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Volatile composition of Mencía wines

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Abstract

Monovarietal Mencía wines from two different Appellation of Origin Controlled (AOC) of Galicia (NW Spain) were analyzed. The content of some varietal and fermentative volatile compounds was determined by gas-chromatography and GC–mass spectrometry. These red wines, independently of their origin, showed terpene profiles, that were typical of their varietal characteristics with an important contribution of linalool, citronellol, α-pinene and β-pinene. Other important varietal compounds were: norisoprenoids (α- and β-ionone, and theaspirane), phenyl-ethanol and benzyl alcohol. The 67 analytical variables were submitted to analysis of variance, and the results showed that only nine volatile compounds were significantly different ($p < 0.05$) among the two AOC (*trans*-3-hexenol, 1-butanol, isobutanol, ethyl acetate, hexyl acetate, butyric acid, γ-butyrolactone, methionol, and *N*-(2-hydroxy-ethyl)-acetamide). Principal component analysis showed the differentiation of wines according to geographical areas.

Keywords: Mencía variety; Red wine; Volatile composition; Characterization; Differentiation

1. Introduction

The winemaking sector is one of the principal economic sources of Galicia (NW Spain). Vineyards are found in all four of the Galician provinces, but 86% of the cultivated area, representing 87.9% of total production, is found in Ourense and Pontevedra. Five Appellations of Origin Controlled (AOC) exist in this area: 'Rías Baixas' in the province of Pontevedra, 'Ribeiro', 'Valdeorras' and 'Monterrei' in the province of Ourense, and 'Ribeira Sacra' between the provinces of Ourense and Lugo.

'Monterrei' is the smallest AOC in Galicia, but it has tripled its production of wine in the last few years. This zone has a continental climate, Mediterranean type, with an average temperature of 12.6 °C and a rainfall of 683 mm/year. Wine production is equally divided between red and white wines, Mencía being the preferential red variety. Its red wines have a purple or cherry-red colour, with evident violet tones, and an aroma of red fruit is noticeable.

'Ribeira Sacra' has doubled its production in the last few years and is the principal AOC producer of red wines of the Galician community. The average temperature of this region is 13–14 °C and rainfall reaches 700–900 mm/year. The Mencía red variety is the most important in this AOC and these wines have a deep and brilliant cherry colour. Their bouquet is fruity (with nuances of raspberry and blackberry), with a medium to long intensity.

During the last few years, there has been a growing interest within the Galician winemaking industry, in the recuperation of the denominated autochthonous or noble varieties, in order to endow differentiated and singular characteristics to the Galician wines (Hernáez Mañas, 1993). In the specific case of red wines produced from the Mencía variety, there are only three studies. Of these, two have studied their content of certain metals (Núñez, Peña, Herrero, & García-Martín, 2000; Peña, Latorre, García, Botana, & Herrero, 1999) and in the third, allusion is made to the volatile fraction as a basis of differentiation between Mencía wines from the AOC 'Ribeira Sacra' and the red wines produced in the rest of Galicia (Rebolo, Peña, Latorre, García, Botana, & Herrero, 2000). Given the lack of existing information, the first objective of this study has been to identify and

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quantify the principal volatile compounds present in the young red wines produced from Mencía grapes. Wines from the AOC 'Monterrei' and the AOC 'Ribeira Sacra' have been analyzed in order to establish an aromatic pattern for the Mencía variety. The second purpose of this study was to check whether or not there are differences between the wines from these two AOC, and a multivariate analysis was carried out in order to evaluate this possible differentiation between the Mencía wines according to the each production area.

2. Materials and methods

2.1. Wine samples

Six different monovarietal Mencía wines investigated in duplicate in this study were kindly donated by some wineries from two Galician AOC ('Ribeira Sacra' and 'Monterrei'). Production techniques were uniform for all wines studied, and all wine samples were collected six months after winemaking and then analyzed. Two sub-samples have been collected in each winery for the same wine, and since no significant differences were found, the mean results were used as a basis for this work.

