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Differences in Odor-Active Compounds of Trincadeira Wines Obtained from Five Different Clones

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Odorant compounds of five young clonal red wines made from *cv.* Trincadeira, a native grape variety of *Vitis vinifera* L. grown in Portugal, were studied using 2001 and 2003 vintages. The study was carried out using gas chromatography−mass spectrometry (GC-MS) for compound identification and the gas chromatography−olfactometry (GC-O) posterior intensity method to detect the potentially most important aroma compounds. Forty-one odorant peaks were detected by GC-O analysis, from which 31 were identified by GC-MS. The odorant compounds with the highest odorant average intensities are 3-methylbutanoic acid, 2-phenylethanol, 2,5-dimethyl-4-hydroxy-3(2H)-furanone, and 4-vinylguaiacol. The GC-O analysis showed odor intensity differences among compounds, which was confirmed by analysis of variance (ANOVA). Principal component analysis (PCA) and hierarchical cluster analysis (HCA) showed that the five clonal wines from the 2001 vintage were more similar than those from the 2003 vintage. Moreover, stepwise linear discriminant analysis (SLDA) demonstrated that the factor vintage has influence on the Trincadeira clonal red wine odorant profile differentiation.

KEYWORDS: GC-O posterior intensity method; odorant compounds; Trincadeira; clonal red wines; vintage

INTRODUCTION

Trincadeira is a neutral red grape variety widely planted in Portugal, and it has been one of the first varieties chosen for the Portuguese clonal selection program, which started in 1978. Nowadays, with this program, the intention has been to develop superior clones based on wine quality rather than yield (*1*). One clone is the certified vegetative descent of one vine chosen for its identity, its phenotypic characteristics, and its sanitary conditions.

Several studies carried out with different GC-O techniques to evaluate the volatile compounds in wines have shown that odorants with major sensory impact resulting from alcoholic fermentation are common to all grape varieties, but are present at different concentration levels and proportions depending on the wine (*2–4*). Besides grape variety, climatic conditions, and viticultural practices, soil and region have an important influence on the aroma and flavor character of each wine (*5, 6*).

Recently, the GC-O analysis has been used to identify odor-active compounds in wines from Chardonnay (*7*), white Riesling and some hybrids (*8*), aged Vidal blanc (*9*), Gewürztraminer (*10–12*) Schreube (*10, 11*), Pinot Noir (*13*), young Merlot and Cabernet Sauvignon (*2–4*), Tempranillo (*14, 15*), Grenache (*2, 14*) and Touriga Nacional (*16*). Furthermore, quantitative GC-O analyses have been carried out by several authors in order to find out key differences in the odor profiles of three monovarietal young red wines (*2*), different Spanish aged red wines (*17*), four Madeira wines from Malvazia, Boal, Verdelho and Sercial cultivars (*18*) or among clonal red wines (*15*). To our knowledge, there are no references in the literature concerning the identification or odorant intensity determination of Trincadeira clonal wines by GC-O analysis. Therefore, the two complementary objectives of this study were the following: first, to characterize five Trincadeira clonal red wines from two different vintages according to their odorant composition using a previously optimized GC-O posterior intensity method (*15*); and second, to differentiate the same wines according to vintage.

MATERIALS AND METHODS

Samples. Grapes of five certified clones (**Table 1**) from the Portuguese variety *Vitis vinifera* L. *cv.* Trincadeira from the Ribatejo Controlled Denomination of Origin were sampled from one experimental vineyard, in the 2001 and 2003 vintages. Harvesting time was determined considering the commercial ripening of grapes in a range between 21.7 and 24.0 °Brix and the pH values were around 3.4.

