Abstract: The aim of this work was to analyze and compare the concentration of higher alcohols, esters, and acids in wines from Tempranillo and Tempranillo Blanco. Tempranillo Blanco is a new and little-studied white variety that originated from Tempranillo by a natural mutation. During three seasons, grapevines of both varieties were harvested, and nine wines were made from each. The volatile composition of the wines was determined by GC-MS. In the wines of both varieties, the content of higher alcohols was higher than those of esters and acids. Wines from Tempranillo Blanco had lower content of 2-phenylethanol, methionol, 1-hexanol, benzyl alcohol, and total higher alcohols, but higher hexyl acetate and ethyl decanoate than Tempranillo wines. Total ethyl esters and total esters were higher in Tempranillo wines due to the higher ethyl lactate and ethyl succinate content derive from the malolactic fermentation that was not made in Tempranillo Blanco. The content of hexanoic and octanoic acids and total acids was also higher in Tempranillo Blanco wines than in Tempranillo. This is one of the first studies carried out on the wine volatile composition of Tempranillo Blanco and therefore contributes to a better understanding of the oenological characteristics of this white variety.

Keywords: fermentative volatile compounds; red wines; white wines; Tempranillo; Tempranillo Blanco; GC-MS

1. Introduction

Aroma is one of the most important sensory attributes of grapes and wines [1,2]. The aroma constituents have been classified [3] according to their origin in: varietal aromas, come from the grape, and pre-fermentative aromas, formed from the harvest until the beginning of the alcoholic fermentation [4,5]; fermentative aromas, synthesized by yeasts in the alcoholic fermentation or by lactic bacteria in the malolactic fermentation [6,7]; and aging aromas, come from the wine conservation stage [8]. Among these compounds, fermentative volatile compounds quantitatively represent most of the constituents of the wine aroma and are mainly grouped in three families: higher alcohols, esters, and acids [9]. The fatty acids and their ethyl esters are, together with the higher alcohols and their acetate esters, the principal responsible for fermentation bouquet [10]. Due to the importance of fermentative volatile composition for wine quality, this parameter has been widely used to classify and authenticate wines mainly according to grape variety and/or geographical origin of the wines [11–18]. However, there are few works focused on differentiating white and red wines according to their fermentative volatile composition [19,20].

Tempranillo (Vitis vinifera L.), a red variety derived from two other varieties: Albillo Mayor and Benedicto, could have been born by a spontaneous hybridization in the last millennium, probably in the environment of the Ebro valley (Spain) [21]; while Tempranillo Blanco (Vitis vinifera L.) was originated from a natural mutation of a Tempranillo grapevine [22], being found in 1988 in an ancient vineyard of Murillo de Rio Leza location...
(La Rioja, Spain). This somatic variant of Tempranillo has been selected and enlisted into the Appellation d’Origine Contrôlée (A.O.C.) Rioja (Spain) as a new grapevine variety to be cultivated by the winegrowers of this region. Nowadays, Tempranillo is the most cultivated variety in the A.O.C. Rioja, and Tempranillo Blanco has become the second white variety most planted in the A.O.C. Rioja (12%), behind the Viura variety (70%). However, there are few scientific studies that focus on the characterization of the wine volatile composition of this grape white variety [23,24], and there is no work that studies the differences between the wine volatile composition of Tempranillo and its mutation, Tempranillo Blanco. There is a previous study, based on advanced machine learning techniques, in order to discriminate grapes and wines from these two grape varieties, using their chemical profiles (nitrogen, phenolic and volatile composition) over two seasons [25]. However, in this work, the fermentative volatile composition of the wines was not characterized in detail. Therefore, the aim of this work was to study the volatile composition of wines elaborated with Tempranillo Blanco grapes and compare it with one of the Tempranillo wines.

