

C₆-alcohols as varietal markers for assessment of wine origin

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Abstract

A significant part of the compounds present in wines having six carbon atoms, the C₆-compounds, derive from grape polyunsaturated fatty acids (primarily originated from membrane lipids), namely linoleic and α -linolenic acids, through a cascade of enzymatic reactions. This biochemical pathway yield C₆-aldehydes, which are subsequently reduced to C₆-alcohols, which can, in turn, be esterified to produce esters. As the C₆-compounds derive from varietal precursors, they could hypothetically contribute to judge wine origin and affiliation. In this way, two C₆-alcohols, (*E*)-3-hexenol and (*Z*)-3-hexenol, have been referred as the most important because its ratio can act as an indicator of the variety of origin.

This study presents the results, concerning the concentration of the three main C₆-alcohols, 1-hexanol, (*E*)-3-hexenol and (*Z*)-3-hexenol, as well as ratios between them, for 43 monovarietal wines from *Vinhos Verdes* demarcated region, belonging to six white – *Alvarinho* (8), *Arinto* (1), *Avesso* (9), *Azal* (1), *Loureiro* (17) and *Trajadura* (4) – and three red – *Amaral* (1), *Borraçal* (1) and *Vinhão* (1) – grape varieties. Wines were produced at experimental scale using slightly different winemaking practices and representing various *terroirs* and vintages, being analyzed after different conservation periods.

The results showed that (*E*)-3-hexenol/(*Z*)-3-hexenol ratio clearly discriminates *Loureiro* wines from those of *Alvarinho*, *Avesso* and *Trajadura*. Moreover, 1-hexanol/(*E*)-3-hexenol and 1-hexanol/(*Z*)-3-hexenol ratios may also be able to discriminate *Vinhos Verdes* monovarietal wines, and can act on a second level differentiation. The remaining monovarietal wines produced results which may be observed as indicative, since only one sample of each was analysed.

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1. Introduction

Wines with Appellation of Origin *Vinhos Verdes* are produced in a large area in Portugal composed by nine sub-regions (Amarante, Ave, Baião, Basto, Cávado, Lima, Monção, Paiva and Sousa). There are seven recommended white grape varieties (*Alvarinho*, *Arinto*, *Avesso*, *Azal*, *Batoca*, *Loureiro* and *Trajadura*) and eight red grape varieties (*Amaral*, *Borraçal*, *Brancelho*, *Espadeiro*, *Padeiro-de-Basto*, *Pedral*, *Rabo-de-Ovelha* and *Vinhão*) to produce these wines. Monovarietal wines play an important role in the economy of *Vinhos Verdes* region, since it is frequent to find it from the white varieties *Alvarinho*, *Arinto*, *Azal*, *Loureiro* and *Trajadura* as well as from the red cultivars, *Espadeiro* and *Vinhão*.

During last decades, several studies were conducted in order to distinguish grape varieties. Monoterpenic compounds both in free or in glycosidically bound form [1–4], amino acids and anthocyanin profiles [5–7] and DNA markers [8–11] were used to achieve this purpose. The evaluation of wine origin in terms of grape variety remains a difficult challenge, although Siret et al. [10] have referred the possibility to analyse residual DNA using microsatellite markers.

On the other hand, Rapp et al. [12] have referred that the content of (*E*)-3-hexenol and its isomer (*Z*)-3-hexenol are the most important analytical parameters to discriminate monovarietal wines of *Riesling*, *Müller-Thurgau*, *Kerner*, *Scheurebe*, *Ehrenfelser* and *Bacchus*. These two compounds seem to be sufficiently stable and remain unaffected by the metabolic activity of yeasts [13,14]. Also, Moret et al. [15], refer these two compounds among the significant parameters discriminating Venetian white wines. However, slight differences in the amounts of these two C₆-alcohols, either absolute or relative, according to *terroir* and winemaking procedures including must protection

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with N₂, skin and enzymatic maceration, type of preservative used and moment of its application, were referred by several authors [16–19]. The scale of fermentation, experimental or industrial, can also influence the results, although vaguely [16].

They belong to the so-called C₆-compounds, which are formed during pre-fermentative steps including harvesting, transport, crushing and pressing, as well as during eventual must heating or grape maceration [20,21]. This group comprises alcohols and aldehydes, which derive from membrane lipids via the lipoxygenase pathway. Firstly, linoleic and α -linolenic acids are produced by the action of an acyl-hydrolase, and then the corresponding 13-hydroperoxides are formed by the lipoxygenase activity, which require the presence of oxygen. Then, a hydroperoxide-lyase leads to the formation of hexanal, from hydroperoxide of linoleic acid, and (*Z*)-3-hexenal and (*E*)-2-hexenal from hydroperoxide of α -linolenic acid; an isomerase can inter-convert the two hexenals. Finally, an alcohol-dehydrogenase reduces the aldehydes to the corresponding alcohols, i.e., 1-hexanol, (*Z*)-3-hexenol and (*E*)-2-hexenol [22–25]. Contrary to (*Z*)-3-hexenol, references about the mechanism leading to (*E*)-3-hexenol in wines, were not found. Nevertheless, this alcohol may be formed from (*E*)-3-hexenal which derived from (*Z*)-3-hexenal as referred by Hatanaka [25] for various fruits.

Since it is observed in a previous work [26] that wines from *Loureiro* and *Alvarinho* varieties present significant differences respecting the ratio between (*E*)- and (*Z*)-isomers of 3-hexenol, this work tries to confirm these observations and to expand the study to other monovarietal wines from the *Vinhos Verdes* region; the possibility to characterize and distinguish monovarietal wines from *Vinhos Verdes* region by the relative abundance of 1-hexanol and both (*Z*)- and (*E*)-isomers of 3-hexenol was also the challenge. For this purpose, 43 wines ranging several ages and taking in account different winemaking procedures – settling conditions, addition of enzymatic preparations, SO₂ levels and fermentation in unlike volume containers, different vintages and different *terroirs* – were analyzed; the development of a simple and fast methodology to obtain aromatic extracts for chromatographic analysis, was intended.

2. Experimental

2.1. Vinifications

All wines were made according to the traditional technology applied in *Vinhos Verdes* region, but three different methodologies were conducted. So, in order to better explain these differences, winemaking procedures were divided into three groups, representing similar characteristics. Group A include *Loureiro* and *Trajadura* varieties, group B comprise *Alvarinho* and *Loureiro* varieties and group C enclose *Alvarinho*, *Amaral*, *Arinto*, *Avesso*, *Azal*, *Borraçal*, *Loureiro*, *Trajadura* and *Vinhão* cultivars (Table 1).