About 60 kg of grapes of each clone in good sanitary conditions at the final stage of ripening were hand harvested and transported to the experimental winery at Estação Vitivinícola Nacional in 20 kg plastic boxes. The grapes were destemmed and crushed on a commercial grape

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Table 1. Codes of Trincadeira Clonal Red Wines

vintage	certified clone	code
2001	T 11 EAN (PT)	1T2
	T 12 EAN (PT)	1T3
	T 13 EAN (PT)	1T4
	T 14 EAN (PT)	1T5
	T 15 EAN (PT)	1T6
2003	T 11 EAN (PT)	3T2
	T 12 EAN (PT)	3T3
	T 13 EAN (PT)	3T4
	T 14 EAN (PT)	3T5
	T 15 EAN (PT)	3T6

destemmer-crusher and then transferred to 60 kg-capacity stainless steel cubes for maceration. A 6% solution containing SO_2 (sulfur dioxide) was added to the musts prior to alcoholic fermentation (30 mg.L^{-1}). All the alcoholic fermentations were completed by the metabolism of spontaneous yeasts at the controlled temperature of 23 °C. The wines were transferred to 20 L glass carboys equipped with fermentation locks, and kept at 24 °C until dry and through malolactic fermentation. Afterward, wines were racked, and transferred to clean 10 L glass carboys, and the free SO_2 was adjusted to 30 mg.L^{-1}. Two weeks after the final rack and SO_2 adjustment, wines were bottled and stored at cellar temperature. The five Trincadeira clonal wines from the 2001 and 2003 vintages were analyzed after equal time of bottling in order to avoid the influence of the time bottling in the obtaining of the analytical and sensory data. Thus, all these wines were kept approximately for eighteen months in bottle before the extraction for further analyses.

Reagents. Dichloromethane and sodium sulfate anhydrous, both analytical grade, were purchased from Merck (Darmstadt, Germany). The dichloromethane was redistilled in a Vigreux column. The GC-O and GC-MS standards were: diacetyl, ethyl butanoate, ethyl 2-methylbutanoate, ethyl 3-methylbutanoate, 2-methyl-1-propanol, 2-methyl-1-butanol, 3-methyl-1-butanol, ethyl hexanoate, ethyl octanoate, benzaldehyde, 2-methylpropanoic acid, γ-butyrolactone, butanoic acid, 3-methylbutanoic acid, hexanoic acid, guaiacol, 2-phenylethanol, 2,5-dimethyl-4-hydroxy-3(2H)-furanone (Furaneol, registered trademark of Firmenich S.A., Geneva, Switzerland), eugenol, 4-ethylphenol, syringol and vanillin were purchased from Fluka Chemie (Buchs, Switzerland); ethyl isobutyrate, isoamyl acetate, 3-(methylthio)-1-propanol, 4-vinylguaiacol, ethyl vanillate and acetovanillone from Aldrich Chem, Co (Gillingham-Dorset); 4-ethylguaiacol and 2-ethyl-4-hydroxy-5-methyl-3(2H)-furanone (homofuraneol) from TCI (Tokyo Chemical Industry Co., Ltd.); β-damascenone was kindly supplied by Symrise (Holzminden, Germany).

Sample Preparation. Volatile compounds were extracted from wine samples (50 mL) using discontinuous ultrasound liquid−liquid extraction with redistilled dichloromethane, dried over sodium sulfate anhydrous and then concentrated to 0.30 mL (*19, 20*) The wine extraction was performed in duplicate and the extracts were stored at −20 °C until analysis.

FTIR Analysis. All the Trincadeira clonal wines were analyzed by FTIR spectrophotometry, in a WineScan FT120 (Foss, Hillerød, Denmark) equipment, by the Analysis Service of the Enology Department of the Estação Vitivinícola Nacional. The infrared measurement range was 926 to 5012 cm. The following analytical parameters were determined: density (g·mL^{-1}), alcohol degree (% vol.), titratable acidity (expressed as g·L^{-1} tartaric acid), and pH.

GC-O Analysis. The GC-O system consisted of an Agilent Technologies 6890 Series chromatograph (Wilmington, DE, USA) equipped with a flame ionization detector (FID) and an Olfactory Detection Port (ODP, Gerstel, Germany). GC effluent was split 1:3 between the FID and the ODP. Each sample (0.6 μL) was injected using the splitless mode into a capillary column (INNOWAX, 30 m length × 0.25 mm i.d. × 0.25 μm film thickness, J&W Scientific, Folsom, CA). Operating conditions were as follows: injector and FID, 250 °C; ODP, 220 °C; carrier gas hydrogen, 2.0 mL.min^{-1}; the oven temperature was held at 45 °C for 5 min and increased to 210 at 3.5 °C min^{-1} and held at 210 °C for 20 min. The linear retention indices (LRI) of the compounds (GC-FID and the olfactometry peaks) were calculated from the retention time of n-alkanes (C_9−C_{26}, C_{28} and C_{30}) by linear interpolation (*21*).