2. Materials and Methods

2.1. Tempranillo and Tempranillo Blanco Wine Samples

The wines were elaborated in the Experimental Winery of the Instituto de Ciencias de la Vid y del Vino (ICVV), following the traditional methods of the A.O.C. Rioja. The grapes were harvested in the ICVV vineyard manually at the optimum moment of technological maturity and transferred to the winery. Then, Tempranillo grapes (23.1 ± 1.0 °Brix; 13.6% ± 0.6% v/v) were destemmed, crushed, and were put into tanks with 50 mg of SO₂/kg of must in each tank. While Tempranillo Blanco grapes (22.8 ± 1.0 °Brix; 13.4% ± 0.7% v/v) were put into a pneumatic press (3.5 bar) to extract the must with which the tanks were filled. In addition, 50 mg/kg of total SO₂ was added to the Tempranillo Blanco musts, which were placed in a temperature-controlled chamber (7 °C for 24 h) to must settling. Thus, three different tanks of 30 L of capacity were used to carry out the alcoholic fermentation (AF) in both Tempranillo and Tempranillo Blanco musts, with the addition of selected Saccharomyces cerevisiae yeasts (Safoeno SC 22, Fermentis, Marcq-en-Barœul, France) at a dosage of 20 g/hL. The vinification process was performed in temperature-controlled rooms, one for Tempranillo at 18–24 °C and another one at 17 °C for Tempranillo Blanco musts. Measurements of temperature and density were carried out daily to follow the AF development. Once the AF was finished (when musts reached <2.5 g/L of residual sugar), the solid parts were removed and, in Tempranillo wines, Oenococcus oeni lactic acid bacteria (Viniflora CiNe, CHR Hansen, Hoersholm, Denmark), at a dosage of 1 g/hL, were inoculated to carry out the malolactic fermentation (MLF) in Tempranillo wines.

In the final wines, alcoholic degree and total acidity were determined according to the OIV official methods [26].

Thus, once the wines were finished (after MLF in the case of Tempranillo and after AF for Tempranillo Blanco), aliquots of each wine were taken and frozen at −20 °C for the subsequent determination of their volatile composition.

Wine production was repeated for three vintages (2017–2019), with three replicates per variety. Consequently, the results of wine volatile composition for each grape variety correspond to the study of 9 vinifications (n = 9).

2.2. Determination of Wine Volatile Compounds by GC-MS

The day before analysis, the Tempranillo and Tempranillo Blanco wines (stored in a Falcon) were removed from the freezer and placed in the refrigerator at a low and controlled temperature, and once defrosted, they were immediately processed in order to study their volatile composition, using the method described by Rubio-Bretón et al. [27], according to the method of Oliveira et al. [28]. In a tube of 10 mL, 8 mL of wine (centrifuged at 3220×g, during 15 min, at 4 °C), 10 µL of internal standard (2-octanol, Sigma-Aldrich, Madrid, Spain; concentration: 2.5 g/L in ethanol), and a magnetic stir bar were added. Extraction of wine volatile compounds was performed by stirring the sample (for 15 min) with 400 µL
of dichloromethane (Merck, Darmstadt, Germany). After cooling for 10 min at 0 °C, the organic phase was separated by centrifugation (5031 x g, 10 min, 4 °C), and the extract was recovered into a vial. Gas chromatographic determination of analytes was performed using a Gas Chromatograph (GC) 7890B with a Mass Detector (MS) 7000C (Agilent, Palo Alto, CA, USA). The volume of injection was 2 µL, in split mode (1:15). A VF-Wax 52 CB (60 m × 0.25 mm i.d. × 0.25 µm) capillary column (Agilent) was used. The temperature of the injector was programmed from 40 to 250 °C, at 180 °C/min. The oven temperature was held for 2 min at 50 °C, then programmed to rise at 3 °C/min from 50 to 250 °C. The detector was operated at electronic impact mode (70 eV), with an acquisition range (m/z) from 29 to 260. The identification of volatile compounds was carried out using the NIST library and by comparison with the mass spectrum of available standards (Sigma-Aldrich, Madrid, Spain). A semi-quantification was carried out, relating the areas of each volatile compound with the area and the known concentration of the internal standard.