2.1.1. Group A

Grapes were crushed and pressed and then 36 mg l⁻¹ of SO₂ were added. The must was cooled by a heat exchanger before being submitted to static sedimentation (12 °C, 16 h). Fermen-

Table 1

Codification and general characterization of samples in terms of winemaking technology, rootstock, sub-region and vintage

Variety	Code	Group	Rootstock	Sub-region	Vintage	
White wines						
<i>Alvarinho</i>	ALV1	B	161-49	Lima	1998	
	ALV2	B	1103-P	Monção	1998	
	ALV3	B	196-17	Monção	1998	
	ALV4	B	1103-P	Monção	1998	
	ALV5	B	1103-P	Monção	1998	
	ALV6	B	196-17	Monção	1998	
	ALV7	B	196-17	Monção	1998	
	ALV8	C	196-17	Sousa	2003	
<i>Arinto</i>	ARI1	C	196-17	Sousa	2003	
	AVE1	C	196-17	Amarante	2001	
	AVE2	C	110-R	Baião	2002	
	AVE3	C	110-R	Amarante	2002	
<i>Avesso</i>	AVE4	C	196-17	Baião	2002	
	AVE5	C	110-R	Baião	2003	
	AVE6	C	110-R	Amarante	2003	
	AVE7	C	196-17	Baião	2003	
	AVE8	C	196-17	Amarante	2003	
	AVE9	C	196-17	Sousa	2003	
<i>Azal</i>	AZA1	C	196-17	Sousa	2003	
	LOU1	A	196-17	Lima	1995	
	LOU2	A	196-17	Lima	1995	
	LOU3	A	196-17	Lima	1995	
	LOU4	B	196-17	Cávado	1998	
	LOU5	B	SO4	Lima	1998	
	LOU6	B	196-17	Cávado	1998	
	LOU7	B	SO4	Lima	1998	
	LOU8	B	196-17	Cávado	1998	
	<i>Loureiro</i>	LOU9	C	196-17	–	2002
		LOU10	C	196-17	Lima	2002
		LOU11	C	1103-P	–	2002
		LOU12	C	1103-P	Lima	2002
		LOU13	C	196-17	–	2003
		LOU14	C	196-17	Lima	2003
		LOU15	C	1103-P	–	2003
		LOU16	C	1103-P	Lima	2003
LOU17		C	196-17	Sousa	2003	
<i>Trajadura</i>	TRA1	A	196-17	Lima	1995	
	TRA2	A	196-17	Lima	1995	
	TRA3	A	196-17	Lima	1995	
	TRA4	C	196-17	Sousa	2003	
Red wines						
<i>Amaral</i>	AMA1	C	161-49	Amarante	2003	
<i>Borraçal</i>	BOR1	C	1103-P	Lima	2003	
<i>Vinhão</i>	VIN1	C	161-69/1103-P	Amarante	2003	

–, not applicable.

tations were conducted by *Saccharomyces cerevisiae bayanus* QA23 (addition of 200 mg l⁻¹) and took place at 18 °C, in 500 l vats; air was supplied to must until density was above 1015 kg m⁻³. SO₂, on the amount of 48 mg l⁻¹, was added to the produced wines. For each variety, three wines were produced: a control wine and other two treated with 50 mg l⁻¹ of a commercial enzymatic preparation, AR2000 (Gist-Brocades) and NOVOFERM 12 (Novo Nordisk), during 30 d. The produced wines were treated with 600 mg l⁻¹ of sodium bentonite – *Volclay KWK Food Grade*, 20–70 mesh, 10% in aqueous solution

– and submitted to cold stabilization (15 d, -3°C) before filtration. Then, 78 mg l^{-1} of SO_2 were added to wines before bottling.

2.1.2. Group B

The must, obtained by crushing, pressing (60 mg l^{-1} of SO_2 were added after pressing) and static sedimentation (7°C , 24 h), was inoculated with the 200 mg l^{-1} of the yeast *Saccharomyces cerevisiae bayanus* QA23. Fermentations took place at 18°C , in 101 vessels, and were done in duplicate for precaution. The produced wines were combined and the blend was treated with 400 mg l^{-1} of sodium bentonite – *Volclay KWK Food Grade*, 20–70 mesh, 10% in aqueous solution. Then, the free SO_2 content was corrected to 35 mg l^{-1} , and finally submitted to cold stabilization (between 0°C and 3°C) before bottling. The conservation of the wines occurred at cellar temperature and light absence. One of the *Alvarinho* wines was submitted to an enzymatic treatment during 20 d, with 25 mg l^{-1} of AR2000 (Gist-Brocades), before fining procedure with bentonite.

2.1.3. Group C

The must, obtained by crushing (60 mg l^{-1} of SO_2 were added after crushing), pressing and static sedimentation (12°C , 24 h). The white and red musts were inoculated with 250 mg l^{-1} of the yeast *S. cerevisiae* “bouquet” (*FERMOL*, *AEB GROUP*) and *S. cerevisiae* “clarifiant” (*FERMOL*, *AEB GROUP*), respectively. Fermentations took place at a temperature around 18°C to 20°C , in 101 vessels; when it starts, 600 mg l^{-1} of bentonite (*BENTOGRAN*[®], *AEB GROUP*) were added. Next, SO_2 was corrected to 30 mg l^{-1} . Finally wines were stabilized at cellar temperature during 2 months and then free SO_2 was set to 35 mg l^{-1} before bottling.

2.2. General analysis

Titrateable and volatile acidity, pH, free and total SO_2 and ethanol were determined using standard procedures [27].

2.3. Extraction of volatiles

In a 10 ml culture tube (*Pyrex*, ref. 1636/26MP), 8 ml of wine, clarified by centrifugation if necessary, $1.068\text{ }\mu\text{g}$ of internal standard (4-nonanol, *Merck* ref. 818773) and a magnetic stir bar ($22.2\text{ mm} \times 4.8\text{ mm}$) were added. Extraction was done by stirring wine with $400\text{ }\mu\text{l}$ of dichloromethane (*Merck*, ref. 1.06050) during 15 min, over a magnetic stirrer. After cooling at 0°C during 10 min, the magnetic stir bar was removed and the organic phase was detached by centrifugation ($\text{RCF} = 5118$, 5 min, 4°C) being the extract recovered into a vial, using a Pasteur pipette. Then, the aromatic extract was dried with anhydrous sodium sulphate (*Merck*, ref. 1.06649) and picked up again into a new vial. Each wine was extracted in triplicate.

2.4. Chromatographic analysis

Gas chromatographic analysis of volatile compounds was performed using a GC–MS constituted by a Varian 3400 Chro-

matograph and an *ion-trap* mass spectrometer Varian Saturn II. A $1\text{ }\mu\text{l}$ injection was made in a capillary column, coated with CP-Wax 52 CB ($50\text{ m} \times 0.25\text{ mm i.d.}$, $0.2\text{ }\mu\text{m}$ film thickness, Chrompack). The temperature of the injector (SPI, septum-equipped programmable temperature) was programmed from 20°C to 250°C , at $180^{\circ}\text{C min}^{-1}$. The oven temperature was held at 40°C , for 5 min, then programmed to rise from 40°C to 250°C , at $3^{\circ}\text{C min}^{-1}$, then held 20 min at 250°C and finally programmed to go from 250°C to 255°C at $1^{\circ}\text{C min}^{-1}$. The carrier gas was helium N60 (Air Liquide) at 103 kPa, which corresponds to a linear speed of 15.5 cm s^{-1} at 150°C . The detector was set to electronic impact mode (70 eV), with an acquisition range from 29 m/z to 360 m/z , and an acquisition rate of 610 ms. Some other analysis were performed on a Chrompack GC-FID, by injecting $3\text{ }\mu\text{l}$ of the sample on a splitter injector, under the same conditions referred above; split vent was set to 16 ml min^{-1} .

2.5. Identification and quantification of 1-hexanol and 3-hexenol isomers

Identification of C_6 -alcohols was performed using the software Saturn version 5.2 (Varian), by comparing mass spectra and retention times with those of pure standard compounds, 1-hexanol (*Fluka*, ref. 73117), (*E*)-3-hexenol (*Aldrich*, ref. 22,471-5) and (*Z*)-3-hexenol (*Fluka*, ref. 53056). The three compounds were quantified as 4-nonanol equivalents.

