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GC-OLFACTOMETRY AND DESCRIPTIVE SENSORY ANALYSIS IN THE STUDY OF CLONAL RED WINES

GC-OLFACTOMETRIA E ANÁLISE SENSORIAL DESCRITIVA NO ESTUDO DE VINHOS TINTOS MONOCLONAIS

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SUMMARY

The aroma profiles of five distinct clonal red wines from Aragonez *Vitis vinifera* L. cultivar were studied by gas chromatography-olfactometry (GC-O) and descriptive sensory analysis. Thirty-two odourant peaks were perceived by the sniffers in at least one of the five clonal wine extracts according to the GC-O posterior intensity method and twenty-nine odourant compounds were identified by gas chromatography-mass spectrometry (GC-MS). The 3-methylbutanoic acid, 2-phenylethanol, 4-vinylguaiacol, furaneol, and homofuraneol were the highest average intensity odourant compounds in all clonal wines. Principal component analysis permitted the establishment of a relationship between the different odourant compound variables and the wines, as well as between the aroma descriptors and the wines. Furthermore, a multiple linear regression model (68.9% explained variance) with a vector grouping 7 odourant compounds associated with aroma defects and a second vector grouping 13 compounds with *fruity-sweet* descriptors was found. The differentiation of the five clonal wines achieved by GC-O and descriptive sensory analysis revealed that these analyses are complementary.

RESUMO

Os perfis de aroma de cinco vinhos tintos monoclonais de *Vitis vinifera* L. var. Aragonez foram estudados por cromatografia gasosa-olfactometria (CG-O) e por análise sensorial descritiva. Foram detectados trinta e dois picos odourantes pelos *sniffers* em pelo menos um dos cinco extractos de vinho conforme a CG-O pelo método de intensidade posterior e vinte e nove compostos odourantes foram identificados por cromatografia gasosa acoplada à espectrometria de massa (CG-EM). O ácido 3-metilbutanóico, o 2-feniletanol, o 4-vinilguaiacol, o furaneol e o homofuraneol foram os compostos que apresentaram os valores mais elevados de intensidade odourante em todos os vinhos. A análise em componentes principais permitiu estabelecer uma relação entre as variáveis compostos odourantes e os vinhos, bem como, entre os descritores de aroma e os vinhos. Além disso, foi encontrado um modelo de regressão linear múltipla (68,9% de variância explicada) com um vector que agrupou 7 compostos odourantes associados a defeitos de aroma e um segundo vector que agrupou 13 compostos com descritores de *frutado-adocicado*. A diferenciação dos cinco vinhos monoclonais conseguida com CG-O e análise sensorial descritiva demonstrou que estas análises são complementares.

Key words: GC-O posterior intensity method; descriptive sensory analysis; clonal wines

Palavras-chave: CG-O pelo método de intensidade posterior; análise sensorial descritiva; vinhos monoclonais

INTRODUCTION

The large variability and diversity of red wines produced with Portuguese *Vitis vinifera* L. cultivars fully justifies their chemical and aroma characterisation. Furthermore, the characterisation of the aroma of clonal wines is of undeniable interest to the winemaking industry, due to its qualitative, productive and financial aspects. The Portuguese Clonal Selection Program was created in 1978 with the objectives of to select the best varieties of grapes and to increase the quality of wines produced in Portugal.

Aragonez cultivar is among the eight most planted red grape varieties in Portugal and some clones of this cultivar have already been certified.

Several papers around the world report the overall identification of odour-active compounds in white and red wines and musts but few referred particularly to clonal wines (Botelho *et al.*, 2007; Botelho *et al.*, 2008). Gas chromatography-olfactometry (GC-O) methodologies strongly contributed to these publications. Most of these studies have focused on red varieties such as Pinot Noir (Moio and Etiévant, 1995), Grenache (Ferreira *et al.*, 1998a, López *et al.*, 1999), Tempranillo (Ferreira *et al.*, 1998b), Cabernet Sauvignon and Merlot (López *et al.*, 1999; Ferreira *et al.*, 2000, Kotseridis and Baumes, 2000, Falcão *et al.*, 2008), Touriga Nacional (Falco, 2004), Aragonez and Trincadeira (Botelho *et al.*, 2007, 2008) and have

shown that specific aroma profiles can be explained by relatively few odourant compounds.

