form, which could again be a differentiating element for this variety. This confers a great interest in this somatic variant from an enological point of view in its use because of its superior and different aromatic profile during the final part of the ripening period compared to the universal varieties studied. During the penultimate week of measurement, there was a higher content of volatile compounds of interest for the sensory profile. In this sense, it could be indicated that thanks to the behavior of Tintilla de Rota during the ripening period, it would be possible to have a more or less extended range in which to set a harvest date according to the enological parameters studied (Table 1) and in comparison with the varieties included in the study.

### *3.4. PCA of Volatile Compounds*

Table 2 shows the loadings of the Principal Component Analysis (PCA) for the 26 volatile glycosylated and free compounds analyzed in the grape musts of the universal varieties studied (Tempranillo, Syrah, Merlot, Cabernet Sauvignon) and the somatic variant Tintilla de Rota. The compound matrix studied as the concentration variable was composed of thirteen alcohols and aldehydes (1-hexanol, trans-2-hexen-1-ol, cis-3-hexen-1-ol, trans-3-hexen-1-ol, hexanal, T-2-hexenal, 1-octanol, T-2-heptenal, T-T-2,4-heptadienal, octanal, 2-octenal, nonanal and T-2-nonenal), two volatile phenols (phenylethyl alcohol and benzyl alcohol), eight terpenic compounds and derivatives (citronellol, trans-geraniol, limonene, *p*-ment-1-en-4-ol, eucalyptol, hydroxylinalool, 2,6-dimethyl-3,7-octadien-2,6-diol and geranic acid) and three norisoprenoids (β-damascenone, β-ionone and ionol).

The PCA analysis extracted three factors which explained 68.79% of the total variance data. Factor 1 (F1) explained 40.07% of the total variance and it was positively correlated, to a greater extent with the phenolic compounds: phenylethyl alcohol and benzyl alcohol, the terpenes: trans-geraniol, limonene, *p*-ment-1-en-4-ol, hydroxylinalool, 2,6-dimethyl-3,7-octadien-2,6-diol and the norisoprenoids β-damascenone, β-ionone, and to a lesser extent with geranic acid. On the other hand, F1 correlated negatively with the alcohols 1-hexanol, trans-2-hexen-1-ol, cis-3-hexen-1-ol, trans-3-hexen-1-ol, hexanal, T-2-hexenal, 1-octanol mainly, and with the aldehydes T-2-heptenal, T-T-2,4-heptadienal, nonanal to a lesser extent. Factor 2 (F2) was positively correlated mainly with octanal and to a lesser extent with trans-2-hexen-1-ol, cis-3-hexen-1-ol, and negatively with T-2-heptenal, citronellol and eucalyptol. It should also be noted that F2 showed a correlation to a similar degree to F1 with the concentration of geranic acid. Finally, Factor 3 (F3) showed a positive correlation with nonanal and ionol mainly and with 1-octanol, T-2-heptenal, 2-octenal, hydroxylinalool and β-damascenone to a lesser degree, and negatively with eucalyptol, T-2-hexenal and T-2-nonenal, although to a moderate degree.

**Table 2.** Principal Component Analysis (PCA) loadings of volatile compounds.

| Compounds | F1 | F2 | F3 |
|---|---|---|---|
| 1-hexanol | −0.883 | −0.056 | 0.080 |
| trans-2-hexen-1-ol | −0.678 | 0.490 | −0.061 |
| cis-3-hexen-1-ol | −0.700 | 0.496 | 0.247 |
| trans-3-hexen-1-ol | −0.788 | −0.219 | 0.289 |
| Hexanal | −0.858 | 0.398 | −0.001 |
| T-2-Hexenal | −0.658 | 0.203 | −0.302 |
| 1-octanol | −0.653 | −0.344 | 0.573 |
| T-2-Heptenal | −0.399 | −0.721 | 0.466 |
| T-T-2,4-Heptadienal | −0.573 | −0.459 | 0.260 |
| Octanal | −0.214 | 0.662 | 0.028 |
| 2-Octenal | 0.286 | 0.067 | 0.549 |
| Nonanal | −0.378 | −0.278 | 0.746 |
| T-2-Nonenal | −0.235 | 0.388 | −0.267 |
| Phenylethyl alcohol | 0.848 | −0.040 | −0.123 |
| Benzyl alcohol | 0.822 | −0.407 | −0.121 |
| Citronellol | 0.162 | −0.703 | −0.102 |
| trans-Geraniol | 0.685 | 0.257 | −0.017 |
| Limonene | 0.877 | 0.024 | −0.015 |
| *p*-ment-1-en-4-ol | 0.771 | 0.123 | 0.073 |
| Eucalyptol | 0.360 | −0.701 | −0.469 |
| Hydroxylinalool | 0.724 | 0.268 | 0.514 |
| 2,6-dimetil-3,7-octadien-2,6-diol | 0.708 | −0.120 | 0.426 |
| Geranic acid | 0.431 | 0.411 | −0.012 |
| β-Damascenone | 0.726 | 0.207 | 0.568 |
| β-Ionone | 0.716 | 0.318 | 0.212 |
| Ionol | 0.166 | 0.276 | 0.795 |

Extraction method: Principal Component Analysis.