The GC-O analysis was carried out using the posterior intensity method (*15*). The sniffing panel was composed by a group of 8 sniffers trained in odor recognition and with a large experience in GC-O analysis (*15, 22*). During all the experiments, the sniffers were asked to assign odor properties to each detected odor peak and to use a memorized five-point intensity interval scale (1, very mild; 2, mild; 3, moderate; 4, strong; 5, very strong) for intensity evaluation. The panel average intensity scores were calculated. The intensity of odors not detected by a sniffer was set to 0 (zero).

GC-MS Analysis. A Finnigan MAT (San Jose, CA, USA) GC-MS equipment (Magnum) was used to analyze the wine extracts. An aliquot of 0.6 μL was injected and volatile compounds were separated using a fused silica capillary column of polyethylene glycol (DB-WAX, 30 m length × 0.25 mm i.d. × 0.25 μm film thickness, J&W Scientific, Folsom, CA, USA). Operating conditions were as follows: injector and interface temperature, 250 °C; carrier gas helium (inlet pressure 12 psi and split ratio 1:60); the temperature gradient used began at 50 °C for 2 min, and was raised to 180 at 3.5 °C min^{-1} and held at this temperature for 25 min. The mass spectrometer was operated in the electron impact mode at 70 eV, scanning the range m/z 39−340. Identification of volatile compounds was systematically confirmed with the retention indices of the available pure standard compounds (determined in the same analysis conditions) and with the comparison between the mass spectra of the volatile compounds and of the pure standard compounds. All mass spectra were also compared with those of the data system libraries (NIST and Wiley).

Statistical Analysis. The software package SPSS release 14.0 for Windows (SPSS Inc., Chicago, IL, USA) was used for analyses of variance (one-way ANOVA), *post hoc* LSD test, principal component analysis (PCA) and hierarchical cluster analysis (HCA). The multivariate data analysis PCA, based on a correlation matrix, was computed using the SPSS factor reduction procedure with Varimax rotation. The average scores of all odorant peaks were introduced as variables (columns) and the clonal wines were introduced as objects (rows). The Varimax rotation is an orthogonal rotation method which simplifies the factor interpretation (*23*). The first principal components (PCs) were retained by the Kaiser criteria and the scree test (*23, 24*). Stepwise linear discriminant analysis (SLDA) is a supervised method used for classification purposes. SLDA renders a number of orthogonal linear discriminant functions equal to the number of categories minus one. This method minimizes the variance within categories and maximizes the variance between categories. The variables included in the analysis are determined with a SLDA using Wilk's Lambda as a selection criterion and an F-statistic factor to establish the significance of the changes in Lambda when a new variable is tested (*24*).

RESULTS AND DISCUSSION

The analytical results obtained by FTIR analysis of the five Trincadeira clonal wines from the 2001 and 2003 vintages are presented in **Table 2**. The clonal wines from the 2001 vintage showed, in general, higher average values for the alcohol degree and lower pH average values than the clonal wines from the 2003 vintage.

The odorant compounds of those clonal wines were evaluated by the GC-O posterior intensity method, and their identification was performed by GC-MS. Forty-one odorant peaks were perceived by the sniffers in at least one of the ten clonal wine extracts according to the posterior intensity method and thirty-one odorant compounds were identified by GC-MS. **Table 3** presents the number attributed to the detected odorant peaks, the linear retention indices (LRI), the identity of the compounds, the reliability of identification, the main odor descriptors, the average intensity scores obtained by the GC-O posterior intensity method, the clonal wine effect on the average intensity scores