On the other side, descriptive sensory analysis is a useful and complementary tool for describing aroma profiles as well as for finding differences between wines. For example Escudero *et al.* (2007) studied the aroma of five premium red wines combining GC-O analysis with sensory descriptive analysis of wines and they have found interesting correlations between data from both analyses.

The knowledge about the relationships between the individual role of each odourant compound and the global role of all aroma compounds is decisive to be able to choose the best clones to produce the best wines.

Therefore the purpose of this study was to differentiate and study the aroma of five distinct clonal red wines from Aragonez *Vitis vinifera* L. cultivar using the GC-O posterior intensity method and descriptive sensory analysis.

MATERIAL AND METHODS

Samples

Grapes of five certified clones (TABLE I) from the Portuguese variety *Vitis vinifera* L. *cv.* Aragonez from the Alentejo Controlled Denomination of Origin were sampled from one experimental vineyard, in the 2001 vintage. Harvesting time was determined considering the commercial ripening of grapes in a range between 22.0 and 25.0 ºBrix and the pH values were around 3.4.

TABLE I

Codes of the five Aragonez *Vitis vinifera* L. clonal wines.
Códigos dos cinco vinhos monoclonais de Aragonez Vitis vinifera L.

Certified clone	2001 Vintage
T 54 EAN (PT)	1AA1
T 56 EAN (PT)	1AA2
T 57 EAN (PT)	1AA3
T 58 EAN (PT)	1AA4
T 60 EAN (PT)	1AA5

About 60 Kg of grapes of each clone in excellent sanitary conditions at the final stage of ripening were hand harvested and transported to the experimental winery at INIA - Dois Portos (Portugal) in 20 kg plastic boxes. The grapes were destemmed and crushed on a commercial grape destemmer-crusher and then transferred to 60 Kg-capacity stainless steel vessels for maceration. A 6 % solution of sulfur dioxide (SO_2) 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 ºC. The wines were transferred to 20 L glass carboys equipped with fermentation locks, and kept at 24 ºC through malolactic fermentation. Afterwards, 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 racking and SO_2 adjustment, wines were bottled and stored at cellar temperature. The five Aragonez clonal wines were kept for approximately eighteen months in bottle before the sampling for further analyses.

Reagents

Dichloromethane and anhydrous sodium sulfate, both analytical grade 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 anhydrous sodium sulfate and then concentrated to 0.30 mL (Cocito *et al.*, 1995; Ribeiro-Corrêa, 1996; Botelho *et al.*, 2008). The wine extraction was performed in duplicate and the extracts were stored at -20 ºC until analysis.

FTIR analysis

All the Aragonez clonal wines were analysed by FTIR spectrophotometry, in a WineScan FT120 (Foss, Hillerød, Denmark), by the Analysis Service of the Enology Department of INIA – Dois Portos. 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 2, Gerstel, Germany). GC effluent was split 1:3 between the FID and the ODP. Each sample (0.8 µL) was injected using the splitless mode into a capillary column (INNOWAX, 30 m length x 0.25 mm i.d. x 0.25 µm film thickness, J&W Scientific, Folsom, CA). Operating conditions were as follows: injector and FID, 250 ºC; ODP, 220 ºC; carrier gas hydrogen, 2.0 mL min^{-1}; the oven temperature was held at 45 ºC for 5 min and increased to 210 ºC at 3.5 ºC min^{-1} and held at 210º C for 20 min. The linear retention indices (LRI) of the compounds (FID and the olfactometry peaks) were calculated from the retention time of n-alkanes (C9-C26, C28 and C30) by linear interpolation (Philips, 1989). Each wine sample was analysed by eight sniffers and no odour descriptions were given in advance. Furthermore, they were asked to describe the attribute of the odour detected, which was recorded (Botelho *et al.*, 2007; Botelho, 2008; Botelho *et al.*, 2008).

Posterior intensity method

A panel of eight sniffers, three men and five women (aged 25-62 years), carried out the sniffing of the extracts. All of them had extensive experience in GC-O analysis. The GC-O analysis was carried out using the posterior intensity method. Each sniffer evaluated the wine extract once using a memorised five-point intensity interval scale (1 - very mild; 2 - mild; 3 - moderate; 4 - strong; 5 - very strong) for intensity evaluation and the average scores of the eight sniffers was calculated for all detected odourants (Botelho *et al.*, 2007; Botelho, 2008; Botelho *et al.*, 2008).