2.6. Statistical treatment of data

Homogeneity of variances was checked with the Levene test and normality of the variables was checked by the parametric Kolmogorov–Smirnov test with Lilliefors correction, both assuming a significance level of 5%. Whenever one of these two conditions fails, the non-parametric Kruskal–Wallis test was applied. Then, statistical differences between monovarietal wines were checked by parametric analysis of variance (ANOVA) or by the non-parametric Mann–Whitney test, for each pair of wines, two by two.

Standard deviation, confidence limits and coefficients of variation (C.V.) were determined with 95% of confidence level. Software SPSS 14.0 for Windows was used.

3. Results and discussion

There were analysed 40 white wines and 3 red wines available in our laboratory, belonging to *Alvarinho* (8), *Arinto* (1), *Avesso* (9), *Azal* (1), *Loureiro* (17), *Trajadura* (4), *Amaral* (1), *Borraçal* (1) and *Vinhão* (1) varieties (Tables 1 and 2). *Alvarinho*'s match to three sub-regions, two vintages, three rootstocks and two different winemaking procedures; additionally for group B technology, one wine was submitted to enzymatic treatment being analysed at three different stages, 0 months, 8 months and 20 months old. *Avesso*'s accounts for one technology and two rootstocks only, but were considered three sub-regions and three vintages. *Loureiro* wines are the most abundant group and represent four vintages, three sub-regions,

Table 2
Age and general physico-chemical parameters of the analysed wines

Variety	Code	Age (months)	pH	Titrateable acidity ^a /(g l ⁻¹)	Volatile acidity ^b /(g l ⁻¹)	Free SO ₂ /(mg l ⁻¹)	Total SO ₂ /(mg l ⁻¹)	Ethanol/(%, v/v)
White wines								
<i>Alvarinho</i>	ALV1	0	–	–	–	–	–	–
	ALV2	0	–	–	–	–	–	–
	ALV3	0	–	–	–	–	–	–
	ALV4	8	3.05	7.7	0.40	25	132	13.5
	ALV5	8	3.03	7.6	0.40	25	130	13.5
	ALV6	8	3.06	6.9	0.40	29	131	13.9
	ALV7	20	–	–	–	–	–	–
	ALV8	17	2.80	9.61	0.62	22	92	12.5
<i>Arinto</i>	ARI1	17	2.99	7.97	0.37	22	101	12.0
	AVE1	8	2.90	8.2	0.38	32	146	9.7
	AVE2	7	2.81	10.65	0.65	11	68	12.4
	AVE3	7	3.07	8.21	0.62	14	108	11.8
<i>Avesso</i>	AVE4	7	2.74	10.66	0.41	15	78	9.5
	AVE5	7	3.08	6.60	0.58	20	77	11.4
	AVE6	7	2.70	11.4	0.55	33	108	9.3
	AVE7	7	2.81	7.60	0.49	17	95	11.3
	AVE8	7	3.04	9.70	0.56	22	70	10.8
	AVE9	17	2.74	9.45	0.53	29	100	12.8
<i>Azal</i>	AZA1	17	2.82	10.78	0.49	20	84	10.6
	LOU1	1.5	3.00	8.25	0.29	4	26	10.8
	LOU2	1.5	–	–	–	–	–	–
	LOU3	1.5	–	–	–	–	–	–
	LOU4	0	–	–	–	–	–	–
	LOU5	0	–	–	–	–	–	–
	LOU6	8	2.87	9.3	0.39	31	134	11.3
	LOU7	8	2.81	10.6	0.33	26	120	10.2
<i>Loureiro</i>	LOU8	20	–	–	–	–	–	–
	LOU9	7	2.70	11.12	0.81	12	51	9.0
	LOU10	7	2.85	10.24	0.40	15	85	10.4
	LOU11	7	2.74	9.94	0.57	15	83	10.3
	LOU12	7	2.94	10.5	0.50	14	140	11.6
	LOU13	7	2.75	9.70	0.54	25	113	10.1
	LOU14	7	2.82	9.05	0.26	28	100	9.7
	LOU15	7	2.78	10.6	0.38	20	83	8.9
	LOU16	7	2.94	9.27	0.31	27	105	9.4
	LOU17	17	2.81	8.64	0.38	20	87	11.9
<i>Trajadura</i>	TRA1	1.5	3.35	7.61	0.24	4	33	9.5
	TRA2	1.5	–	–	–	–	–	–
	TRA3	1.5	–	–	–	–	–	–
	TRA4	17	3.24	6.57	0.38	22	95	10.8
Red wines								
<i>Amaral</i>	AMA1	17	2.81	14.0	0.32	28	103	7.3
<i>Borraçal</i>	BOR1	17	3.41	11.43	0.55	10	41	9.2
<i>Vinhão</i>	VIN1	17	3.28	8.91	0.49	20	64	9.0

(–) Not determined. ALV2, ALV4 (8 months old) and ALV5 (8 months old, treated with AR2000) correspond to the same base wine; this is also valid for ALV3, ALV6 (8 months old) and ALV7 (20 months old). LOU1, LOU2 (treated with AR2000) and LOU3 (treated with NOVOFERM 12) correspond to the same must; LOU4, LOU6 (8 months old) and LOU8 (20 months old) correspond to the same base wine; this is also valid for LOU5 and LOU7 (8 months old). TRA1, TRA2 (treated with AR2000) and TRA3 (treated with NOVOFERM 12) correspond to the same must.

^a As tartaric acid.

^b As acetic acid.

three rootstocks, and three winemaking procedures; additionally, like for *Avarinho*'s, there were considered wines submitted to enzymatic treatment and the same wine with distinct bottle conservation periods. Finally, *Trajadura* wines were obtained from grapes harvested in two distinct years representing two sub-regions but accounting for the same rootstock; for the

first year, the wine was treated with two different enzymatic preparations.

Considering the 43 samples, the age varies from 0 months to 20 months, corresponding to wines analysed immediately after alcoholic fermentation and with 20 months of bottle conservation, respectively. Taking in account fermentation scale, group

A wines were produced using 500l vats while groups B and C ones were fermented in 101 vessels. Respecting the amount and moment of SO₂ added, the three groups were treated differently, being 36 mg l⁻¹ after pressing for group A and 60 mg l⁻¹ for groups B and C, this last after crushing. Settling conditions also differ slightly: 12 °C during 16 h for group A, 7 °C during 24 h for group B and 12 °C during 24 h for group C. Moreover, contrary to groups A and B wines, bentonite was added to fermenting must during production of group C ones.

It must be noted that only one wine of *Arinto*, *Azal*, *Amaral*, *Borraçal* and *Vinhão* varieties was analyzed, due to unavailability of samples. So, the obtained results must be look as indicative only. However, in the absence of a great number of analyses for these monovarietal wines, their inclusion could be justified as the results are able to be a start point for future work and indicate their tendency respecting the amounts and ratios between C₆-alcohols. On the other hand, in spite of *Avesso* wines are not often commercially available, this variety has a great potential to produce monovarietal quality white wines, justifying the number of analysis performed.

None of the wines were submitted to aging processes because *Vinhos Verdes* wines are characterized by their freshness and their floral and fruity flavours and, in this way, generally consumed young.