Table 2. Analytical Results of the Five Trincadeira Clonal Wines ($n = 4$) from the Two Vintages by FTIR Analysis

clonal wines		volumic mass ($g.mL^{-1}$)	alcohol degree (% vol.)	titratable acidity ($g.L^{-1}$ tartaric acid)	pH
vintage 2001					
1T2	x[a]	0.9910	13.35	5.75	3.42
	SD[b]	*0.00*	*0.64*	*0.49*	*0.05*
1T3	x	0.9912	13.35	5.70	3.42
	SD	*0.00*	*0.49*	*0.71*	*0.05*
1T4	x	0.9903	14.30	6.00	3.36
	SD	*0.00*	*0.14*	*0.14*	*0.01*
1T5	x	0.9927	13.60	5.55	3.39
	SD	*0.00*	*0.85*	*0.07*	*0.10*
1T6	x	0.9926	13.60	5.50	3.41
	SD	*0.00*	*0.57*	*0.42*	*0.04*
vintage 2003					
3T2	x	0.9941	12.70	5.40	3.59
	SD	*0.00*	*0.00*	*0.28*	*0.06*
3T3	x	0.9916	13.10	5.20	3.53
	SD	*0.00*	*0.00*	*0.14*	*0.04*
3T4	x	0.9923	12.55	4.75	3.69
	SD	*0.00*	*0.07*	*0.07*	*0.00*
3T5	x	0.9920	13.30	5.00	3.62
	SD	*0.00*	*0.00*	*0.14*	*0.03*
3T6	x	0.9922	13.00	5.05	3.56
	SD	*0.00*	*0.00*	*0.07*	*0.04*

[a] x: average. [b] SD: standard deviation.

for each vintage and the vintage effect on the average intensity scores differences among the vintages.

According to the GC-O analysis, 3-methylbutanoic acid (P15), 2-phenylethanol (P22), 2,5-dimethyl-4-hydroxy-3(2*H*)-furanone (P27), and 4-vinylguaiacol (P35) were the odorants with the highest average intensities in all clonal wines and in both vintages.

In the five clonal wines from the 2001 vintage, statistically significant differences (ANOVA) were observed among the average intensity scores for seven odorant compounds. Applying the same data analysis to the clonal wines from the 2003 vintage, fifteen odorant compounds showed statistically significant differences. These results indicate that the five clonal wines from the 2001 vintage were more similar than those from the 2003 vintage. The effect of vintage was very important owing to the number of statistically significant differences found. Indeed, fourteen odorant compounds presented statistically significant differences due to the effect of the vintage factor.

Seven esters, well-known as important constituents of young wine aroma and referred to as key compounds in the fruity flavors of wines (*25, 26*) were detected in our GC-O experiments, like ethyl isobutyrate (P1), ethyl butanoate (P3), ethyl 2-methylbutanoate (P4), ethyl 3-methylbutanoate (P5), isoamyl acetate (P7), ethyl hexanoate (P9), and ethyl octanoate (P10). Among these esters, all produced by yeast during alcoholic fermentation, isoamyl acetate had an important role in the vintage differentiation among clonal wines ($p < 0.001$). Indeed, in the five wines from the 2001 vintage, the average intensity of this volatile compound was zero. In opposition, the average intensity of isoamyl acetate varied from 0.8 to 1.0 score, in all clonal wines from the 2003 vintage.

The odorant compound 2-phenylethanol (P22) reached a high intensity average score in all Trincadeira clonal wines. This compound has been detected in wines from different provenience by GC-O (*4, 15, 27*). Even though the presence of odorant compound 2-phenylethanol (P22) in grapes, as well as in precursor hydrolysates, has been previously reported in GC-O experiments (*27, 28*), this compound is mostly produced by yeasts during alcoholic fermentation.

Among C_{13}-norisoprenoid compounds, only β-damascenone (P17) was detected in both vintages. Clonal wine 1T6 was the only one with an average intensity score of zero. In all the other nine wines, the average intensity score varied in range between 0.9 and 2.0. This compound has been described as an important odor-active compound found in musts (*27*) and wines (*4, 15, 17*).

Monoterpenic compounds were not detected by GC-O or GC-MS in all wines analyzed in this study. This result indicates that the aroma of this cultivar is not influenced by monoterpenic compounds.

Among the lactones family, only γ-butyrolactone (P13) was detected by GC-O analysis, with an average intensity score ranging from 0.0 to 0.9, which denotes its very low odor intensity when compared to the other compounds found in Trincadeira clonal wines.