2.3. Statistical Analysis

The statistical elaboration of the data was performed using SPSS Version 21.0 (IBM, Chicago, IL, USA). Volatile compounds data were processed using the variance analysis (ANOVA) (p ≤ 0.05). Boxplots were performed to support analyses of the volatile composition variability among and within Tempranillo (T) and Tempranillo Blanco (TB) wines since these plots are powerful graphical representations that provide an overview of the data set distribution [29].

3. Results and Discussion

The basic characteristics of the wines were: Tempranillo: alcoholic degree 14.4 ± 0.5 (% v/v) and total acidity 4.7 ± 0.6 (as g/L of tartaric acid); Tempranillo Blanco: alcoholic degree 13.3 ± 0.3 (% v/v) and total acidity 6.4 ± 0.1 (as g/L of tartaric acid).

3.1. Volatile Composition of Tempranillo and Tempranillo Blanco Wines

Figure 1 shows the concentration of higher alcohols in the wines made with the Tempranillo and Tempranillo Blanco grape varieties. All the alcohols were found in higher mean concentrations in the wines elaborated with the Tempranillo grape variety than in those corresponding to the Tempranillo Blanco ones. This difference was significant in the case of 2-phenylethanol, methionol, 1-hexanol, and benzyl alcohol (Figure 1c–e,g). Therefore, the total alcohol content was also significantly higher in red wines than in whites (Figure 1h). Aznar and Arroyo [19] carried out an exhaustive study of 17 white wines and 23 red wines, made from different white and red grape varieties and from 7 Spanish regions, observing that the red wines had a higher concentration of higher alcohols than the white wines. Likewise, Weldegergis et al. [20] analyzed 334 South African wines (110 white and 224 red) and also observed a higher concentration of higher alcohols in red wines. These results are in agreement with those observed for Tempranillo versus Tempranillo Blanco (Figure 1).

This group of compounds was the most abundant of the three studied, followed by esters and acids (Figures 1–3). Higher alcohols can be formed anabolically from sugars as well as catabolically from amino acids via the Ehrlich pathway [30]. Among the seven alcohols found in the wines (Figure 1a–g), only the concentration (relative to internal standard concentration) of 2-phenylethanol (Figure 1c) was found above its perception threshold (14 mg/L) in both white and red wines, providing rose aroma to the wines [31]. Regarding the total alcohols (Figure 1h), at concentrations below 300 mg/L, these compounds contribute to the desirable complexity of wine [32]; however, if its concentrations exceed 400 mg/L, they have a negative influence on wine aroma. In our wines, the concentration of total alcohols (relative to internal standard concentration) was much lower than this value, which is considered positive for wine quality.
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**Figure 1.** Higher alcohols concentration (mg/L relative to internal standard concentration) in Tempranillo and Tempranillo Blanco wines: (a) isobutanol; (b) isoamyl alcohols; (c) 2-phenylethanol; (d) methionol; (e) 1-hexanol; (f) (E)-3-hexenol; (g) benzyl alcohol; (h) total alcohols. All parameters are given as average values ± the standard deviations ($n = 9$). Different letters indicate significant differences between wines elaborated with the two grape varieties ($p \leq 0.05$). If there are no letters, it means that there were no significant differences between samples ($p > 0.05$).
Figure 2. Esters concentration (mg/L relative to internal standard concentration) in Tempranillo and Tempranillo Blanco wines: (a) isoamyl acetate; (b) 2-phenylethyl acetate; (c) hexyl acetate; (d) total acetate esters; (e) ethyl hexanoate; (f) ethyl octanoate; (g) ethyl decanoate; (h) total C6, C8, C10 ethyl esters; (i) ethyl lactate; (j) ethyl succinate; (k) total ethyl esters; (l) total esters. All parameters are given as average values ± the standard deviations (n = 9). Different letters indicate significant differences between wines elaborated with the two grape varieties (p ≤ 0.05). If there are no letters, it means that there were no significant differences between samples (p > 0.05).