According to Figure 2, F1 showed a clear trend in glycosylated compounds, with a positive displacement, mainly due to increases in the concentration of phenolic compounds such as phenylethyl alcohol and benzyl alcohol, terpenes such as trans-geraniol, limonene, *p*-ment-1-en-4-ol, hydroxylinalool, 2,6-dimethyl-3,7-octadien-2,6-diol and the norisoprenoids β-damascenone, or β-ionone. Syrah was the one that showed the greatest displacement towards positive values in F1, followed by the somatic variant Tintilla de Rota and, in third place, the rest of the universal varieties in a similar way. On the other hand, the free compounds were more displaced to the negative values of F1, mainly due to the increase in alcohols and aldehydes (Table 2). Regarding Factor 2 (F2), a completely different behavior was observed between the somatic variant Tintilla de Rota and the universal varieties. Moreover, Tintilla de Rota was displaced in F2 towards negative values and both glycosylated and free compounds were positioned at a similar distance in F2 (Figure 6). This behavior could probably be attributed to the increase of T-2-heptenal, citronellol and eucalyptol mainly and due to T-T-2,4-heptadienal, benzyl alcohol and some alcohols such as 1-octanol and trans-3-hexen-1-ol to a lesser extent in Tintilla de Rota grape must. Universal varieties were positioned more positively in F2, probably due to the increase of some alcohols such as trans-2-2-hexen-1-ol and cis-3-hexen-1-ol and the aldehydes octanal and T-2-nonenal (Table 2). Concerning Factor 3 (F3), it was observed how, in general, the glycosylated volatile compounds were positioned towards more negative values. Moreover, free compounds were positioned positively towards this factor, with the exception of Syrah (S_g), which moved towards positions similar to the free compounds, possibly due to the increase in the concentration of compounds such as ionol, β-damascenone, nonanal or 2-octenal.

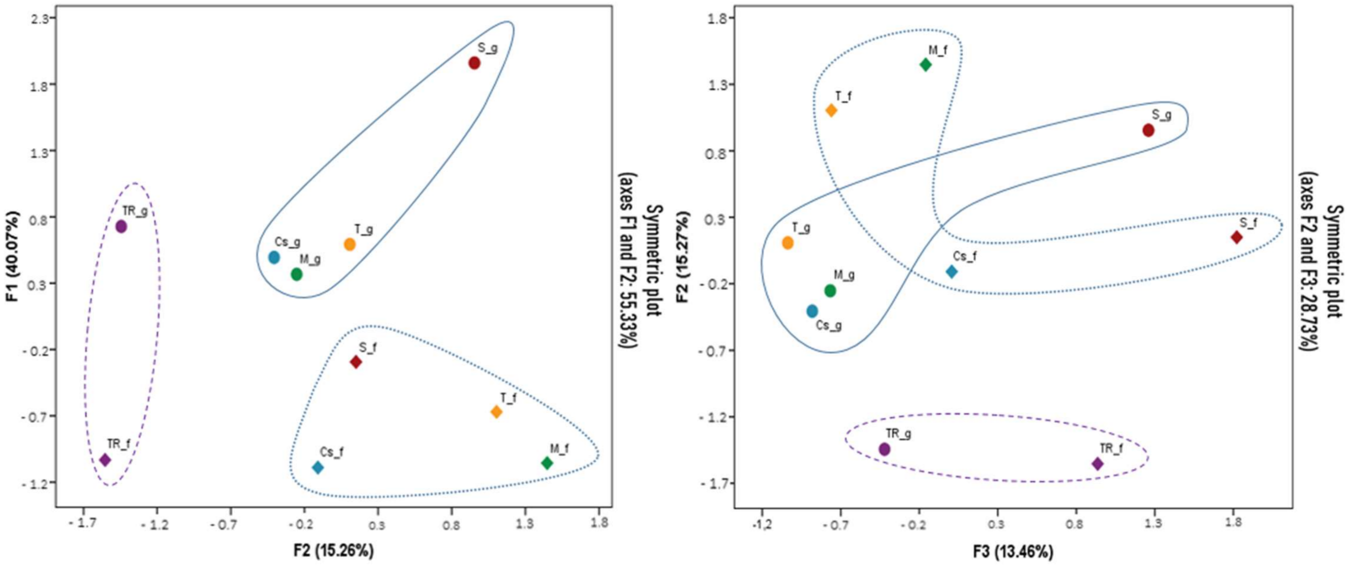


**Figure 6.** Principal Component Analysis (PCA) score plots of Tempranillo (T), Cabernet Sauvignon (Cs), Merlot (M), Syrah (S) and Tintilla de Rota (TR) grape musts glycosylated (g) and free (f), volatile compounds.

## 4. Conclusions

The results of the physicochemical analysis suggested an interesting potential for the production of young Tintilla de Rota red wines with low alcohol content and high total polyphenol content and color intensity compared to the universal varieties studied. On the other hand, in terms of total acidity and pH values, in general, differences were not observed between the different cultivars and Tintilla de Rota.

Furthermore, the evolution of volatile compounds in grape musts during the ripening period showed that the somatic variant Tintilla de Rota has a differentiated and interesting aromatic profile compared to the rest of the varieties. It includes a moderate content in C6 compounds and a high content in alcohols and aldehydes families, mainly due to the presence of 1-octanol associated with fruity aromas. In relation to aromatic alcohols, the somatic variant studied showed higher values for this family of compounds, and the higher content both in total and in the free form, mainly due to the high content of benzyl alcohol, related to floral aromas such as roses and sweets. Regarding terpenes and their derivatives, it should be noted that Tintilla de Rota presented an interesting value with a similar content to Syrah and Tempranillo, and considerably higher than Cabernet Sauvignon, with outstanding citronellol content. In terms of norisoprenoid content, Tintilla de Rota has values well below those of Syrah, although it has values similar to those of the other universal varieties. All these facts would suggest that the somatic variant Tintilla de

Rota could be presented as an interesting alternative with a different aromatic profile and optimal ripening and adaptability in a warm climate zone.

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