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Multivariate analysis for the classification and differentiation of Madeira wines according to main grape varieties

José S. Câmara [a,*], M. Arminda Alves [b], José C. Marques [a]

[a] *Centro de Química da Madeira, Depto. de Química da Universidade da Madeira, Campus Universitário da Penteada, 9000-390 Funchal, Portugal*
[b] *Depto. de Enga Química, Faculdade de Engenharia da Universidade do Porto, Rua Roberto Frias, 4099 Porto, Portugal*

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Abstract

In order to differentiate and characterize Madeira wines according to main grape varieties, the volatile composition (*higher alcohols*, *fatty acids*, *ethyl esters* and *carbonyl compounds*) was determined for 36 monovarietal Madeira wine samples elaborated from *Boal*, *Malvazia*, *Sercial* and *Verdelho* white grape varieties. The study was carried out by headspace solid-phase microextraction technique (HS-SPME), in dynamic mode, coupled with gas chromatography–mass spectrometry (GC–MS). Corrected peak area data for 42 analytes from the above mentioned chemical groups was used for statistical purposes. Principal component analysis (PCA) was applied in order to determine the main sources of variability present in the data sets and to establish the relation between samples (objects) and volatile compounds (variables). The data obtained by GC–MS shows that the most important contributions to the differentiation of *Boal* wines are benzyl alcohol and (*E*)-hex-3-en-1-ol. Ethyl octadecanoate, (*Z*)-hex-3-en-1-ol and benzoic acid are the major contributions in *Malvazia* wines and 2-methylpropan-1-ol is associated to *Sercial* wines. *Verdelho* wines are most correlated with 5-(ethoxymethyl)-furfural, nonanone and *cis*-9-ethyldecenoate. A 96.4% of prediction ability was obtained by the application of stepwise linear discriminant analysis (SLDA) using the 19 variables that maximise the variance of the initial data set.

Keywords: Wine; Volatile compounds; HS-SPME/GC–MS analysis; PCA; SLDA

1. Introduction

Madeira wines are characterized by a typical vinification and ageing procedures, including fortification in order to obtain an ethanol content of about 18% (v/v), followed by a baking process known as "*estufagem*" during which the wine is submitted to rather high temperatures (45–50 °C) for about three months. The four basic types of Madeira wines are named according the main grape varieties from which they are prepared. *Malvazia* is fortified in an early stage of fermentation in order to produce a distinctive sweet wine with a sugar content of approximately 110 g l^{-1}. *Boal* is fortified in order to obtain a medium sweet wine (90 g l^{-1}) and the *Verdelho* ferments still further to produce a medium dry wine (65 g l^{-1} of sugar). *Sercial* is allowed to complete fermentation and originates dry wines with less than 25 g l^{-1} of sugar content. The baking process plays an important role in the definition of the *bouquet*, as the temperature can change the chemical profile and, consequently, the organoleptic character of the wines, by the increase of the kinetics of chemical and enzymatic reactions occurring during wine conservation, such as it happens with other foods and beverages [1–5]. In addition, it must be noted that old Madeira wines are maintained for long periods of aging, frequently more than 20 years, in cellars which temperatures as high as 30–35 °C and humidity levels of 70–75%. The quality and value of the wine is closely related with the characteristic aroma developed during this long maturation period.

The aroma is one of the most important factors in the determination of wine character and quality. The volatile fraction of a wine can be composed by over 800 different compounds

* Corresponding author. Tel.: +351 291705112; fax: +351 291705149.
E-mail address: jsc@uma.pt (J.S. Câmara).

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[6,7] but only some tens are odour-active [8,9] and must be considered for differentiation purposes. These compounds belong to very heterogeneous groups such as *monoterpenes*, *higher alcohols*, *aldehydes*, *ketones*, *ethyl esters* and *fatty acids*. Some of these compounds come from the grapes and are typical of the variety, but most of them are formed during fermentation process and wine ageing. This great variety of volatile compounds, with different polarities, volatilities and a wide range of concentrations, is responsible for the complexity of wine *bouquet* and ensures the specificity and character.

The wine aroma composition is influenced by many factors such as grape variety, edafoclimatic conditions and winemaking process but also depends on yeast strain used, pH of the medium, content and type of nitrogen available, sugars, fermentation temperature and aeration. Each one of these parameters has a significant influence on the quality of the wine and affects the characterisation and differentiation of different wines.