2.2. Volatile compound analysis

Monoterpene alcohols, hexenols and hexanols were extracted as follows: a sample of 100 ml of wine was adjusted to pH 7, by the addition of NaOH, and 1 ml of 3-octanol (10 mg/l) was added as an internal standard. The sample was extracted three times (10, 5 and 5 ml) with diethyl ether-pentane (1:1, v/v). This organic extract was concentrated to 0.5 ml, under nitrogen. A Hewlett–Packard 5890 gas chromatograph, with flame ionization detector (FID) and equipped with a HP-Innowax (60 m × 0.25 mm i.d., film thickness 0.25 μm) capillary column was used. One μl sample of the extract was injected in splitless mode (30 s). Temperature program: Held 1 min at 45 °C, raised at 3 °C/min to 230 °C, and held for 25 min. Helium was used as the carrier gas (18 psi). Temperature of the injector and detector was 230 °C.

Methanol and the higher alcohols were determined by direct injection, using a Carbowax 1540 column (7.5 m × 2.3 mm i.d.) in a Hewlett–Packard 5890 gas chromatograph (FID detection) at 80 °C isothermic temperature (carrier gas, hydrogen at 8 psi), following the method described by Bertrand (1968).

Fatty acids and their ethyl esters, and acetates were extracted according to the method described by Bertrand (1981): 2 ml of 3-octanol (50 mg/l) as internal standard and 1 ml of sulphuric acid (1/3) were added to 50 ml of wine. These were extracted three times (4, 2 and 2 ml) with diethyl ether–hexane (1:1, v/v). One μl of the organic extract was injected into Hewlett–Packard 5890 gas chromatograph, with FID, under the same capillary column and chromatographic conditions indicated for the terpenes.

Volatile phenols were extracted according to the method proposed by Chatonnet and Boidron (1988). After adjustment of the ionic strength of the medium with ammonium sulphate and addition of an internal standard (3,4-dimethylphenol), liquid–liquid extraction with dichloromethane was performed. The organic extract obtained was washed, and then extracted with NaOH (pH 13). Hydrochloric acid was added to the aqueous phase and this was extracted three times with diethyl ether. The final organic extract was concentrated under nitrogen and injected in the same capillary column and chromatographic conditions as the compounds cited above.

Samples containing the internal standard and the standard compounds at similar concentration of wine were also treated in the same way as the wine samples, and the final calculations are referred to the basis of the concentration of this reference solution. Determination of the different compounds was made in triplicate. The identification of the volatile compounds was confirmed by comparing either their mass spectra (MS Chemstation Wiley 7N library) and their retention times with those of standards. Three μl of each concentrated organic extract was injected in duplicate in splitless mode (purge time, 30 s; purge rate, 70) in a Hewlett–Packard HP 5890-II gas chromatograph coupled to a Hewlett–Packard HP 5970 mass spectrometer. Spectra were recorded in the electron impact mode (ionization energy, 70 eV; source temperature, 250 °C), using an HP-Innowax column (60 m × 0.25 mm i.d.; film thickness 0.25 μm). The carrier gas was helium (18 psi). The temperature program was isothermal at 45 °C for 1 min, then 3 °C/min to 230 °C with a final isotherm of 25 min. The acquisition was made in scanning mode (mass range, 30–300 amu, 1.9 spectra/s).

2.3. Statistical analysis

Significant differences of the triplicate analysis for each sample, among the two wines from the same winery and among the wines from different AOC were carried out by analysis of variance (ANOVA) by using the Excel 5.0 program. Statistical comparisons between the triplicate analysis for each wine and between the two sub-samples of each wine were made using the Student's *t*-test. Fischer's least significant differences (LSD) was used to evaluate the significance of the analysis between the wines from different geographical area of production. Principal component analysis (PCA) was performed using Statistica 5.0. program (StatSoft, Inc.) in order to find the possible differentiation between wines according to geographical origin.

3. Results and discussion

The average concentrations and the standard deviations for the 56 variables or the normalized peak area for the other 11 compounds are illustrated in Tables 1–4, and 5 respectively, which correspond to the average of the three wines from each AOC. The wines sampled from this red Galician variety showed a similar volatile profile and, only nine volatile compounds showed significant differences at the 95% level according to geographical area of origin (*trans*-3-hexenol, 1-butanol, isobutanol, ethyl acetate, hexyl acetate, butyric acid, γ-butyrolactone, methionol, and *N*-(2-hydroxy-ethyl)-acetamide). Samples from 'Ribeira Sacra' AOC possess higher isobutanol, ethyl acetate, butyric acid and γ-butyrolactone contents than do Mencía wines from 'Monterrei' AOC, whereas their contents in *trans*-3-hexenol, 1-butanol, hexyl acetate, methionol and *N*-(2-hydroxy-ethyl)-acetamide are lower.