3.1. Wines general characterization

Since the Portuguese legislation for the Appellation of Origin *Vinhos Verdes* imposes ethanol contents between 8.0% and 11.5%, except for *Alvarinho* wines which must be comprised between 11.5% and 13.0%, and fix acidity, expressed as tartaric acid, to be at least 6.0 g l⁻¹ (4.5 g l⁻¹ for *Alvarinho*), the wines ALV4, ALV5, ALV6 and AMA1 disrespect the rules (Table 2). It must be referred that two wines, LOU1 and TRA1, present low levels of free SO₂.

As can be seen in Table 2, some wines were not characterised in terms of titratable and volatile acidity, free and total SO₂, pH and ethanol content. Nevertheless, as they only differ in age or by the application of slightly winemaking practices, as indicated at the baseboard of the Table 2, they may have similar characteristics of the related base wines (e.g., the uncharacterised ALV3 and ALV7 wines may be analogous to ALV6 wine, as they only correspond to a different conservation stage).

3.2. Determination of C₆-alcohols

Due to the higher number of samples to be analysed, a simple and fast methodology for extraction of the volatile compounds was developed. To optimize it, several conditions were tested: wine volume 8 ml and 9 ml; CH₂Cl₂ volume 300 µl, 400 µl and 500 µl; extraction time 15 min, 30 min and 45 min; salting-out addition of 1 g and 2 g of NaCl or (NH₄)₂SO₄ and 3 g of NaCl. Considering quality of GC–MS chromatograms in terms resolution and peak area respecting 1-hexanol, (*E*)-3-hexenol and (*Z*)-3-hexenol, the best results were obtained using 8 ml of wine – without salting-out – 400 µl of CH₂Cl₂ and an extraction time of 15 min. Then, accuracy was determined making six

extractions of the same *Alvarinho* wine, being obtained the following results: 1-hexanol = 419.2 ± 52.1 µg l⁻¹, C.V. = 11.8%; (*E*)-3-hexenol = 13.1 ± 1.4 µg l⁻¹, C.V. = 10.4%; (*Z*)-3-hexenol = 22.3 ± 3.0 µg l⁻¹, C.V. = 13.0%; (*E*)-3-hexenol/(*Z*)-3-hexenol = 0.6 ± 0.1, C.V. = 12.8%; 1-hexanol/(*E*)-3-hexenol = 32.4 ± 5.0, C.V. = 14.8%; 1-hexanol/(*Z*)-3-hexenol = 18.9 ± 1.3, C.V. = 6.5%. An example of a GC–MS chromatogram section can be observed in Fig. 1.

Since the main scope of this work is to compare monovarietal wines respecting relative amounts of C₆-alcohols, the quantification of compounds as 4-nonanol equivalents may be reasonable. Moreover, confidence limits and C.V. are small enough to consider the proposed methodology a suitable way to compare wines.

3.3. Discrimination of wines using C₆-alcohols

In order to assess the possibility to discriminate *Vinhos Verdes* monovarietal wines, the concentration of (*E*)- and (*Z*)-isomers of 3-hexenol were determined and the ratio between them calculated (Table 3). A third C₆-compound, 1-hexanol, in spite of its double origin, pre-fermentative and fermentative, was also determined as it can be useful in a second level discrimination through the calculation of 1-hexanol/(*E*)-3-hexenol and 1-hexanol/(*Z*)-3-hexenol ratios. Quantitatively, these three C₆-compounds are the most relevant ones.

From Table 3, it can be seen that *Alvarinho* and *Loureiro* wines were analysed by GC–MS while C₆-compounds from *Trajadura*, *Azal*, *Arinto*, *Amaral*, *Borraçal* and *Vinhão* wines were determined by GC–FID. Wines from *Avesso* variety were analysed by the two methodologies. In all the cases, C₆-alcohols were determined in a semi-quantitative approach, as 4-nonanol equivalents. It must be referred that GC–FID done concentrations slightly higher than GC–MS for the three compounds, as can be observed for sample AVE7; however, because the ratio between them remains almost constant, the results obtained with different detectors can be compared. Additionally, the low values obtained for confidence limits respecting the three replicates of the same sample indicate little fluctuations in concentrations regarding the three compounds studied, as already mentioned above.

Application of Kolgomorov–Smirnov test showed that variables do not follow a normal distribution, for white wines. In this way, the existence of significant differences between wines was confirmed by means of Kruskal–Wallis test; then, Mann–Whitney test was used to compare medians, permitting direct comparison between wines, two by two. Respecting red wines, variables behave as normal but homogeneity of variances fails, except for 1-hexanol/(*Z*)-3-hexenol. For each variable – 1-hexanol/(*E*)-3-hexenol, 1-hexanol/(*Z*)-3-hexenol and (*E*)-3-hexenol/(*Z*)-3-hexenol – different letters were assigned in Table 3 to indicate significant differences between wines. Since the intention of the proposed methodology is to discriminate monovarietal wines, white wines and red wines were compared independently.

Respecting white wines, (*E*)-3-hexenol/(*Z*)-3-hexenol ratio permits the discrimination between all the wines except for the