Wine production is actually spread all over the world and brand names and processes are sometimes subject of adulteration or reproduction, increasing the demand for quality studies and authenticity investigation [10]. Identification of wine aroma components and the relationships between their relative content may be a useful tool in differentiating the wines from different varieties and establishing criteria of genuineness to improve the quality of the wines, prevent fraud and guarantee their origin. In general, analysis of volatile compounds can be used to characterise different varieties such as the contribution of ethyl esters of fatty acids and acetates of higher alcohols, from neutral grape varieties [11]. Moreover, volatile wine compounds can be used to differentiate wines with different geographic origins [12]. Kwan and Kowalski [13] performed a good differentiation of samples from French and American *Pinot Noir* on the basis of hexan-1-ol and cyclo-hexane. García-Jares et al. [14] differentiate white wines from Rias Baixas (Spain) using 19 volatile compounds. Sugars, organic acids and amino acids were used by Arminda Alves [15] in the differentiation of Porto wines and Guedes de Pinho used the multivariate technique for characterisation and differentiation varieties from different regions [16]). Trace elements were used by Day et al. [17] for the identification of the geographical origin of wines using stepwise discriminant analysis. Despite the work carried out on this area, the performance achieved is always dependent on the data set available as some components are present in high concentration (hundreds of $mg\,l^{-1}$) and can be easily analysed by GC but the majority is found at the low $ng\,l^{-1}$ level and need to be extracted and concentrated before analysis. In addition, major compounds from the complex wine matrix can cause interference increasing the difficulty of analysis of trace compounds.

Several classical analytical methods such as liquid–liquid extraction [18,19], simultaneous distillation–solvent extraction [20], solid-phase extraction [21,22], supercritical fluid extraction [23], microwave extraction [24] and ultrasound extraction [25], have been developed for the analysis of the minor volatile compounds in wines. These classical analytical methods have some drawbacks such as the relatively low reproducibility, possibility of solvent cross-contamination, insufficient selectivity and time consuming procedures. At the beginning of the 1990s a new technique, solid-phase micro-extraction (SPME), was proposed by Pawliszyn and coworkers [26,27] offering two main advantages: no extraction solvent required and combination of extraction and pre-concentration in a single step without pre-treatment of samples. Moreover, the procedure showed to be fast, inexpensive, requiring low sample volumes and good automation. This technique has been successfully used in wine samples [28–31] to characterise a wide range of aroma compounds, including monoterpenes and C_{13} norisoprenoids [32], esters [33] and sulphides [34].

Grape variety, vintage year and winery procedures are the three main sources of variation in the chemical composition of wine. Although the main purpose of this study was to determine which components could differentiate Madeira wines according variety, an attempt was made to determine whether the variables selected for this purpose could also reveal other sources of differentiation such as the harvesting year. To achieve this objective, the content of *higher alcohols*, *acetates*, *fatty acids*, *ethyl esters* and *carbonyl compounds* of 36 monovarietal Madeira wines samples produced from the four white grape varieties *Boal*, *Malvazia*, *Sercial* and *Verdelho*, was determined over three consecutive harvests (1998–2000). Multivariate techniques of data analysis – principal component analysis (PCA) and stepwise linear discriminant analysis (SLDA) – were employed in order to establish differentiation criteria as a function of the varieties used in the preparation of these wines.

The analytes were extracted by HS-SPME using a polyacrylate fibre (PA-85 μm) and analysed by GC–MS. The influence of climatic conditions and winemaking techniques on the differentiation of wine varieties was not considered in the study as grapes had been harvested in the same site and the same technology was applied for all wines.

2. Materials and methods

2.1. Sample wines

Grapes of *Vitis vinifera* cultivars *Boal*, *Malvazia*, *Sercial* and *Verdelho*, supplied for the *Instituto do Vinho Madeira* (IVM), collected at the final stage of ripening were used. Production techniques were similar to all wines studied, with the exception of the fermentation period before fortification. The musts were treated with SO_2 ($50\,mg\,l^{-1}$) and fermentation, carried out in oak casks with spontaneous yeasts, was stopped by addition of ethanol when the appropriate amount of natural grape sugars has been converted. The 36 wines stayed in casks for eight months before sample collection

and storage at −28 °C until use. The HS-SPME extraction was always carried out in triplicate.