The terpene content of a wine is considered to be a positive quality factor. This is because they contribute to its varietal aroma, serve to differentiate it from other varieties, and supply floral nuances to the wine (Marais, 1991). These concentrations were not significantly different between the two AOC. Among the most relevant monoterpene alcohols found in Mencía wines, is linalool (Table 1), with contents that range from 229 to 109 µg/l. In all cases, this is well above its perception threshold of 50 µg/l (Ribéreau-Gayon, Boidron, & Terrier, 1975). The content of this compound was higher in the wines from the AOC 'Ribeira Sacra'.

Citronellol is also present in all the samples at concentrations above or equal to its perception threshold of 18 µg/l (Ribéreau-Gayon et al., 1975) and will certainly also contribute to the aroma of the Mencía wines. The wines from the AOC 'Monterrei' have much higher concentrations of nerol than samples from AOC 'Ribeira Sacra', and α-terpineol, geraniol and terpene-4-ol were

Table 1
Concentration in terpenes and norisoprenoids (µg/l) in Mencía wines

Compound	'Monterrei' AOC		'Ribeira Sacra' AOC	
	Average[a]	SD	Average[a]	SD
Linalool	142	24	182	44
Nerol	164	26	58	5
Geraniol	54	3	62	8
Citronellol	24	3	28	8‘
α-Terpineol	66	8	80	23
Terpinen-4-ol	28	6	25	0
Citronellal	106	23	100	4
α-Pinene	195	45	156	36
β-Pinene	171	14	65	17
Theaspirane	40	8	35	11
α-Ionone	7	5	6	4
β-Ionone	35	15	32	5

[a] Average of three different wine samples and SD (standard deviation).

Table 2
Concentration in alcohols (mg/l) in Mencía wines

Compound	'Monterrei' AOC		'Ribeira Sacra' AOC	
	Average[a]	SD	Average[a]	SD
1-Hexanol	3.35	0.49	3.09	0.07
3-Hexanol	21	1	26	1
trans-3-Hexenol*	0.16	0.01	0.10	0.02
cis-3-Hexenol	0.31	0.07	0.27	0.01
trans-2-Hexenol	0.03	0.00	0.02	0.00
Metanol	121	8	138	10
1-Butanol*	6	1	1	1
2-Butanol	n.d.	–	1	1
1-Propanol	34	5	27	2
Isobutanol*	41	2	64	4
Isoamyl alcohols	254	42	264	1
2-Phenyl-ethanol	25	8	16	3
Benzyl alcohol	8	0	8	1

n.d., not detected.
[a] Average of three different wine samples and SD (standard deviation).
* Significantly different at $p < 0.05$.

Table 3
Concentration in acetates, ethyl esters and fatty acids (mg/l) in Mencía wines

Compound	'Monterrei' AOC		'Ribeira Sacra' AOC	
	Average[a]	SD	Average[a]	SD
Ethyl acetate*	45	1	56	4
Isoamyl acetate	0.368	0.106	0.273	0.004
Hexyl acetate*	0.044	0.015	n.d.	–
Phenyl-ethyl acetate	0.289	0.048	0.057	0.028
Ethyl butyrate	0.46	0.01	0.48	0.06
Ethyl hexanoate	0.19	0.00	0.18	0.03
Ethyl octanoate	0.21	0.00	0.20	0.02
Ethyl decanoate	0.07	0.00	0.06	0.00
Ethyl dodecanoate	0.52	0.37	0.38	0.27
Ethyl tetradecanoate	0.14	0.01	0.13	0.01
Ethyl lactate	37	8	56	7
Diethyl succinate	1.84	0.80	2.80	0.13
Isobutyric acid	1.30	0.21	1.28	0.07
Butyric acid*	1.58	0.04	2.60	0.34
Isovaleric acid	0.84	0.04	0.60	0.13
Valeric acid	n.d.	–	n.d.	–
Hexanoic acid	1.11	0.04	1.00	0.15
Heptanoic acid	n.d.	–	0.16	0.11
Octanoic acid	1.00	0.06	0.88	0.07
Nonanoic acid	0.45	0.32	0.33	0.23
Decanoic acid	0.27	0.01	0.32	0.02
Dodecanoic acid	n.d.	–	0.03	0.00
Tetradecanoic acid	0.21	0.15	0.09	0.01
Hexadecanoic acid	1.29	0.49	0.59	0.25

[a] Average of three different wine samples and SD (standard deviation).
* Significantly different at $p < 0.05$.