The concentration of esters in the wines made with the two grape varieties, Tempranillo and Tempranillo Blanco, is shown in Figure 2. Acetate esters, with the exception of isoamyl acetate (Figure 2a), showed higher mean concentration in white wines, although this difference was only significant in the case of hexyl acetate (Figure 2c). The content of total acetate esters was practically the same in both types of wines (Figure 2d). This same trend was observed for the ethyl esters, whose content was higher in Tempranillo Blanco wines than in Tempranillo ones, and the difference increased as the ester chain increased, so that it became significant for the ethyl decanoate (Figure 2g). It is important to note that the sum of ethyl hexanoate, octanoate, and decanoate was higher in Tempranillo Blanco wines than in Tempranillo ones (Figure 2h), although the difference was not significant. These results are in agreement with those reported by other authors, who have observed that, in general, most of the ethyl and acetate esters are found to be at similar or higher contents in white wines compared to red ones [33,34].

In the white grape variety, malolactic fermentation (MLF) was not carried out; thus, the two esters related to this fermentation were practically not found in white wines, compared to the red wines (Figure 2i,j), in which malolactic fermentation was carried out. In this sense, the formation of ethyl lactate is directly related to the amount of lactic acid produced during the MLF [35,36]. Therefore, the concentration of total ethyl esters and total esters was higher in Tempranillo than in Tempranillo Blanco wines (Figure 2k,l).
The principal esters of wine are synthesized enzymatically by yeast during the alcoholic fermentation from alcohols and acids. Thus, acetyl-CoA is condensed with higher alcohols by the enzyme alcohol acetyltransferase to form acetate esters, and ethyl esters are formed by reaction between ethanol and fatty acids [10]. These esters are primarily responsible for the wine aroma, providing floral and fruity notes. However, ethyl esters of organic acids seem to play only a limited role in the organoleptic qualities of wines. Among the three acetate esters found in the wines (Figure 2a–c), both isoamyl acetate and 2-phenylethyl acetate were found at concentrations (relative to internal standard concentration) above their perception thresholds (0.03 and 0.25 mg/L, respectively) [37] in both types of wines (Figure 2a,b), giving notes of banana and roses, respectively. As for the ethyl esters (Figure 2e–g), two of them, ethyl hexanoate and ethyl octanoate (Figure 2e,f), presented concentrations (relative to internal standard concentration) above their olfactory detection thresholds (0.014 and 0.005 mg/L, respectively) [38], with pleasant floral and fruity notes. However, neither the ethyl lactate nor the ethyl succinate contributed to the wine aroma, as noted above.

In Figure 3, the concentrations of acids in the wines made with the Tempranillo and Tempranillo Blanco varieties are shown. Both the content of hexanoic and octanoic acids (Figure 3a,b) was significantly higher in white wines than in reds; therefore, the sum of both was higher in Tempranillo Blanco than in Tempranillo wines (Figure 3c). This result agrees with that reported by Weldegergis et al. [20], who observed that C6 and C8 acids were found in higher concentrations in white wines than in red ones.

The synthesis of these acids by yeasts is related to the metabolism of carbohydrates since glucose is the main source of its precursor, acetyl-CoA [10]. These compounds contribute with a fresh flavor to wine or an unpleasant flavor if they are in excess. The total acids content (relative to internal standard concentration) in both types of wines was so far lower than 20 mg/L (Figure 3c), that is the concentration where it is stated that these compounds impair wine aroma [35]. Both acids, hexanoic and octanoid acids.
(Figure 3a,b), showed, in both white and red wines, a concentration (relative to internal standard concentration) higher than their perception thresholds: 0.42 and 0.50 mg/L, respectively [38].

3.2. Boxplot Analysis of Tempranillo and Tempranillo Blanco Wine Volatile Composition

As can be seen in Figure 4, the variability in the content of higher alcohols was greater in Tempranillo wines than in Tempranillo Blanco ones. Likewise, the average content of these compounds was lower in all cases in the white variety, being their distribution practically symmetric, unlike what occurred for the red variety. The concentration of isobutanol, isoamyl alcohols, 2-phenylethanol, 1-hexanol, and total alcohols (Figure 4a–c,e,h) was higher than the mean concentration in most of the wines, while the opposite occurred for methionol and benzyl alcohol (Figure 4d,g), while the distribution for (E)-3-hexenol was symmetric (Figure 4f).