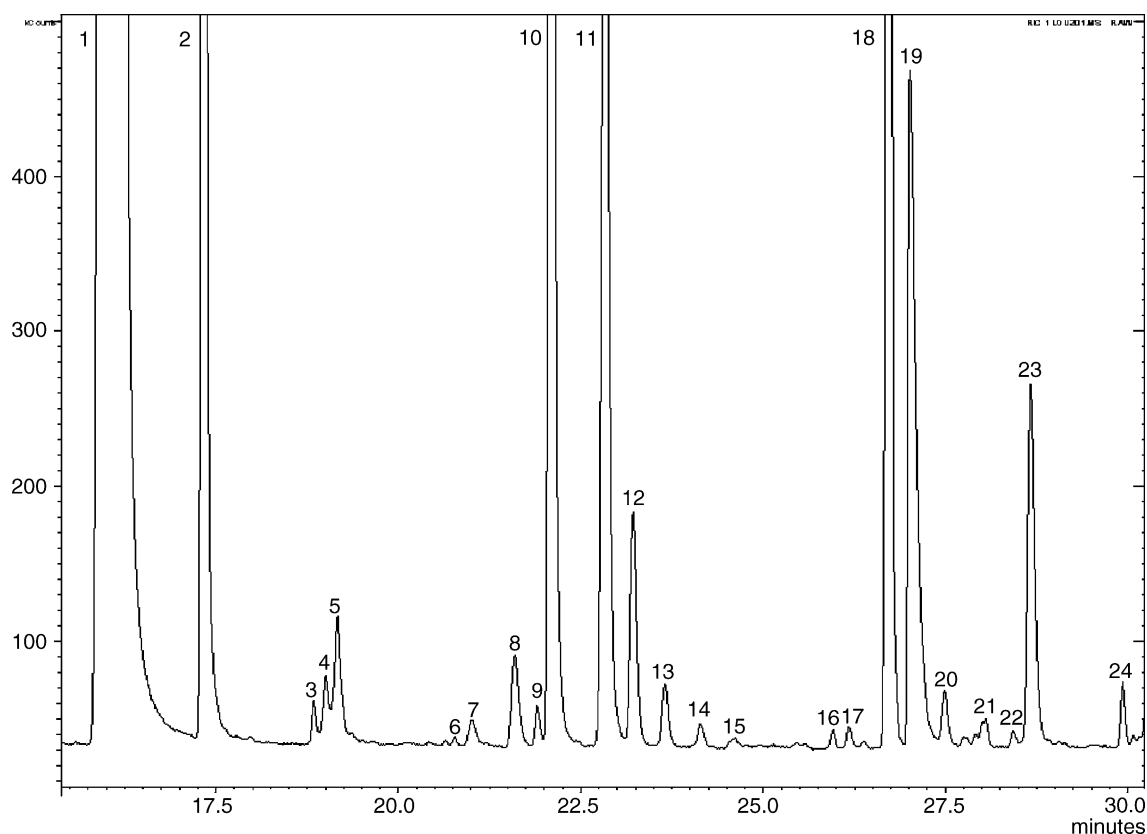


Fig. 1. Example of a GC–MS chromatogram section obtained from a *Loureiro* wine. (1) 2-Methyl-1-butanol + 3-methyl-1-butanol, (2) ethyl hexanoate, (3) ethyl pyruvate, (4) unknown, (5) hexyl acetate + acetoin, (6) (*Z*)-3-hexenyl acetate, (7) 4-methyl-1-pentanol, (8) 3-methyl-1-pentanol, (9) unknown, (10) ethyl lactate, (11) 1-hexanol, (12) (*E*)-3-hexenol, (13) 3-ethoxy-1-propanol, (14) (*Z*)-3-hexenol, (15) 2-nonanone, (16) unknown, (17) unknown, (18) ethyl octanoate, (19) acetic acid, (20) 1-heptanol, (21) furan linalool oxide + neroloxide, (22) unknown, (23) 4-nonanol, (24) ethyl 3-hydroxybutanoate.

pair *Avesso/Azal*. Considering 1-hexanol/(*E*)-3-hexenol ratio, the differentiation was also possible but there are three exceptions, *Alvarinho/Avesso*, *Avesso/Azal* and *Trajadura/Azal*. For the third ratio, 1-hexanol/(*Z*)-3-hexenol, the exceptions are *Avesso/Arinto*, *Trajadura/Arinto* and *Avesso/Azal*. Due to the small number of wines evaluated (only one), the mentioned statistical methodology was not able to discriminate *Azal* wines from *Arinto* ones. This difficulty is also valid for *Amaral*, *Borraçal* and *Vinhão* varieties, although results may suggest the possibility of using these three ratios to discriminate respective monovarietal wines.

As previously reported [12–15,18], the (*E*)-3-hexenol/(*Z*)-3-hexenol ratio seems also to be a varietal marker for *Alvarinho*, *Loureiro*, *Avesso* and *Trajadura* wines, as the obtained values are quite constant with low standard deviations (Table 3); results obtained for *Azal*, *Arinto*, *Amaral*, *Borraçal* and *Vinhão* varieties must be confirmed by analysing a great number of wines. Analysis were carry out in wines from different vintages and diverse *terroirs* as well as in wines having different ages (Tables 1 and 2); additionally, diverse winemaking procedures including SO₂ amount and moment of application to the must, different settling conditions (time and temperature) and different fermentation volumes, were used.

Wines from *Loureiro* grape variety accounts for the large number of analysis belonging to group A wines (LOU1–LOU3),

group B (LOU4–LOU8) and group C (LOU9–LOU17); the first group represents one sub-region, one rootstock and one vintage having the three wines identical age; the second group represents the same vintage, but accounts for two rootstocks and two sub-regions and three different ages; the third group accounts for two vintages, two sub-regions and two rootstocks having wines two distinct stages of conservation. Results show that later addition of SO₂ (after pressing for groups A and B) seem to originate higher amounts of 1-hexanol, and the two isomers of 3-hexenol for group B wines, influencing both 1-hexanol/(*E*)-3-hexenol and 1-hexanol/(*Z*)-3-hexenol ratios; however, (*E*)-3-hexenol/(*Z*)-hexenol ratio remains almost unchangeable. These facts may be also correlated with fermentation volume (500l for group A and 10l for groups B and C) as well as with amount of SO₂ added, 36 mg l⁻¹ for group A and 60 mg l⁻¹ for groups B and C. These observations agree with Nicolini et al. [16,18]. On the other hand, the three ratios may not be significantly influenced either by the adopted settling conditions or by bentonite addition during fermentation (group C), although levels of the three alcohols may be smaller for the last feature. Different conservation periods present similar results, e.g., for LOU4 (0 months), LOU6 (8 months) and LOU8 (20 months) as well as for LOU5 (0 months) and LOU7 (8 months). It is known that C₆-alcohols may be present in a glycosidically bound form, susceptible of being liberated by means of specific

Table 3
Mean levels (C) with 95% confidence limits for 1-hexanol, (*E*)-3-hexenol and (*Z*)-3-hexenol, and mean values for 1-hexanol/(*E*)-3-hexenol, 1-hexanol/(*Z*)-3-hexenol and (*E*)-3-hexenol/(*Z*)-3-hexenol ratios