GC-MS analysis

Finnigan MAT (San Jose, CA, USA) GC-MS equipment (Magnum) was used to analyse 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 x 0.25 mm i.d. x 0.25 µm film thickness, J&W Scientific, Folsom, CA, USA). Operating conditions were as follows: injector and interface temperature, 250 ºC; carrier gas helium (inlet pressure 12 psi and split ratio 1:60); the temperature gradient began at 50 ºC for 2 min, and was raised to 180 ºC at 3.5 ºC 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 (Botelho, 2008, Botelho *et al.*, 2008). All mass spectra were also compared with those of the data system libraries (NIST and Wiley).

Descriptive sensory analysis

The sensory panel was composed by the eight judges who integrated the group of GC-O sniffers. Seven olfactory descriptors were used: *sweet*, *red fruits*, *dried fruits*, *spicy*, *woody*, *herbaceous* and *animal*. The judges were asked to score these attributes as well as the aroma quality of each wine on a structured scale (0: no perception to 5: highest perception). The training of the judge panel consisted of descriptive sensory analysis of several red wines, during three months. After the training period, the five Aragonez clonal wines were evaluated twice in different sessions to assess panel and judge performance (Botelho, 2008), and they were presented to the judges in random order to eliminate first order carry-over effects (Williams, 1949). An amount of 30 mL of wine samples was given to each judge in wine tasting glasses at 20 ºC, under white natural lighting (ISO 3591:1977). For in-mouth evaluation, judges sipped the samples and were required not to swallow it after determination of the attributes' intensities. Water was provided for mouth rinsing between samples.

Statistical analysis

The software package SPSS release 14.0 for Windows (SPSS Inc., Chicago, IL, USA) was used for Spearman's rank correlation test, analysis of variance (one-way ANOVA), *post hoc* LSD test, principal component analysis (PCA) and multiple linear regression analysis. Since the analysis of variance test suggested that a difference existed among populations, a multiple comparison test was used to rank the means and to identify the means that were different. The Fisher LSD multiple comparison test was applied to the means because it is the least conservative test in comparison with the Tukey and Duncans tests and should produce the highest difference (Maroco, 2003).

The principal component analysis (PCA), based on a correlation matrix, was computed using the SPSS factor reduction procedure with Varimax rotation for the GC-O posterior intensity method average scores of all odourant compounds detected. The Varimax rotation is an orthogonal rotation method which simplifies the factor interpretation (Pardo and Ruiz, 2001). The first principal components (PCs) were retained by the Kaiser criteria and the scree test (Maroco, 2003; Pardo and Ruiz, 2001). Significant loadings with an absolute value >0.700 represented a strong influence (Siebert, 1999).

The multiple linear regression analysis was done between the GC-O scores of odourant compounds

and the aroma quality scores of the five clonal wines.

RESULTS AND DISCUSSION

The results obtained by FTIR analysis of the five Aragonez clonal wines from the 2001 vintage are presented in TABLE II. The five clonal wines were statistically significantly different considering the four analytical parameters (volumic mass, alcohol degree, titratable acidity and pH). The clonal wine 1AA3 presented the highest alcohol degree (13.85 % vol.) and in opposition, the 1AA1 showed the lowest average value (12.85 % vol.). The pH values of all clonal wines varied from 3.76 to 4.08.

TABLE II

Analytical results of the five Aragonez clonal wines (n=4) by FTIR analysis.
Resultados analíticos por análise FTIR dos cinco vinhos monoclonais de Aragonez.

Clonal wines		**Volumic mass ($g.mL^{-1}$)**	**Alcohol degree (% vol.)**	**TA ($g.L^{-1}$ tartaric acid)**	**pH**
1AA1	*x*	0.9911d	12.85a	4.25a	4.08e
	S.D.	*0.00*	*0.07*	*0.07*	*0.01*
1AA2	*x*	0.9903b	13.45b	4.55b	3.90c
	S.D.	*0.00*	*0.07*	*0.07*	*0.01*
1AA3	*x*	0.9901a	13.85c	4.95d	3.76a
	S.D.	*0.00*	*0.07*	*0.07*	*0.01*
1AA4	*x*	0.9912d	13.35b	4.45bc	3.87b
	S.D.	*0.00*	*0.07*	*0.07*	*0.01*
1AA5	*x*	0.9908c	13.75c	4.35ac	4.06d
	S.D.	*0.00*	*0.07*	*0.07*	*0.01*
Clonal effect		***	***	**	***

x: average; *S.D.:* standard deviation*; ns*: not significant; *Significant ($p < 0.05$); **Highly significant ($p < 0.01$); ***Very highly significant ($p < 0.001$); average values followed by the same letter, in the same column, are not significantly different (LSD, 0.05).