However, the mean content of esters was higher in the Tempranillo Blanco wines than in those of Tempranillo ones (Figure 5), with the exception of ethyl lactate and diethyl succinate (Figure 5i,j), which is logical due to these compounds are related to the MLF, as indicated above. Furthermore, the variability in the content of the esters was not as different between the two grape varieties as it was for the higher alcohols. Most of the wines, both red and white, had a higher concentration of acetate esters than the average content (Figure 5a–d). This same trend was observed for ethyl esters (Figure 5e–h), with the exception of ethyl decanoate in Tempranillo Blanco, whose concentration in most of the wines was lower than the average content (Figure 5g).

Regarding ethyl lactate and diethyl succinate in Tempranillo (Figure 5i,j), the first showed a fairly symmetric distribution, while for the second ester, its concentration was lower than the average content in most of the wines.

In the content of total ethyl esters and total esters (Figure 5k,l), ethyl lactate and diethyl succinate have an important weight, so their concentrations were higher in the red wines than in the white ones.

Finally, as can be seen in Figure 6, the average content of hexanoic acid was clearly higher in Tempranillo Blanco wines than in Tempranillo wines (Figure 6a), while in the case of octanoic acid, its average concentration was practically the same in both wines (Figure 6b), so that the total acids mean content was higher in white wines than in red ones (Figure 6c). The distribution in the concentration of the acids was inverse, since the majority of the Tempranillo wines showed a lower concentration than the average content and, on the contrary, in the majority of the Tempranillo Blanco wines, the concentration of the acids was higher than their average content (Figure 6).

![Figure 4. Cont.](image-url)
Figure 4. Boxplots for the higher alcohols concentration (mg/L relative to internal standard concentration) in the Tempranillo (T) and Tempranillo Blanco (TB) wines: (a) isobutanol; (b) isoamyl alcohols; (c) 2-phenylethanol; (d) methionol; (e) 1-hexanol; (f) (E)-3-hexenol; (g) benzyl alcohol; (h) total alcohols.

However, the mean content of esters was higher in the Tempranillo Blanco wines than in those of Tempranillo ones (Figure 5), with the exception of ethyl lactate and diethyl succinate (Figure 5i,j), which is logical due to these compounds are related to the MLF, as indicated above. Furthermore, the variability in the content of the esters was not as different between the two grape varieties as it was for the higher alcohols. Most of the wines, both red and white, had a higher concentration of acetate esters than the average content (Figure 5a–d). This same trend was observed for ethyl esters (Figure 5e–h), with the exception of ethyl decanoate in Tempranillo Blanco, whose concentration in most of the wines was lower than the average content (Figure 5g).
The white variety Tempranillo Blanco has been presented as a promising variety for making quality wines. However, it would be necessary to conduct more studies from this white variety to know the desired wines to be produced in the wineries or demanded by the consumers. Thus, during three consecutive vintages, grapes from plants of both varieties that were planted in different growing areas in order to observe the consistency of the wine volatile composition from its variety and its adaptability to the environmental conditions and to the desired wines to be produced in the wineries or demanded by the consumers. Therefore, this study adds valuable information that might contribute to knowing the Tempranillo Blanco wines' volatile composition.

On the other hand, the malolactic fermentation carried out on the red wines marked the difference in total esters between the two varieties, being higher in Tempranillo than in Tempranillo Blanco wines, mainly with respect to their volatile compounds. In this work, the fermentation of red Tempranillo Blanco and Tempranillo wines was studied and compared. There are few available studies that allow knowing the potential of Tempranillo Blanco wines, mainly with respect to their volatile compounds. In this work, the fermentation of red Tempranillo Blanco and Tempranillo wines was studied and compared.