Table 4
Concentration in volatile phenols (mg/l) in Mencía wines

Compound	'Monterrei' AOC		'Ribeira Sacra' AOC	
	Average[a]	SD	Average[a]	SD
Eugenol	0.034	0.010	0.032	0.015
4-Ethyl-phenol	1.568	0.191	0.557	0.153
Siringol	0.238	0.169	0.448	0.002
Vanillin	0.109	0.050	0.187	0.108
Ethyl-vanillate	0.319	0.098	0.202	0.032
Acetovanillone	0.346	0.091	0.298	0.260
4-Ethyl-guaiacol	0.171	0.121	0.144	0.102

[a] Average of three different wine samples and SD (standard deviation).

Table 5
Normalized peak area[a] in lactones, aldehydes and sulphur compounds in Mencía wines

Compound	'Monterrei' AOC		'Ribeira Sacra' AOC	
	Average[b]	SD	Average[b]	SD
γ-Butyrolactone*	552	3	694	10
γ-Ethoxybutyrolactone	n.d.	–	8	6
Pantolactone	27	1	30	21
γ-Hexalactone	n.d.	–	3	2
γ-Heptalactone	n.d.	–	15	3
Benzaldehyde	23	12	57	2
Furfural	27	3	48	34
Methionol*	1797	99	351	163
N-(2-Hydroxy-ethyl) acetamide*	84	10	n.d.	–
N-(3-Methyl-buthyl) acetamide	953	108	1437	112
N-(2-Phenyl-ethyl) acetamide	266	21	608	184

n.d., not detected.

[a] (Compound surface/internal standard surface) × 100.

[b] Average of three different wine samples and SD (standard deviation).

* Significantly different at $p < 0.05$.

present in similar concentrations in all the analyzed Mencía wines.

The only terpenic aldehyde that has been quantified, citronellal (strongly scented, but more aggressive with regard to aroma than its corresponding alcohol), has been detected at the same level in wines from the two AOC. As for the terpenic hydrocarbons, the highest content of α-pinene and its isomer, β-pinene, appeared in the wines from the AOC 'Monterrei'.

In general, the presence of norisoprenoids is also considered to be a quality factor and typical from each variety, as they supply an agreeable scent of tobacco, fruits, tea, etc. (Schreier, 1984). Also, although usually present in very low amounts (a few μg/l), as their perception threshold is very low, they play an important part in the aroma (Etiévant, Issanchou, & Bayonove, 1983; Razungles, Bayonove, Cordonnier, & Sapis, 1988). Among the norisoprenoids that were identified (Table 1), β-ionone stands-out with a much higher content than its perception threshold of 0.007 ng/l in water (Bayonove, Baumes, Crouzet, & Günata, 2000) and supplies an aroma of violets. α-ionone has only been identified in two of the six analyzed wine samples. Theaspirane, excepting for one sample from the AOC 'Ribeira Sacra', was present in higher contents in the samples from the AOC 'Monterrei'.

The alcohols with six carbon-atoms, hexenols and hexanols, supply "vegetal" and "herbaceous" nuances to the wine. This, depending on their concentration, could constitute a defect. In the case of 1-hexanol (Table 2), the highest content of this compound were present in wines from the AOC 'Monterrei'. The contents of 3-hexanol were around 25 mg/l in almost all the samples, independently of the AOC. As for the hexenols, the highest concentrations of *trans*-3-hexenol (significantly different at the 95% level) were obtained in the samples from the AOC 'Monterrei'. Its isomer, *cis*-3-hexenol was present also at a highest concentration in samples from the AOC 'Monterrei'. *trans*-2-Hexenol was present at lowest concentrations, but at similar levels in the six samples analyzed.