The odourant compounds of those clonal wines were evaluated by the GC-O posterior intensity method, and their identification was performed by GC-MS. TABLE III presents the number attributed to the detected odourant peaks, the linear retention indices (LRI), the identity of the compounds, the reliability of identification, the main odour descriptors, the average intensity scores obtained by the posterior intensity method, and the clonal wine effect on average intensity score differences among the wines.

Thirty-two odourant peaks were perceived by the sniffers in at least one of the five clonal wine extracts according to the posterior intensity method and twenty-nine odourant compounds were identified by GC-MS. Accordingly to the results presented in TABLE III, 3-methylbutanoic acid (P14), 2-phenylethanol (P20), 4-vinylguaiacol (P27), furaneol (P22) and homofuraneol (P23) were the highest average intensity odourant compounds in all clonal wines.

Each clonal wine showed few differences, particularly in the number of odourant peaks detected. In fact, clonal wines 1AA2 and 1AA3 presented the highest number of odourant peaks, twenty-nine, while the other three 1AA1, 1AA4, and 1AA5, showed twenty-six odourant peaks. The five profiles were very similar as confirmed by LSD test (TABLE III). In only 25% of the odourant peaks statistically significant differences were found, regarding the average intensities.

The PCA was applied to the posterior intensity method (GC-O) data of the five clonal wines in order to verify if it could be possible to clearly differentiate the wines. This multivariate analysis permitted the establishment of a relationship between the different odourant compound variables and the wines, and finding the most important factors of variability. The four principal components explained 100% of the total variance observed. Figure 1 shows in a two dimensional plot of PC1 against PC2 the locations of the thirty-two GC-O peaks and the five wines. The percentage value corresponding to each PC, presented in

TABLE III

Odourant compounds found in five Aragonez clonal wines: peak number, gas chromatographic retention data, compound identification, olfactory description, average olfactory intensity scores and significance level.

Compostos odourantes encontrados nos cinco vinhos monoclonais de Aragonez: número de pico, dados da retenção em cromatografia gasosa; identificação de cada composto; descritor olfactivo; pontuação média da intensidade olfactiva e nível de significância.