The 2,5-dimethyl-4-hydroxy-3(2*H*)-furanone (P27) and homofuraneol (P28), both described with the odor descriptors burnt sugar and candy cotton, were detected by GC-O analysis. The average intensity scores of the first compound were always higher in all clonal wines than those of the second one. The 2,5-dimethyl-4-hydroxy-3(2*H*)-furanone was identified in juice and wines from *Vitis labrusca* hybrid grapes (*29, 30*). Recently, it has also been detected in *Vitis vinifera* wines (*4, 10, 11, 31*) Homofuraneol was first reported in *Vitis vinifera* wines (*10*) and, since then, has been considered as an odor-active compound in wines (*4, 17, 31*).

Three volatile acids, butanoic acid (P14), 3-methylbutanoic acid (P15) and hexanoic acid (P19), were also determined by GC-O analysis. The last one had basically no odorant importance since it was detected only in the 3T4 clonal wine extract.

Six volatile phenols guaiacol (P20), 4-ethylguaiacol (P26), eugenol (P33), 4-ethylphenol (P34), 4-vinylguaiacol (P35) and syringol (P37) have been considered odor-active compounds of red wines (*4, 17, 32*) All of them were identified in the odorant fraction of Trincadeira, except 4-ethylguaiacol which was not detected in the five clonal wines from the 2001 vintage, revealing the high statistical effect of vintage ($p < 0.001$) on the occurrence of this volatile phenol in these wines.

Vanillin (P39), ethyl vanillate and acetovanillone (P40) were also detected in the GC-O analysis. The first volatile compound was not detected in all wines from the 2001 vintage which indicates the effect of the vintage ($p < 0.001$) on vanillin detection in Trincadeira wines.

PCA was applied to the GC-O posterior intensity method data of the five clonal wines in order to verify if it could be possible to clearly differentiate the wines from the 2001 and 2003 vintages. This multivariate analysis permitted the establishment of a relationship between the different odorant compound variables and the wines, and the finding of the most important factors of variability.

Figure 1 shows in the two-dimensional plot of PC1 against PC2, the locations of the forty-one GC-O peaks and the clonal wines. The percentage value corresponding to each PC, presented in **Figure 1**, indicates the percentage of variation in the data explained by the PC's. Clonal wines from the 2001 vintage (1T2 to 1T6), localized on the negative side of PC2, show a great proximity among them and represent a well defined group of wines. This similarity was previously demonstrated by the ANOVA and LSD results (as shown in **Table 3**).

These five clonal wines were influenced by the same group of odorant compounds. Specifically, the main odorants correlated with these wines are ethyl isobutyrate (P1), diacetyl (P2), ethyl 2-methylbutanoate (P4), benzaldehyde (P11), 3-methylbutanoic acid (P15), and 2-phenylethanol (P22). Wines 3T3 and 3T5 from

Table 3. Odorant Compound Intensity Scores Determined by GC-O Posterior Intensity Method in Trincadeira Clonal Wines. Clone and Vintage Effect on Average Intensity Score Differences of Odorant Compounds among Clonal Wines