Identical values of isoamyl alcohol were observed in all the analyzed wines from the AOC 'Ribeira Sacra' (264 mg/l), while in those from AOC 'Monterrei' these values were slightly lower. The highest contents of isobutanol (significantly different at the 95% level) correspond to the samples from the AOC 'Ribeira Sacra', while those from the wines from the AOC 'Monterrei' were only around 40–45 mg/l. 1-butanol has practically only been identified in the samples from the AOC 'Monterrei' (significantly different at the 95% level) and 2-butanol was only detected in one sample from the AOC 'Ribeira Sacra'. The methanol content, at levels above 130 mg/L, was high in any of the two AOC.

The contents of 2-phenyl-ethanol were notably higher in the AOC 'Monterrei' and the other varietal alcohol, benzyl alcohol, appeared in concentrations that were similar in all the samples. There are no important differences between the different AOC, and so the nuance of "blackberry" that this supplies appears to be typical of the variety Mencía.

The ethylic esters of the fatty acids and the acetates of the higher alcohols are two groups of compounds of undoubted importance in the wine aroma, as their nuances coincide with the fruity descriptors of the wines and, in the case of the red wines, play a modulating role in the quality (Ferreira, Fernández, Peña, Escudero, & Cacho, 1995). The content of these two families of compounds are shown in Table 3. The concentration of ethyl acetate was significantly different at the 95% level and was slightly lower in wines from AOC 'Monterrei'. Isoamyl acetate, with a characteristic odour of "banana", was found at similar values and above its perception threshold in all the samples (0.16–1 mg/l) (Etiévant, 1991). The values obtained for the AOC 'Monterrei' were slightly higher than those of the other AOC. Hexyl acetate was only detected (significantly different at the 95% level) in the wine samples from the AOC 'Monterrei' and phenyl-ethyl acetate was also present at much higher levels in the samples from this AOC.

Between the ethylic esters that it has been possible to identify, ethyl butyrate, hexanoate, octanoate and dodecanoate, were those found at the highest concentrations in the six analyzed wines, although at levels scarcely above 0.5 mg/l. They have similar concentrations independently of the AOC of origin. However, the highest concentrations of ethyl lactate and diethyl succinate correspond to the wines from AOC 'Ribeira Sacra'.

Within the family of fatty acids (Table 3) isobutyric, butyric, hexanoic and octanoic acids were notable for their higher concentrations. The butyric acid was the only one that showed a pattern of higher concentrations (significantly different at the 95% level) in the wines from the AOC 'Ribeira Sacra', although, as a group, the samples from the AOC 'Monterrei' present a higher concentration of fatty acids.

The volatile phenols are another family of substances that play an important role in the aroma and are responsible for certain nuances reminiscent of "species". As can be seen in Table 4, the content of eugenol was similar in the wines from the two AOC and, in all the analyzed samples, this phenol was found at concentrations higher than its perception threshold of 5–15 μg/l (Chatonnet, Barbe, Canal-Llauberes, Dubourdieu, & Boidron, 1992). For this reason a nuance of "cloves" will almost certainly be noted in Mencía wine. Ethyl-vanillate and acetovanillone were also present at concentrations higher than their perception threshold in the wines from the AOC 'Monterrei'. 4-Ethyl-phenol was also present at a much higher level than its perception threshold (0.650 mg/l, for red wines) (Boidron, Chatonnet, & Pons, 1988) in these samples. Etiévant, Issanchou, Marie, Ducruet, and Flanzy (1989) have demonstrated the positive contribution of this compound to red wines at concentrations of between 1.2 and 2.4 mg/l. 4-Ethyl-guiacol has only been detected in three wines, but at contents lower than its perception threshold of 350 μg/l (Boidron et al., 1988). The content of vanillin was higher in the wines from the AOC 'Ribeira Sacra', although lower than its perception threshold of 400 μg/l (Boidron et al., 1988).

In Table 5, the results are expressed as the normalized peak area, which is the ratio between the areas of each substance and the peak of the internal standard. The γ-butyrolactone was the only lactone identified in all the analyzed samples. The wines from the AOC 'Monterrei' showed similar normalized peak area values between themselves that were significantly lower ($p < 0.05$) than those in the wines from the AOC 'Ribeira Sacra'. Of the rest of the lactones identified, pantolactone, which was detected in three samples, can be highlighted. The normalized peak area levels of benzaldehyde and furfural were also higher in the samples from the AOC 'Ribeira Sacra'. On the contrary, the only sulphur compound identified by mass spectrometry in all the analyzed wines, methionol (3-methylthio-propanol), was detected at higher and significantly different ($p < 0.05$) levels in the wines from the AOC 'Monterrei'.