Variety	Code	C/($\mu\text{g l}^{-1}$)			Mean value		
		1-Hexanol	(<i>E</i>)-3-Hexenol	(<i>Z</i>)-3-Hexenol	1-Hexanol/ (<i>E</i>)-3-hexenol	1-Hexanol/ (<i>Z</i>)-3-hexenol	(<i>E</i>)-3-Hexenol/ (<i>Z</i>)-3-hexenol
White wines							
<i>Alvarinho</i>	ALV1 ^a	446.4 ± 68.5	16.5 ± 3.0	28.3 ± 6.6	27.1 ± 2.2	15.8 ± 1.6	0.6 ± 0.0
	ALV2 ^a	410.7 ± 49.2	15.8 ± 3.2	22.7 ± 2.3	26.1 ± 2.1	18.1 ± 0.8	0.7 ± 0.1
	ALV3 ^a	326.0 ± 41.6	14.6 ± 3.6	18.1 ± 4.0	22.4 ± 2.9	18.1 ± 1.9	0.8 ± 0.0
	ALV4 ^a	452.0 ± 41.9	17.7 ± 3.8	25.6 ± 4.1	25.6 ± 3.3	17.7 ± 1.3	0.7 ± 0.1
	ALV5 ^a	437.9 ± 40.7	17.8 ± 3.7	23.5 ± 5.2	24.7 ± 3.3	18.7 ± 2.9	0.8 ± 0.0
	ALV6 ^a	396.9 ± 9.5	15.9 ± 1.4	19.9 ± 3.5	25.0 ± 2.3	20.0 ± 3.3	0.8 ± 0.2
	ALV7 ^a	443.7 ± 44.2	16.8 ± 2.3	19.7 ± 1.3	26.4 ± 1.3	22.5 ± 1.0	0.9 ± 0.1
	ALV8 ^a	216.2 ± 15.9	11.8 ± 0.6	17.5 ± 3.1	18.3 ± 2.0	12.4 ± 1.8	0.7 ± 0.2
	Mean				24.5a	17.9a	0.7a
	S.D.				2.9	2.9	0.1
<i>Arinto</i>	ARI1 ^b	305.9 ± 22.9	19.4 ± 2.6	21.6 ± 3.0	15.8 ± 1.0	14.2 ± 1.3	0.9 ± 0.1
	Mean				15.8b	14.2b,d	0.9b
	S.D.				0.4	0.5	0.0
<i>Avesso</i>	AVE1 ^a	154.5 ± 24.7	4.5 ± 0.9	35.3 ± 5.6	34.2 ± 2.1	4.4 ± 0.1	0.1 ± 0.0
	AVE2 ^a	228.3 ± 38.9	6.7 ± 3.9	23.0 ± 4.2	34.9 ± 12.8	9.9 ± 0.3	0.3 ± 0.1
	AVE3 ^a	117.8 ± 15.0	5.4 ± 0.2	13.6 ± 1.0	21.8 ± 2.2	8.6 ± 0.7	0.4 ± 0.0
	AVE4 ^a	171.4 ± 32.0	4.2 ± 2.1	31.3 ± 5.4	41.5 ± 16.5	5.5 ± 1.9	0.1 ± 0.1
	AVE5 ^a	169.2 ± 5.6	5.6 ± 0.2	13.5 ± 1.2	30.0 ± 1.3	12.5 ± 1.0	0.4 ± 0.0
	AVE6 ^a	238.0 ± 42.3	10.4 ± 2.4	17.0 ± 3.4	23.0 ± 3.0	14.0 ± 0.7	0.6 ± 0.1
	AVE7 ^a	204.1 ± 58.3	8.6 ± 0.7	17.4 ± 1.7	23.8 ± 8.8	11.7 ± 2.2	0.5 ± 0.1
	AVE7 ^b	294.8 ± 73.9	14.3 ± 0.4	28.8 ± 8.9	20.6 ± 4.7	10.2 ± 0.6	0.5 ± 0.1
	AVE8 ^b	278.4 ± 30.5	5.7 ± 0.8	12.6 ± 0.5	49.0 ± 2.0	22.0 ± 3.0	0.5 ± 0.1
	AVE9 ^b	144.1 ± 5.9	11.1 ± 1.2	17.2 ± 3.5	13.0 ± 1.0	8.4 ± 1.6	0.6 ± 0.2
	Mean				29.8a	10.6b	0.4c
	S.D.				11.1	5.1	0.2
<i>Azal</i>	AZA1 ^b	408.3 ± 36.3	16.6 ± 1.5	43.2 ± 5.0	24.6 ± 0.1	9.5 ± 0.4	0.4 ± 0.0
	Mean				24.6a,b,d	9.5b	0.4b,c
	S.D.				0.0	0.2	0.0
<i>Loureiro</i>	LOU1 ^a	747.0 ± 30.5	47.7 ± 2.9	4.7 ± 1.3	15.7 ± 0.8	160.3 ± 44.8	10.3 ± 3.4
	LOU2 ^a	772.6 ± 184.6	52.3 ± 6.6	5.0 ± 1.2	14.8 ± 2.3	155.1 ± 27.5	10.5 ± 1.2
	LOU3 ^a	719.2 ± 96.5	51.1 ± 9.1	5.1 ± 0.8	14.2 ± 4.2	141.2 ± 27.1	10.0 ± 1.5
	LOU4 ^a	450.1 ± 18.3	76.8 ± 4.1	7.4 ± 0.9	5.9 ± 0.2	60.8 ± 5.0	10.4 ± 0.8
	LOU5 ^a	615.2 ± 105.8	85.8 ± 18.0	9.4 ± 2.7	7.2 ± 0.3	65.9 ± 8.5	9.2 ± 1.1
	LOU6 ^a	445.7 ± 64.1	72.9 ± 2.0	6.7 ± 1.1	6.1 ± 1.0	66.8 ± 15.2	10.9 ± 2.2
	LOU7 ^a	624.3 ± 131.3	69.4 ± 29.4	7.7 ± 4.6	9.1 ± 1.8	82.7 ± 30.9	9.1 ± 1.8
	LOU8 ^a	484.1 ± 13.9	77.7 ± 4.1	6.8 ± 0.7	6.2 ± 0.2	71.5 ± 8.9	11.5 ± 1.5
	LOU9 ^a	271.5 ± 2.6	31.5 ± 2.4	4.5 ± 1.2	8.6 ± 0.7	61.5 ± 17.1	7.1 ± 2.1
	LOU10 ^a	275.5 ± 4.8	27.5 ± 1.7	3.7 ± 0.9	10.0 ± 0.5	75.1 ± 18.4	7.5 ± 2.1
	LOU11 ^a	247.3 ± 7.8	32.3 ± 2.0	3.8 ± 0.3	7.7 ± 0.3	65.8 ± 3.9	8.6 ± 0.5
	LOU12 ^a	226.1 ± 11.8	14.6 ± 1.9	2.7 ± 0.2	15.5 ± 1.5	83.9 ± 11.0	5.4 ± 1.2
	LOU13 ^a	283.6 ± 17.7	31.9 ± 1.6	3.5 ± 0.5	8.9 ± 0.2	80.9 ± 15.3	9.1 ± 1.6
	LOU14 ^a	249.2 ± 29.9	35.4 ± 9.9	3.6 ± 1.4	7.1 ± 1.3	69.9 ± 24.6	9.9 ± 3.5
	LOU15 ^a	272.9 ± 45.8	32.1 ± 8.1	4.8 ± 1.3	8.5 ± 1.1	56.6 ± 8.4	6.6 ± 0.2
	LOU16 ^a	319.2 ± 16.1	47.0 ± 4.9	4.1 ± 0.8	6.8 ± 0.4	78.2 ± 15.5	11.5 ± 2.5
	LOU17 ^a	174.4 ± 18.9	52.5 ± 2.3	6.2 ± 0.8	3.3 ± 0.5	28.0 ± 1.8	8.4 ± 1.4
	Mean				9.1c	82.6c	9.2d
	S.D.				3.7	35.7	1.8
<i>Trajadura</i>	TRA1 ^b	1101.0 ± 7.5	25.2 ± 0.7	35.6 ± 1.3	43.6 ± 1.5	30.9 ± 1.0	0.7 ± 0.0
	TRA2 ^b	987.0 ± 39.9	18.4 ± 0.7	35.7 ± 2.8	53.7 ± 4.3	27.7 ± 1.4	0.5 ± 0.0
	TRA3 ^b	947.5 ± 52.5	17.5 ± 0.7	32.7 ± 2.2	54.3 ± 1.9	29.0 ± 0.7	0.5 ± 0.0
	TRA4 ^b	395.6 ± 13.1	21.5 ± 1.3	39.6 ± 0.8	18.4 ± 0.6	10.0 ± 0.3	0.5 ± 0.0
	Mean				42.5d	24.4d	0.6e
	S.D.				15.2	8.8	0.1

Table 3 (Continued)

Variety	Code	C/($\mu\text{g l}^{-1}$)			Mean value		
		1-Hexanol	(E)-3-Hexenol	(Z)-3-Hexenol	1-Hexanol/ (E)-3-hexenol	1-Hexanol/ (Z)-3-hexenol	(E)-3-Hexenol/ (Z)-3-hexenol
Red wines							
<i>Amaral</i>	AMA1 ^b	877.2 \pm 21.0	25.0 \pm 1.3	10.2 \pm 1.0	35.0 \pm 1.0	85.8 \pm 7.7	2.4 \pm 0.2
	Mean				35.0	85.8	2.4
	S.D.				0.4	3.1	0.1
<i>Borraçal</i>	BOR1 ^b	1868.2 \pm 124.6	25.7 \pm 2.8	87.1 \pm 14.0	72.7 \pm 3.0	21.5 \pm 2.0	0.3 \pm 0.0
	Mean				72.7	21.5	0.3
	S.D.				1.2	0.8	0.0
<i>Vinhão</i>	VIN1 ^b	1336.6 \pm 57.9	20.0 \pm 0.9	13.1 \pm 0.9	66.9 \pm 0.5	102.0 \pm 2.7	1.5 \pm 0.0
	Mean				66.9	102.0	1.5
	S.D.				0.2	1.1	0.0

For the each variable – 1-hexanol/(E)-3-hexenol, 1-hexanol/(Z)-3-hexenol and (E)-3-hexenol/(Z)-3-hexenol – different letters (a–e) mean significant differences between monovarietal wines ($p < 0.05$). The mean global levels for the ratios as well as their standard deviations (S.D.) are also presented for each group of monovarietal wines.