				2001 vintage						2003 vintage						
peak no.	LRI[a]	odorant compound	odor description	1T2	1T3	1T4	1T5	1T6	clone effect	3T2	3T3	3T4	3T5	3T6	clone effect	vintage effect
P1	971	ethyl isobutyrate[b]	fruity	2.1	2.4	2.1	2.4	2.8	ns[d]	2.0	2.4	2.1	2.5	2.3	ns	ns
P2	975	diacetyl[b]	caramel, butter	2.6	2.6	2.8	2.4	2.8	ns	2.0	2.6	2.8	2.1	2.9	ns	ns
P3	1028	ethyl butanoate[b]	fruity	0.4ab	0.6b	0.0a	0.5b	0.0a	**[f]	1.3	0.0	1.0	1.4	0.8	ns	**
P4	1048	ethyl 2-methylbutanoate[b]	fruity	1.4	1.8	1.3	1.6	1.6	ns	0.8	1.0	1.1	1.3	1.4	ns	ns
P5	1064	ethyl 3-methylbutanoate[b]	fruity	1.5	2.0	1.6	1.6	1.6	ns	1.3	1.9	1.9	1.6	1.4	ns	ns
P6	1086	2-methyl-1-propanol[b]	pungent, herbaceous	0.0	0.0	0.0	0.0	0.0	ns	0.0a	0.8b	0.0a	0.0a	0.0a	*[e]	ns
P7	1121	isoamyl acetate[b]	fruity, banana	0.0	0.0	0.0	0.0	0.0	ns	0.9	0.8	1.0	1.0	0.9	ns	***[g]
P8	1217	2 + 3-methyl-1-butanol[b]	stinky	2.5	2.8	2.6	2.5	2.3	ns	2.6	2.6	2.1	2.4	2.6	ns	ns
P9	1232	ethyl hexanoate[b]	fruity	0.8	0.9	0.0	1.0	1.1	ns	0.8	0.6	1.4	1.0	0.9	ns	ns
P10	1433	ethyl octanoate[b]	fruity, floral	0.9ac	1.3bc	1.0ab	0.9a	0.0a	*	1.3	0.8	0.8	1.0	1.1	ns	ns
P11	1502	benzaldehyde[b]	plastic	2.3	2.3	2.0	1.8	2.1	ns	1.0	1.9	1.8	1.9	1.6	ns	ns
P12	1581	2-methylpropanoic acid[b]	cheese	1.1	1.3	1.0	1.1	1.4	ns	1.0ab	0.9ab	1.9b	0.0a	0.0a	**	ns
P13	1626	γ-butyrolactone[b]	smoky, hot	0.5	0.0	0.0	0.6	0.9	ns	0.8	0.6	0.5	0.0	0.0	ns	ns
P14	1637	butanoic acid[b]	rancid butter, cheese	2.8	2.6	2.0	2.1	2.3	ns	2.9	2.5	2.8	2.0	2.9	ns	ns
P15	1680	3-methylbutanoic acid[b]	stinky, cheese	3.9	4.5	4.0	3.8	4.0	ns	3.9	4.0	3.8	3.8	3.8	ns	ns
P16	1715	3-(methylthio)propanol[b]	raw potatoes	2.5	2.1	1.5	2.3	1.8	ns	2.3	2.5	2.3	1.4	2.4	ns	ns
P17	1814	β-damascenone[b]	floral, fruity, cooked apple	1.4b	1.3b	1.0b	0.9ab	0.0a	*	1.8	1.4	2.0	1.4	1.8	ns	**
P18	1839	unknown[c]	floral	2.6	2.5	1.6	2.8	2.6	ns	2.4	2.1	2.5	2.1	2.1	ns	ns
P19	1854	hexanoic acid[b]	musty, wet cloth	0.0	0.0	0.0	0.0	0.0	ns	0.0a	0.0a	0.6b	0.0a	0.0a	*	ns
P20	1862	guaiacol[b]	smoky, medicinal	2.6	3.0	2.6	1.9	2.5	ns	2.6	2.4	2.3	2.8	2.4	ns	ns
P21	1882	unknown[c]	floral	0.0	0.0	0.0	0.0	0.0	ns	1.1b	0.0a	1.4b	0.0a	0.0a	**	**
P22	1915	2-phenylethanol[b]	floral, roses	3.4	3.9	3.9	3.9	3.9	ns	3.6	3.3	3.4	3.4	3.3	ns	ns
P23	1959	unknown[c]	floral, medicinal	0.0	0.0	0.0	0.0	0.0	ns	0.9b	0.0a	0.0a	0.0a	0.0a	***	*
P24	1998	unknown[c]	spicy	0.0	0.0	0.0	0.0	0.0	*	1.0	0.5	0.8	0.0	0.8	ns	***
P25	2023	unknown[c]	sweet, burnt	2.0b	1.3ab	0.9a	2.1b	2.0b	***	0.0a	1.3b	1.1b	0.0a	0.8ab	***	
P26	2033	4-ethylguaiacol[b]	floral, carnation, clove	0.0	0.0	0.0	0.0	0.0	ns	1.3b	1.6b	0.0a	1.1b	0.0a	***	***
P27	2037	2,5-dimethyl-4-hydroxy-3(2H)-furanone[b]	burnt sugar, candy cotton	3.4b	3.8b	2.0a	3.1b	3.6b	**	4.0	3.3	4.0	4.0	3.6	ns	**
P28	2078	homofuraneol[b]	burnt sugar, candy cotton	1.8	1.5	0.8	0.9	1.4	ns	2.9c	1.3b	1.8b	0.0a	0.0a	***	ns
P29	2084	unknown[c]	floral, medicinal	0.0	0.0	0.0	0.0	0.0	ns	0.0a	0.0a	0.0a	0.9b	0.0a	*	ns
P30	2091	unknown[c]	burnt, spicy	0.0	0.0	0.0	0.0	0.0	ns	0.0a	0.0a	0.8b	0.0a	0.0a	**	ns
P31	2113	unknown[c]	horse stable, horse sweaty	0.0	0.0	0.0	0.0	0.0	ns	0.0a	1.0b	0.0a	0.9b	0.8ab	*	***
P32	2128	unknown[c]	fruity, floral	0.0a	0.0a	0.0a	0.6b	0.8b	*	1.0b	0.0a	0.9ab	0.0a	1.3b	*	ns
P33	2167	eugenol[b]	floral, spicy	1.0	1.0	0.9	0.0	1.0	ns	1.9b	1.0ab	1.9b	0.6a	1.8b	*	**
P34	2183	4-ethylphenol[b]	animal, horse stable	1.0b	1.1b	2.6c	0.5a	1.6b	**	0.0a	2.0c	2.3c	0.5ab	1.1b	***	ns
P35	2203	4-vinylguaiacol[b]	burnt, curry	3.6	4.0	3.8	3.8	3.8	ns	4.0	3.5	3.8	3.9	3.5	ns	ns
P36	2257	unknown[c]	spicy	0.0	0.0	0.0	0.0	0.0	ns	1.1	1.3	1.4	1.3	0.0	ns	***
P37	2269	syringol[b]	medicinal, smoky	1.8	1.4	2.1	1.6	1.6	ns	1.8	2.0	1.8	1.6	1.9	ns	ns
P38	2352	unknown[c]	floral	0.0	0.0	0.0	0.0	0.0	ns	1.6	0.0	1.3	1.0	1.1	ns	***
P39	2566	vanillin[b]	vanilla	0.0	0.0	0.0	0.0	0.0	ns	1.1b	0.9b	0.9b	0.8b	0.0a	*	***
P40	2576	ethyl vanillate[b] + acetovanillone[b]	vanilla, floral	2.8	2.5	3.0	2.9	2.9	ns	2.6	2.5	1.6	2.6	3.3	ns	ns
P41	>2600	unknown[c]	burnt, unpleasant	1.5	1.8	1.8	0.9	1.1	ns	1.6	1.9	1.3	1.5	1.1	ns	ns