Peak no.	**LRI**[a]	**Compound**	**odour descriptor**	**1AA1**	**1AA2**	**1AA3**	**1AA4**	**1AA5**	***Sig.***
P1	971	ethyl isobutyrate[b]	fruity	2.3	1.8	1.6	2.0	1.9	*ns*
P2	975	diacetyl[b]	caramel, butter	0.0a	1.8b	2.3b	2.3b	1.9b	***
P3	1028	ethyl butanoate[b]	fruity	0.9	0.5	0.8	0.4	0.9	*ns*
P4	1048	ethyl 2-methylbutanoate[b]	fruity	0.6	1.3	1.1	0.9	1.4	*ns*
P5	1064	ethyl 3-methylbutanoate[b]	fruity	1.6	1.1	1.3	1.3	1.1	*ns*
P6	1121	isoamyl acetate[b]	fruity, banana	0.0	0.5	1.0	0.8	1.0	*ns*
P7	1217	2+3-methyl-1-butanol[b]	pungent	2.6	2.5	2.3	1.6	2.1	*ns*
P8	1232	ethyl hexanoate[b]	fruity	1.6	1.5	1.4	0.6	1.5	*ns*
P9	1433	ethyl octanoate[b]	fruity, floral	1.1	1.3	1.5	0.0	1.0	*ns*
P10	1502	benzaldehyde[b]	plastic	1.3	1.4	2.0	1.3	1.4	*ns*
P11	1581	2-methylpropanoic acid[b]	cheese	1.1	0.9	1.3	1.3	1.6	*ns*
P12	1626	γ-butyrolactone[b]	smoky, hot, burnt	0.6b	0.0a	0.5b	0.0a	0.0a	*
P13	1637	butanoic acid[b]	rancid butter, cheese	3.4	3.0	3.0	2.9	3.4	*ns*
P14	1680	3-methylbutanoic acid[b]	stinky, cheese	4.3	4.3	3.9	4.3	4.3	*ns*
P15	1715	3-(methylthio)-1-propanol[b]	raw potatoes	3.0	2.1	2.8	1.9	2.5	*ns*
P16	1731	unknown[c]	onion, burnt	0.0a	1.0b	0.0a	0.0a	0.0a	*
P17	1814	β-damascenone[b]	floral, fruity, cooked apple	1.6	0.9	1.9	2.0	1.9	*ns*
P18	1839	unknown[c]	floral	2.8	1.8	2.4	2.5	3.0	*ns*
P19	1862	guaiacol[b]	smoky, medicinal-like	3.1	2.4	2.1	2.8	3.0	*ns*
P20	1915	2-phenylethanol[b]	floral, roses	4.3	4.1	4.1	3.5	4.1	*ns*
P21	2033	4-ethylguaiacol[b]	floral, carnation, clove	0.0a	1.6b	0.0a	0.0a	0.0a	*
P22	2037	furaneol[b]	burnt sugar, candy cotton	4.1	3.3	4.3	3.8	4.1	*ns*
P23	2078	homofuraneol[b]	burnt sugar, candy cotton	3.5	3.3	3.0	3.0	3.3	*ns*
P24	2128	unknown[c]	fruity, floral	0.0a	1.0b	0.0a	0.0a	0.9b	**
P25	2167	eugenol[b]	floral, spicy	1.4b	0.0a	0.9bc	0.6ac	0.6ac	**
P26	2183	4-ethylphenol[b]	animal, horse stable	1.3	2.0	0.9	1.0	1.1	*ns*
P27	2203	4-vinylguaiacol[b]	burnt, curry	4.3	3.8	4.0	3.3	4.3	*ns*
P28	2269	syringol[b]	medicinal-like, smoky	3.0	2.8	2.9	2.4	2.4	*ns*
P29	2494	unknown[c]	burnt, unpleasant	0.9	0.6	2.6	0.5	0.0	*ns*
P30	2566	vanillin[b]	vanilla	1.5b	0.0a	0.8ab	1.3b	0.0a	**
P31	2576	ethyl vanillate[b] + acetovanillone[b]	vanilla, floral	2.3	2.5	3.1	2.8	3.0	*ns*
P32	>2600	unknown[c]	burnt, unpleasant	0.0a	1.0b	1.5b	0.0a	0.0a	***

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

Figure 1, indicates the percentage of variation explained by the PCs.

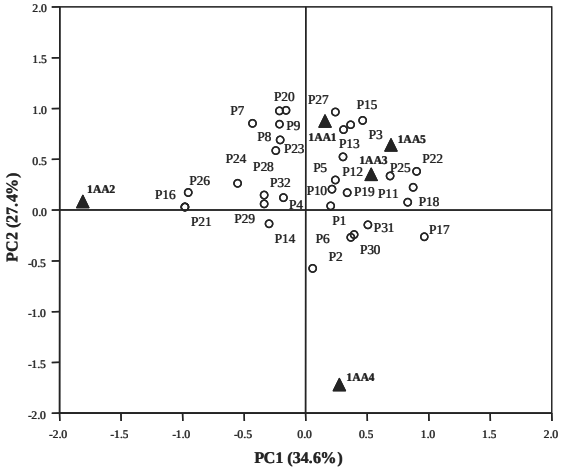

Figure 1 - Plot of the first and second principal components (PCs) of the GC-O data and the five Aragonez clonal wines. The percentage of variation explained by each PC is indicated between brackets. Peaks number P1 to P32 refers to those in TABLE III.

Representação da primeira e segunda componentes principais (CP) dos dados de GC-O e dos cinco vinhos monoclonais de Aragonez. A percentagem de variação explicada por cada CP encontra-se indicada entre parêntesis. Os picos P1 até P32 referem-se aos da TABELA III.

When we use the values of variance analysis from Table III to explain the distribution of wines in the PCA (Figure 1) obtained from the values of the correlation matrix, it can be concluded that the wines 1AA1, 1AA3 and 1AA5, are closely located on the positive side of PC1 and PC2, which might indicate their similarity previously demonstrated by the LSD results (TABLE III) in which statistically significant differences were found among the average intensity of the odourant compounds: diacetyl (P2), γ-butyrolactone (P12), unknown (P24), eugenol (P25), vanillin (P30) and unknown (P32). The 1AA4 wine is located on the PC1 positive side and PC2 negative side, while the 1AA2 wine is located on the negative side of PC1 and positive side of PC2. These two wines are distant from one another and both are distant from the group of the other three wines. The variables which can explain the separation of 1AA2 and 1AA4 wines are one unknown odourant (P16), 4-ethylguaiacol (P21), unknown (P24), vanillin (P30) and unknown (P32). Finally, the separation between the wines 1AA1 and 1AA4 can be explained with the variables diacetyl (P2), γ-butyrolactone (P12) and eugenol (P25).