Regarding ethyl lactate and diethyl succinate in Tempranillo (Figure 5i,j), the first ester shows a fairly symmetric distribution, while for the second ester, its concentration was lower than the average content in most of the wines. Furthermore, there was a significant difference in total esters between the two varieties, being higher in Tempranillo than in Tempranillo Blanco wines, mainly with respect to their volatile compounds. In this work, the fermentation of red Tempranillo Blanco and Tempranillo wines was studied and compared.

In the content of total ethyl esters and total esters (Figure 5k,l), ethyl lactate and diethyl succinate have an important weight, so their concentrations were higher in the red wines than in the white ones. Finally, as can be seen in Figure 6, the average content of hexanoic acid was clearly presented higher 2-phenylethanol (with rose aroma) content than its perception threshold. In the majority of the Tempranillo wines, the concentration of the acids was higher than their average content (Figure 6).

There are few available studies that allow knowing the potential of Tempranillo Blanco wines, mainly with respect to their volatile compounds. In this work, the fermentation of red Tempranillo Blanco and Tempranillo wines was studied and compared. There are few available studies that allow knowing the potential of Tempranillo Blanco wines, mainly with respect to their volatile compounds. In this work, the fermentation of red Tempranillo Blanco and Tempranillo wines was studied and compared. There are few available studies that allow knowing the potential of Tempranillo Blanco wines, mainly with respect to their volatile compounds. In this work, the fermentation of red Tempranillo Blanco and Tempranillo wines was studied and compared. There are few available studies that allow knowing the potential of Tempranillo Blanco wines, mainly with respect to their volatile compounds. In this work, the fermentation of red Tempranillo Blanco and Tempranillo wines was studied and compared.

**Figure 5.** Boxplots for the esters concentration (mg/L relative to internal standard concentration) in the Tempranillo (T) and Tempranillo Blanco (TB) wines: (a) isoamyl acetate; (b) 2-phenylethyl acetate; (c) hexyl acetate; (d) total acetate esters; (e) ethyl hexanoate; (f) ethyl octanoate; (g) ethyl decanoate; (h) total C6, C8, C10 ethyl esters; (i) ethyl lactate; (j) ethyl succinate; (k) total ethyl esters; (l) total esters.

**Figure 6.** Boxplots for the acids concentration (mg/L relative to internal standard concentration) in the Tempranillo (T) and Tempranillo Blanco (TB) wines: (a) isoamyl acetate; (b) 2-phenylethyl acetate; (c) hexyl acetate; (d) total acetate esters; (e) ethyl hexanoate; (f) ethyl octanoate; (g) ethyl decanoate; (h) total C6, C8, C10 ethyl esters; (i) ethyl lactate; (j) ethyl succinate; (k) total ethyl esters; (l) total esters.

**Figure 6. Cont.**
Regarding the volatile composition, higher alcohols were more abundant in both Tempranillo Blanco and Tempranillo wines than esters and acids. Tempranillo Blanco wines had lower content of some higher alcohols, such as 2-phenylethanol, methionol, 1-hexanol, and benzyl alcohol, than Tempranillo wines, but wines from both varieties presented higher 2-phenylethanol (with rose aroma) content than its perception threshold. On the other hand, the malolactic fermentation carried out on the red wines marked the difference in total esters between the two varieties, being higher in Tempranillo than in the white variety. However, the average concentration of esters, especially acetate esters and C6, C8, and C10 ethyl esters, was higher in Tempranillo Blanco than in Tempranillo wines, contributing to floral and fruits perception on these wines. In addition, the mean concentration of the acids increased in Tempranillo Blanco wines with respect to those from Tempranillo.

Tempranillo Blanco has been presented as a promising variety for making quality white wines from a new short-cycle variety. Therefore, this study adds valuable information that might contribute to knowing the Tempranillo Blanco wines’ volatile composition. However, it would be necessary to conduct more studies from this white variety planted in different growing areas in order to observe the consistency of the wine volatile composition from its variety and its adaptability to the environmental conditions and to the desired wines to be produced in the wineries or demanded by the consumers.

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