[a] Linear retention index on INNOWAX capillary column (30 m × 0.25 mm × 0.25 μm). [b] Identification based on the coincidence of gas chromatographic retention indices and mass spectrometric data with those of the pure standards available in the laboratory. [c] Not identified. [d] ns: not significant. [e] * Significant ($p < 0.05$). [f] ** Highly significant ($p < 0.01$). [g] *** Very highly significant ($p < 0.001$); average values followed by the same letter, in the same line, are not significantly different (LSD, 0.05).

the 2003 vintage are both located on the negative side of PC1 and positive side of PC2 which indicates their similarity. The other three wines are located on the positive side of PC1 and very dispersed.

To better visualize the similarities and dissimilarities among the five Trincadeira clonal wines from the two vintages, a HCA was also done with the same data used in PCA. **Figure 2** shows the dendogram obtained using the Ward method. The dendogram displays three clusters of wines in which the wines with similar odorant profiles were included. In the cluster with more elements, all the five Trincadeira clonal wines from the 2001 vintage were grouped.

The five clonal wines from the 2003 vintage were grouped in two distinct clusters. Wines 3T2 and 3T4 represent one cluster, while 3T3, 3T5 and 3T6 compose the other cluster.

The HCA demonstrated that there was a clear and well defined separation between the 2001 and 2003 vintage wine odorant profiles.

A SLDA using the odorant compounds data was performed in order to discriminate the five clonal wines under study. **Table 4** presents the number of steps, the selected variables, the value of F-to-remove of selected variable, the significance level (*Sig.*), and the standardized coefficients of discriminant functions (DFs).