Seven wine aroma descriptors evaluated by the judges, *sweet*, *red fruits*, *dried fruits*, *spicy*, *woody*, *herbaceous* and *animal* were used for aroma profile characterisation of the five Aragonez clonal wines (TABLE IV). Statistically significant differences in the four aroma descriptors average of the five Aragonez clonal wines were found: *sweet*, *herbaceous*, *animal* and *dried fruits*. The clonal wines 1AA3 and 1AA4 presented the highest average values for the *sweet* descriptor. Related to the *herbaceous* descriptor, wine 1AA5 had the highest score (1.4). The average scores of the *animal* and *woody* descriptors were low (below 1.0) for all the clonal wines. Finally, wine 1AA2 which presented the highest odourant intensity average score in 4-ethylphenol also presented the highest *animal* score at descriptive sensory results.

The *animal*, *horsy*, *horse sweat* or *barnyard* descriptors have been used do describe 4-ethylphenol in red wines (Chatonnet and Boidron, 1988; Chatonnet *et al.*, 1992; Chatonnet *et al.*, 1993; Towey and Waterhouse, 1996). The production of 4-ethylphenol by *Brettanomyces/Dekkera* spp. in red wines is only regarded as spoilage when this secondary metabolite is present at levels higher than about 620 $\mu g.L^{-1}$ (Chatonnet *et al.*, 1992; Chatonnet *et al.*, 1993).

In relation to the *red fruits* descriptor, it can be underlined that this descriptor presented the highest average score among overall descriptors. The clonal wine 1AA3 differs from the other wines due to its high average score (2.4) of *red fruits* descriptor. Moreover, the 1AA3 wine showed the highest average score of the *sweet* descriptor. Both descriptors are greatly associated with the high aroma quality of wine (Botelho, 2008). The descriptive sensory evaluation of the five clonal wines showed an inverse relationship between red fruits and animal aroma perception. In fact, a significant negative correlation coefficient r = -0.947 (p = 0.05) between the average scores of animal and red fruits descriptors was found.

The PCA was applied to the average attribute scores of each wine in order to describe the group of sensory data, to establish the relationships between the different sensory variables and wines, and to detect the most important factors of variability. Some authors applied the PCA to study the aroma attributes of wines. For example, Presa-Owens and Noble (1995), using eight aroma attributes to characterise three Spanish white wines, performed a PCA analysis and found three principal components that accounted for 70% of the total variance. Figure 2 shows in the two dimensional plot of PC1 against PC2, the locations of the seven aroma attributes and the five clonal wine samples. Two PCs were found accounting for 85.6% of the total variance. The first principal component (PC1) was characterised by the contrast of *spicy*, *dried fruits* and *woody* attributes having a positive loading, while the *animal* attribute displayed a negative loading. As to the second PC, the attributes *red fruits* and *sweet* showed a positive

TABLE IV

Average intensities (*x* ± *standard deviation*) of the aroma descriptors and quality and identification of statistically significant differences among clonal wines.
Intensidades médias (x ± desvio padrão) dos descritores e qualidade do aroma e identificação das diferenças estatisticamente significativas entre os vinhos monoclonais.