The primary amines that are present in the fermenting medium are acetylated into acetamides by the yeast (Schreier, Drawert, & Junker, 1975). As can be seen in Table 5, the *N*-(2-hydroxy-ethyl)-acetamide has only been identified in samples from the AOC 'Monterrei'. On the contrary, the highest values of normalized peak area of *N*-(3-methyl-buthyl)-acetamide and *N*-(2-phenyl-ethyl)-acetamide were obtained in the samples from the AOC 'Ribeira Sacra'.

Principal component analysis was performed on the complete data set. When a two-dimensional plot (Fig. 1) was drawn, a natural separation of the wines according to their geographical origin was achieved. The two principal components accounted for 82.07% and 15.91% of the variance, respectively. The second PC was responsible for the separation between Mencía wines from 'Monterrei' and 'Ribeira Sacra' AOC.

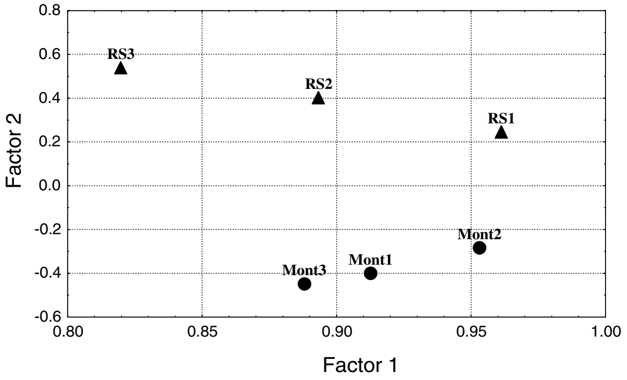

Fig. 1. First and second principal components representation of Mencía wines (Mont, wines from AOC 'Monterrei'; RS, wines from AOC 'Ribeira Sacra').

4. Conclusions

The results obtained from the analysis of the red Galician variety Mencía (from the Appellations of Origin Controlled 'Ribeira Sacra' and 'Monterrei') showed that these monovarietal wines present a high content of diverse families of varietal compounds; substances that can confer typical aromatic connotations, which allows their differentiation from other varieties of red wines. The content of this group of substances is very similar in the wines from both of the AOC, as is also the presence of monohydroxilated alcohols (such as linalool and citronellol) that are found at levels higher than their perception thresholds; the diverse terpenic hydrocarbons such as α- and β-pinene; the presence of various norisoprenoids, such as theaspirane, α- and β-ionone; and the content at which 2-phenyl-ethanol, that provides an aroma of "roses" and benzyl alcohol that endows the wine with a nuance of "blackberry", was detected. Likewise, the presence of other pre- and fermenting aromatic compounds, such as hexanols and hexenols, other alcohols, acetates, ethyl esters, fatty acids, and some volatile phenols, is also important, as many of them surpass their respective perception thresholds and therefore also contribute to the bouquet of these wines.

With respect to each of the AOC, the wines from the AOC 'Ribeira Sacra' are richer in compounds of varietal origin (terpenes, norisoprenoids and varietal alcohols), while the samples from AOC 'Monterrei' show higher contents of the compounds of pre-ferment or fermenting origin, with the exception of ethyl acetate, ethyl lactate, diethyl succinate and the aldehydes (benzaldehyde and furfural).

The volatile compounds quantified in this wine variety (which implies varietal, soil and climatic differences) do not depend on the geographic origin of the wines, but when all volatile compounds were analyzed by PCA, a geographic differentiation of two distinct groups has been achieved according to some aroma compounds (*trans*-3-hexenol, 1-butanol, isobutanol, ethyl acetate, hexyl acetate, and butyric acid) depending on viticultural and enological practices, such as harvesting, crushing, yeast or fermentation temperature (winery practices).

In order to corroborate to which substances is due the typical aroma presented by wines produced from the Mencía red grape variety, subsequent studies will be directed to the determination of the aromatic descriptors by a panel of wine-tasters. Simultaneously, a study will be made of the aroma using gas chromatography–olfactometry. Although a good classification according to geographical origin was obtained by PCA, this may be the result of a small number of wines, so a larger sample of wines should be examined in order to confirm the results obtained in this work.

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