According to these results, six variables, isoamyl acetate (P7), 2-methyl-1-propanol (P6), unknown (P24), 2-methylpropanoic acid (P12), hexanoic acid (P19) and ethyl hexanoate (P9), were found to be discriminating variables. **Table 5** presents the percentage of correctly classified clonal wines and shows that 100.0% of the original grouped cases were correctly classified. The discriminant function obtained allowed the classification of all the wines of both vintages in their correct groups. Consequently, the SLDA achieved a good clonal wine separation regarding vintage year.

This study demonstrated the importance of GC-O posterior intensity method for the odorant characterization of Trincadeira clonal red wines. Moreover, as the clones are the same in the

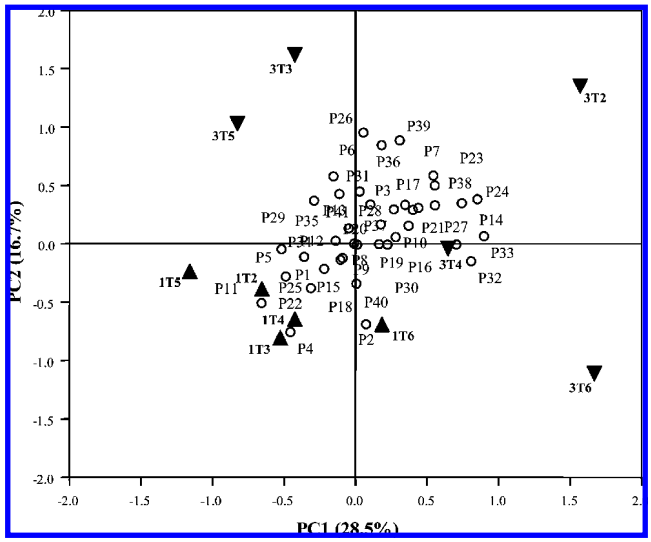

Figure 1. Plot of the first and second principal components (PCs) of the GC-O data and Trincadeira clonal wines from 2001 and 2003 vintages (the percentage of variation explained by each PC is indicated within parentheses).

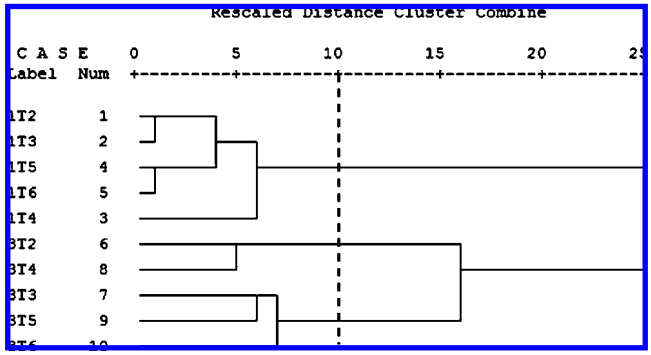

Figure 2. Dendogram of Trincadeira clonal wines using the Ward method.

Table 4. Stepwise Linear Discriminant Analysis According to Vintage (Years 2001 and 2003)

step	selected variable	F-to-remove of selected variable	standardized coefficients of DF
1	P7	370.286	31.588
2	P6	13.984	27.588
3			
4	P24	5.053	19.027
5	P12	26.846	−9.150
6			
7	P19	17.671	−7.768
8	P9	4.934	1.591
Eigenvalues of DFs			62474.692
p-values of DFs			0.000

Table 5. Percentage of Correctly Classified Trincadeira Clonal Wines

			predicted group membership		
	wine year		2001	2003	total
original	count	2001	5	0	5
		2003	0	5	5
	%	2001	100.0	0.0	100.0
		2003	0.0	100.0	100.0

two vintages, have the same origin (vineyard) and similar fermentation conditions, the differences found in the wines between the two vintages could be due to their different climatic conditions. The odorant compounds with the highest odorant average intensities are 3-methylbutanoic acid, 2-phenylethanol, 2,5-dimethyl-4-hydroxy-3(2H)-furanone and 4-vinylguaiacol. Coinciding results were obtained with ANOVA, HCA and LSDA when considering two vintages. According to these statistical analyses, the odorant profiles of the five clonal wines from the 2001 vintage were more similar among them than those of the same clones from the 2003 vintage. Moreover, the results showed that the vintage factor has influence on the Trincadeira clonal red wine odorant profile differentiation.

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