	1AA1	**1AA2**	**1AA3**	**1AA4**	**1AA5**	***Sig.***
Descriptors	*x* ± *S.D.*	*x* ± *S.D.*	*x* ± *S.D.*	*x* ± *S.D.*	*x* ± *S.D.*	
sweet	0.3a ± 0.4	0.5a ± 0.5	1.2b ± 0.4	1.1b ± 0.4	0.6a ± 0.5	*
red fruits	1.8a ± 0.4	1.4a ± 0.6	2.4b ± 0.5	1.8a ± 0.4	1.6a ± 0.5	*
dried fruits	0.7ab ± 0.6	0.3b ± 0.4	0.9a ± 0.4	1.3a ± 0.7	0.8ab ± 0.4	**
spicy	0.6 ± 0.5	0.2 ± 0.4	0.5 ± 0.5	0.7 ± 0.5	0.5 ± 0.5	*ns*
woody	0.1 ± 0.3	0.0	0.1 ± 0.3	0.2 ± 0.4	0.0	*ns*
herbaceous	0.6b ± 0.5	0.7b ± 0.5	0.0ac	0.5bc ± 0.5	1.4d ± 0.6	***
animal	0.2ab ± 0.4	0.6b ± 0.5	0.0a	0.1a ± 0.3	0.1a ± 0.3	*
Aroma quality	2.9a ± 0.7	2.3b ± 0.7	3.4c ± 0.6	3.6c ± 0.6	3.1a ± 0.6	***

ns: not significant; *Significant ($p < 0.05$); **Highly significant ($p < 0.01$); ***Very highly significant ($p < 0.001$).
Average values followed by the same letter, in the same line, are not significantly different (LSD, 0.05).

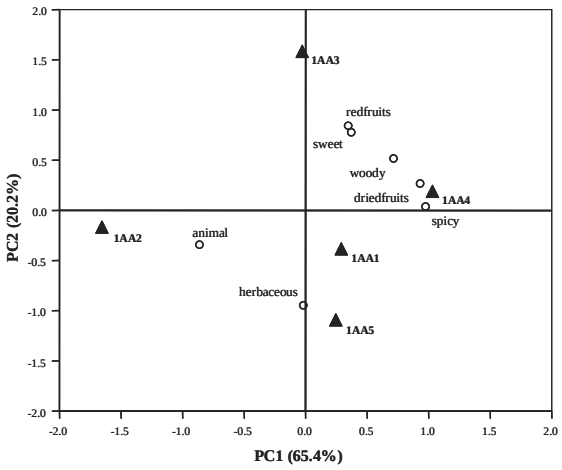

Figure 2 - Plot of the first and second principal components (PCs) of the aroma descriptors data and the five Aragonez clonal wines. The percentage of variation explained by each PC is indicated between brackets.
Representação da primeira e segunda componentes principais (CP) dos descritores de aroma e dos cinco vinhos monoclonais de Aragonez. A percentagem de variação explicada por cada CP encontra-se indicada entre parêntesis.

loading, while *herbaceous* attribute was loading negatively on PC2. Moreover, the PCA analysis showed that the positive side of PC1 and PC2 in the PCA plot was characterised by aroma descriptors positively correlated with wine quality (Botelho, 2008). For this reason, the wines located in this quadrant presented higher aroma quality than the others, due to their positive correlation with those descriptors. Besides, the negative side of PC1 and PC2 of the PCA plot was characterised by the *animal* descriptor which is positively correlated with low aroma quality of wines. Thus, when a wine is located in that quadrant, it means that the wine has a high average intensity in *animal* descriptor.

According to the distribution of clonal wines from the 2001 vintage, in the product space (PC1 x PC2), wines 1AA1 and 1AA5 are close to one another and are located on the positive side of PC1 and on the negative side of PC2. In fact, the aroma profile of these wines is very similar, as demonstrated by the LSD test (TABLE IV) in which only the *herbaceous* descriptor showed statistically significant differences between both wines. Wine 1AA4 is also on the positive side of PC1 but on the positive side of PC2. Wine 1AA2 is located on the negative side of PC1 and negative side of PC2.

Finally, wine 1AA3 is on the PC1 axis and on the positive side of PC2. The wines 1AA3 and 1AA4 present high positive correlation with the descriptors *red fruits*, *sweet*, *woody*, *dried fruits* and *spicy* which seems to indicate that both wines have higher aroma quality than the others. In fact, these two wines showed a very high significant difference ($p<0.001$) in their aroma quality attributes from the other wines. Wine 1AA2 is positively correlated to the *animal* descriptor and 1AA5 is positively correlated with the *herbaceous* descriptor. These two last correlations indicate that both wines have a lower aroma quality. Moreover, according to TABLE IV, they have the highest average intensities for the descriptors *animal* and *herbaceous*, 0.6 and 1.4, respectively. In conclusion, according to the TABLE IV, the wines 1AA1, 1AA2 and 1AA5 presented the lower aroma quality average scores, particularly the wine 1AA2 that revealed a very high significant difference ($p<0.001$) from the other two with an average score of 2.3.