^a Determined by GC–MS.

^b Determined by GC–FID.

enzymes [1,26,28]. Nevertheless, enzymatic treatment of wines applied to LOU2 and LOU3 does not alter significantly either amounts of C₆-alcohols or the ratios between them, when compared with the control wine LOU1. Respecting *Alvarinho* wines, only winemaking procedures B and C could be compared, the first one accounting for three different stages of conservation, one vintage and two sub-regions; group C includes one wine only. The effect of bentonite addition and enzymatic treatment of wines could also be compared. Results show basically the same information as for *Loureiro* ones. In this way, considerable differences respecting wine age were not found, i.e., between ALV2 (0 months) and ALV4 (8 months) or between ALV3 (0 months), ALV6 (8 months) and ALV7 (20 months); also ALV5, treated with enzymatic preparation, was similar to control wine ALV4. *Trajadura* wines seem to behave similarly to *Loureiro*'s and *Alvarinho*'s respecting groups A and C procedures (TRA1 and TRA4) and enzymatic treatments (TRA1 against TRA2 and TRA3). Finally, *Avesso* wines account for only one winemaking procedure but three different vintages, two rootstocks, three sub-regions and two different ages for the last vintage. However, results were not conclusive enough.

It is interesting to note that (E)-3-hexenol/(Z)-3-hexenol ratio is greater than unity for *Loureiro* (ranging from 6.6. to 11.5, $n = 17$) and lower than unity for *Alvarinho* (0.6 to 0.9, $n = 8$), *Trajadura* (0.5 to 0.7, $n = 4$) and *Avesso* (0.1 to 0.6, $n = 9$). These results are in agreement with those found for *Loureiro* and *Alvarinho* wines using another extraction procedure, i.e., desorbing volatile compounds from a XAD-2 resin with an azeotropic mixture of pentane-dichloromethane [26]. Versini et al. [29] also found mean values of 2.5 and 0.3 for the congeners *Loureira* and *Albariño* wines (Galicia, Spain), respectively; additionally, Lema et al. [30] presented values of 0.3 and 0.6 for *Albariño* wines produced from two *sub-zonas*, Condado and Rosal, respectively. Other varieties like *Garganega* [15], *Müller-Thurgau* [12,16,31], *Gewürztraminer* [32], *Bac-*

chus [12] and *Muscat of Alexandria* [33] originate wines with predominance of the (E)-isomer. Contrarily, *Riesling*, *Kerner*, *Scheurebe* and *Ehrenfelser* [12] as well as *Godello* [29], *Tannat* [34], *Airén* [35] and *Emir* [17] varieties seem to produce wines with (E)-3-hexenol/(Z)-3-hexenol ratio lower than the unity. In spite of the diminutive number of analysis carried out, *Arinto* and *Avesso* wines give the impression to behave like *Alvarinho* but the red varieties *Amaral* and *Vinhão* seem to be similar to *Loureiro*, respecting abundance of 3-hexenol isomers.

From these results, it becomes clear that (E)-3-hexenol/(Z)-3-hexenol ratio may be an interesting tool for white *Vinhos Verdes* discrimination. Additionally, 1-hexanol/(E)-3-hexenol and 1-hexanol/(Z)-hexenol ratios permitted the differentiation of some wines and can act as a second level variable for this purpose.

In the case of *Avesso* and *Azal* wines, the three mentioned ratios are unable to discriminate wines; however, concentrations of 1-hexanol, (E)-3-hexenol or (Z)-3-hexenol seem to be able for the required differentiation. Therefore, it seems to be evident that the level of C₆-alcohols may be useful whenever it was not possible the discrimination using the ratios between them. Other compounds with varietal origin, like monoterpenols (including oxides and diols), may be useful for this propose as it was proved that they can differentiate *Vinhos Verdes* grape varieties [1]; however, these compounds must be taken with care since their concentration may undergo more or less significant changes during fermentation, the conservation period and aging [26,36–39], contrary to C₆-compounds.

The fact that *Amaral*, *Vinhão* and *Loureiro* wines presented higher levels of (E)-3-hexenol than the (Z)-isomer, may be attributed to the specificity of enzymes involved in the sequence of C₆-alcohols formation. In this way Rowan et al. [40] referred the inter-conversion between C₆-aldehydes – (E)-3-hexenal, (Z)-3-hexenal and (E)-2-hexenal – which then originate the corresponding alcohols, in apples. Previously, Hatanaka [25] also indicated the possible formation of (E)-3-hexenol from (E)-3-

hexenal for various fruits. Therefore, if the same mechanism occurs in grapes, the found values in this work can be hypothetically attributed to the activity or the inhibition of the involved enzymes.

In spite of the interesting results obtained, this study must be complemented in the future by analysing much more samples of each monovarietal wines in order to make possible the confirmation of the presented results. Moreover, the technological choices adopted during winemaking may influence the relative abundance of 1-hexanol and isomers of 3-hexenol. In this way, for *Müller-Thurgau* wines, Nicolini et al. refer changes in isomers ratio if SO₂ is added immediately after grape pressing instead of after static sedimentation (4.8 to 2.6) or when ascorbic acid was used before sedimentation instead of SO₂ (4.3 to 5.2) [18]. Also, the alternative use of standard vinification, cold skin maceration (9 °C to 14 °C, 21 h), hot skin maceration (18 °C to 24 °C, 3.5 h) or enzymatic maceration conducts to different values, 4.8, 3.7, 4.3 and 2.1, respectively (2.6, 2.9, 3.3 and 1.4 for a different *terroir*); experimental and industrial procedures also produce different results, 3.4 and 1.9, respectively [16]. Cabaroğlu et al. [17] found values of 0.8 and 1.0 when control wines were compared with those obtained after skin contact. The use of pesticides can also influence the referred ratio [33]. Moio et al. [19] refer, however, that must protection with N₂ produces less levels of (*E*)-3-hexenol but similar values for the isomers ratio, when compared to a control wine. Furthermore, the 1-hexanol/(*E*)-3-hexenol and 1-hexanol/(*Z*)-3-hexenol ratios can be strongly affected by the referred winemaking alternatives. Mauricio et al. [41] refer a depletion of 1-hexanol, (*E*)-3-hexenol and (*Z*)-3-hexenol during the first days of fermentation concluding that semi-anaerobic conditions results in lesser contents of the compounds when compared with anaerobic conditions.

So, future work must be focused in industrial wines produced with a large range of winemaking practices in order to confirm these preliminary results; type of environment, oxidant or not, skin contact, enzymatic maceration, carbonic maceration, kind of must and wine clarification and type and amount of preservative added, are some of the variables to be studied. Furthermore, it must be considered the moment of preservative application and the temperatures adopted during grapes and musts handling.

4. Conclusions

The present study showed that C₆-alcohols, namely the ratio between (*E*)-3-hexenol and (*Z*)-3-hexenol, may be a useful tool to assess wine origin concerning the *Vinhos Verdes* monovarietal wines from *Alvarinho*, *Avesso*, *Loureiro* and *Trajadura* grape varieties. This ratio discriminate evidently *Loureiro* wines from those of *Alvarinho*, *Avesso* and *Trajadura*; the former presents values substantially higher than the unity while for the other three, the isomer (*Z*) prevails. Another two ratios, 1-hexanol/(*E*)-3-hexenol and 1-hexanol/(*Z*)-3-hexenol, may be helpful in distinguish monovarietal wines whenever the former ratio is unable to do it alone. Additionally, when the three mentioned ratios are unsuccessful, concentrations of individual C₆-alcohols possibly may be used for this purpose.

Since the number of analysed wines is reduced and the wine-making options were not fully explored, the obtained results may be considered as a start point for future work. Furthermore, the validation of the proposed method will be crucial when concentration of C₆-alcohols have to be measured.

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References

- [1] J.M. Oliveira, I.M. Araújo, O.M. Pereira, J.S. Maia, A.J. Amaral, M.O. Maia, *Anal. Chim. Acta* 513 (2004) 269.
- [2] J. Marais, G. Versini, C.J. van Wyk, A. Rapp, *S. Afr. J. Enol. Vitic.* 13 (1992) 71.
- [3] J. Marais, A. Rapp, *S. Afr. J. Enol. Vitic.* 12 (1991) 51.
- [4] A. Rapp, in: G. Charalambous (Ed.), *Food Flavors: Generation, Analysis and Process Influence—Eighth International Flavor Conference*, Cos, Greece, 6–8 July, Elsevier, 1995, p. 1703.
- [5] K.R. Kim, J.H. Kim, E.-J. Cheong, C.-M. Jeong, *J. Chromatogr. A* 722 (1996) 303.
- [6] E.H. Soufleros, E. Bouloumpasi, C. Tsarchopoulos, C.G. Biliaderis, *Food Chem.* 80 (2003) 261.
- [7] A. de Villiers, G. Vanhoenacker, P. Majek, P. Sandra, *J. Chromatogr. A* 1054 (2004) 195.
- [8] M.A. Faria, R. Magalhães, M.A. Ferreira, C.P. Meredith, F.F. Monteiro, *J. Agric. Food Chem.* 48 (2000) 1096.
- [9] J.R. Vidal, S. Moreno, Y. Gogorcena, A. Masa, J.M. Ortiz, *Am. J. Enol. Vitic.* 50 (1999) 69.
- [10] R. Siret, O. Gigaud, J.P. Rosec, P. This, *J. Agric. Food Chem.* 50 (2002) 3822.
- [11] E. García-Beneytez, M.V. Moreno-Arribas, J. Borrego, M.C. Polo, J. Ibáñez, *J. Agric. Food Chem.* 50 (2002) 6090.
- [12] A. Rapp, C. Volkmann, H. Niebergall, *Vitis* 32 (1993) 171.
- [13] R. Di Stefano, G. Ciolfi, *Riv. Vitic. Enol.* 35 (1982) 431.
- [14] T. Herraiz, M. Herraiz, G. Reglero, P.J. Martin-Alvarez, M.D. Cabezedo, *J. Agric. Food Chem.* 38 (1990) 969.
- [15] I. Moret, G. Scarponi, P. Gescon, *J. Sci. Food Agric.* 35 (1984) 1004.
- [16] G. Nicolini, G. Versini, E. Amadei, *Riv. Vitic. Enol.* 49 (1996) 37.
- [17] T. Cabaroğlu, A. Canbas, R.L. Baumes, C.L. Bayonove, J.P. Lepoutre, Y.Z. Günata, *J. Food Sci.* 62 (1997) 680.
- [18] G. Nicolini, G. Versini, E. Amadei, M. Marchio, *Vitis* 35 (1996) 147.
- [19] L. Moio, M. Ugliano, A. Genovese, A. Gambuti, R. Pessina, P. Piombino, *J. Agric. Food Chem.* 52 (2004) 891.
- [20] D.D. Ramey, A. Bertrand, C.S. Ough, V.L. Singleton, E. Sanders, *Am. J. Enol. Vitic.* 37 (1986) 99.
- [21] W.S. Joslin, C.S. Ough, *Am. J. Enol. Vitic.* 29 (1978) 11.
- [22] J. Crouzet, C. Flanz, Y.Z. Günata, P. Pellerin, J.C. Sapis, in: C. Flanz (Ed.), *(Enologie—Fondements Scientifiques et Technologiques, Part 10, Lavoisier Tec & Doc, Paris, 1998, p. 361.*
- [23] S. Prestage, R.S.T. Linforth, A.J. Taylor, E. Lee, J. Speirs, W. Schuch, *J. Sci. Food Agric.* 79 (1999) 131.
- [24] J. Sanchez, J.L. Harwood, *Eur. J. Lipid Sci. Technol.* 104 (2002) 564.
- [25] A. Hatanaka, *Phytochemistry* 34 (1993) 1201.
- [26] J.M. Oliveira, Ph.D. Thesis, Universidade do Minho, Braga, Portugal, 2000.

- [27] Commission Regulation (EEC) No. 2676/90, Official Journal of the European Union L 272, 03/10/1990, p. 1.
- [28] Z. Günata, I. Dugelay, J.C. Sapis, R. Baumes, C. Bayonove, in: P. Schreier, P. Winterhalter (Eds.), *Progress in Flavour Precursor Studies—Analysis, Generation, Biotechnology*, Allured Publ., Carol Stream, 1993, p. 219.
- [29] G. Versini, I. Orriols, A. Dalla Serra, *Vitis* 33 (1994) 165.
- [30] C. Lema, C. Garcia-Jares, I. Orriols, L. Angulo, *Am. J. Enol. Vitic.* 47 (1996) 206.
- [31] G. Nicolini, G. Versini, A. Dalla Serra, A. Seppi, M. Falcetti, in: A. Lonvaud-Funel (Ed.), *Œnologie 95—5^{ème} Symposium International d'Œnologie*, Lavoisier Tec & Doc, Paris, 1996, p. 539.
- [32] G. Versini, A. Dalla Serra, A. Scienza, P. Barchetti, *Atti Simposio sul Traminer Aromatico*, Bolzano, 1990, p. 59.
- [33] C. Aubert, R.L. Baumes, Y.Z. Günata, J.P. Lepoutre, J.F. Cooper, C.L. Bayonove, *J. Int. Sci. Vigne Vin.* 31 (1997) 57.
- [34] E. Boido, A. Lloret, K. Medina, L. Farina, F. Carrau, G. Versini, E. Dellacassa, *J. Agric. Food Chem.* 51 (2003) 5408.
- [35] M.S. Perez-Coello, M.A. Sanchez, E. Garcia, M.A. Gonzalez-Viñas, J. Sanz, M.D. Cabezero, *J. Agric. Food Chem.* 48 (2000) 885.
- [36] R. Di Stefano, M. Castino, *Riv. Vitic. Enol.* 5 (1983) 245.
- [37] I. Dugelay, Ph.D. Thesis, *École Supérieure Agronomique de Montpellier*, Montpellier, France, 1993.
- [38] Y.Z. Günata, C.L. Bayonove, R.L. Baumes, R.E. Cordonnier, *Am. J. Enol. Vitic.* 37 (1986) 112.
- [39] P.J. Williams, C.R. Strauss, B. Wilson, *J. Agric. Food Chem.* 28 (1980) 766.
- [40] D.D. Rowan, J.M. Allen, S. Fielder, M.B. Hunt, *J. Agric. Food Chem.* 47 (1999) 2553.
- [41] J.C. Mauricio, J. Moreno, L. Zea, J.M. Ortega, M. Medina, *J. Sci. Food Agric.* 75 (1997) 155.