In order to perform multiple linear regression analysis, two groups of GC-O scores of odourant compounds (TABLE V) were defined according to their potential contribution to aroma quality of the five clonal wines. Only well identified odourant compounds were considered in this statistical analysis. The A group (high aroma quality) consisted of odourant compounds related to *fruity-sweet* descriptors: P1 to P6, P8, P9, P17, P22, P23, P30 and P31. The B group (aroma *defects*) was constituted by odourant compounds related to low aroma quality: P7, P11, P13, P14, P19, P26 and P27.

By multiple linear regression analysis between GC-O scores of odourant compounds (group A and B) and aroma quality scores of the five clonal wines (TABLE V), a significant regression model ($p<0.05$) with a total explained variance of 68.9% was found. According to these results, quality could be satisfactorily explained by a multiple linear regression model with just two variables: a vector grouping 7 odourant compounds associated with aroma *defects* and a second vector grouping 13 compounds with *fruity-sweet* descriptors.

TABLE V

Groups of odourant compounds used in the multiple linear regression analysis.
Grupos dos compostos odourantes utilizados na análise de regressão linear múltipla.

Peak no.	Group A	Odour descriptor	Peak no.	Group B	odour descriptor
P1	ethyl isobutyrate	fruity	P7	2+3-methyl-1-butanol	pungent
P2	diacetyl	caramel, butter	P11	2-methylpropanoic acid	cheese
P3	ethyl butanoate	fruity	P13	butanoic acid	rancid butter, cheese
P4	ethyl 2-methylbutanoate	fruity	P14	3-methylbutanoic acid	stinky, cheese
P5	ethyl 3-methylbutanoate	fruity	P19	guaiacol	smoky, medicinal-like
P6	isoamyl acetate	fruity, banana	P26	4-ethylphenol	animal, horse stable
P8	ethyl hexanoate	fruity	P27	4-vinylguaiacol	burnt, curry
P9	ethyl octanoate	fruity, floral			
P17	β-damascenone	floral, fruity, cooked apple			
P22	furaneol	burnt sugar, candy cotton			
P23	homofuraneol	burnt sugar, candy cotton			
P30	vanillin	vanilla			
P31	ethyl vanillate + acetovanillone	vanilla, floral			

Some authors tried to find clear relationships between fruity esters and fruity notes in red wines (Ferreira *et al.*, 1995). In other studies the models built for the fruity notes of red wines were not very satisfactory (Aznar *et al.*, 2003). An additive effect in aroma intensity between furaneol and homofuraneol was observed by Ferreira *et al.* (2002) in red wines. In the same way, although neither 3-mercaptohexan-1-ol nor dimethyl sulphide exhibits any red- or black-berry character both may enhance the perception of fruity aroma in red wine (Bouchilloux *et al.*, 1998; Ferreira *et al.*, 1998b; Segurel *et al.*, 2004). More recently, a study conducted by Pineau *et al.* (2009) showed that ethyl propanoate, ethyl 2-methylpropanoate and ethyl 2-methylbutanoate were involved in black-berry aroma, whereas ethyl butanoate, ethyl hexanoate, ethyl octanoate and 3-hydroxybutanoate conferred red-berry aroma to red wines.

As the above mentioned studies show, the task of determining the direct impact of odourant compounds in red wines aroma is particularly complex in terms of both the number of aroma compounds involved and the existence of complex interactions between odourant compounds. Nevertheless, the present study demonstrates that remarkable findings can be found when combining GC-O posterior intensity method and descriptive sensory analysis.

The knowledge about the global aroma of cultivars through the study of their clones certified or under certification process reveals their great usefulness, since it allows the viticultural agents of productive sector to do a critical and well scientific supported choice of clones in order to obtain higher quality wines.

CONCLUSIONS

The 3-methylbutanoic acid, 2-phenylethanol, 4-vinylguaiacol, furaneol, and homofuraneol were the highest average intensities odourant compounds in the five Aragonez clonal wines. Both PCA and the

multiple linear regression analysis of the scores of GC-O and descriptive sensory analysis of the five Aragonez clonal wines revealed that these sensory analyses are complementary and allow establishing relationships among them. In fact, a multiple linear regression model (68.9% explained variance) with a vector grouping 7 odourant compounds associated with aroma *defects* and a second vector grouping 13 compounds with *fruity-sweet* descriptors was found in order to explain their contribution to clonal wines aroma quality.

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