2.2. Sample extraction conditions

Volatile wine compounds were extracted by headspace solid-phase micro-extraction (HS-SPME) after optimisation of the major parameters with influence in the extraction process [32,35]: fibre type, time and temperature of adsorption, ionic strength and pH. Optimal conditions of extraction were obtained using the following procedure: 2.4 ml of wine were transferred to a 4 ml vial (headspace volume was 1.6 ml, according to the phase ratio $1/\beta = 0.6$) [36], the ionic strength was adjusted to 30% with NaCl and the pH was maintained at 3.3–3.5 (pH of the wine). The samples (50 ml) were spiked with 0.422 μg l^{-1} of octan-3-ol (Sigma–Aldrich) as internal standard, by addition of 50 μl of alcoholic solution at 422 mg l^{-1}. The vial was sealed and headspace extraction was performed for 120 min at 40 °C with a 85 μm PA fibre, keeping the sample under continuous stirring (1200 rpm). The fibre was maintained in the GC injector for 5 min for complete desorption.

2.3. GC–MS conditions

The wine extracts were analysed by gas chromatography coupled with mass spectrometry (GC–MS) using a Varian STAR 3400Cx series II gas chromatograph, equipped with a 30 m × 0.25 mm i.d., 0.25 μm film thickness, Stabilwax (JW Scientific) fused silica capillary column, connected to a Varian Saturn III mass selective detector and operated according to the method described by Câmara et al. [32]. Splitless injection mode was used. The initial oven temperature was set to 40 °C for 1 min. The temperature was increased in three steps: 40–120 °C, at 1° min^{-1}; 120–180 °C at 1.7 °C min^{-1} and 180–220 °C, at 25 °C min^{-1}. Each step was preceded by a small period at constant temperature of 2, 1 and 10 min, respectively. The injector temperature was 250 °C and the transfer line was held at 220 °C. Mass spectra were recorded after electronic impact (EI) ionisation at 70 eV. The mass-to-charge ratio range (m/z) used was 30–300 (1.9 spectra s^{-1}). The ion source and mass ion trap temperatures were set to 180 °C.

2.4. Statistical analysis

Significant differences among the four Madeira wines varieties for each of the constituents were determined by one-way analysis of variance (*Anova*) using a SPSS Program, version 11.0 (SPSS Inc., 2003). Principal component analysis and stepwise linear discriminant analysis were performed using the same SPSS program. These techniques were applied to the normalized relative amounts of the volatile compounds.

Principal component analysis is an unsupervised technique that reduces the dimensionality of the original data matrix retaining the maximum amount of variability [37], allowing the visualisation of the different wines in a two dimensional space and identifying the directions in which most of the information is retained. It is therefore possible to explain the differences between various wines by means of factors obtained from the data sets and, at the same time, to determine which variables contribute the most for such differences.

Stepwise linear discriminant analysis 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 minimises the variance within categories and maximises the variance between categories [38]. The variables included in the analysis are determined with a stepwise-LDA using a 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. The prediction capacity of the discriminant models was studied by "*cross validation*" in order to determine the stability of the model.

3. Results and discussion

The HS-SPME/GC–MS method developed was found to be fully suitable for the analysis of volatile compounds in wine due to its selectivity and sensitivity. A total ion current (TIC) chromatogram obtained for a *Malvazia* wine sample with the 85 μm fibre at the optimal extraction conditions [31], can be seen in Fig. 1. Careful analysis of the chromatograms allowed the assignment of clearly different mass spectra for about eighty peaks (Table 1). The compounds were identified by comparison with mass spectra obtained from the sample with those from pure commercially available standards injected in the same conditions, by comparing the Kovats indexes and the mass spectra included in the NIST library.

The average values from three consecutive harvests showed that these wines have characteristic profiles. Higher alcohols, fatty acids and ethyl esters are the major groups in all studied varieties. *Boal* wines are characterised by the highest content of C_{13} norisoprenoids, higher alcohols and carbonyl compounds. *Malvazia* wines show the highest concentrations of monoterpenes. *Sercial* wines present important levels of acetates, fatty acids and ethyl esters and the highest content of fix acids and phenols while *Verdelho* wines are characterised by the highest content of ethyl esters and furan compounds. Fig. 2 shows the relative amount of total free fraction for each chemical group in the Madeira wines under study.

The compounds clearly identified were used for statistical treatments. All statistical treatments were performed on corrected peak area data (peak area/internal standard area).

3.1. Principal component analysis (PCA)

The 42 analytical variables used for statistical purposes were gathered into four different groups (Table 2): *higher*

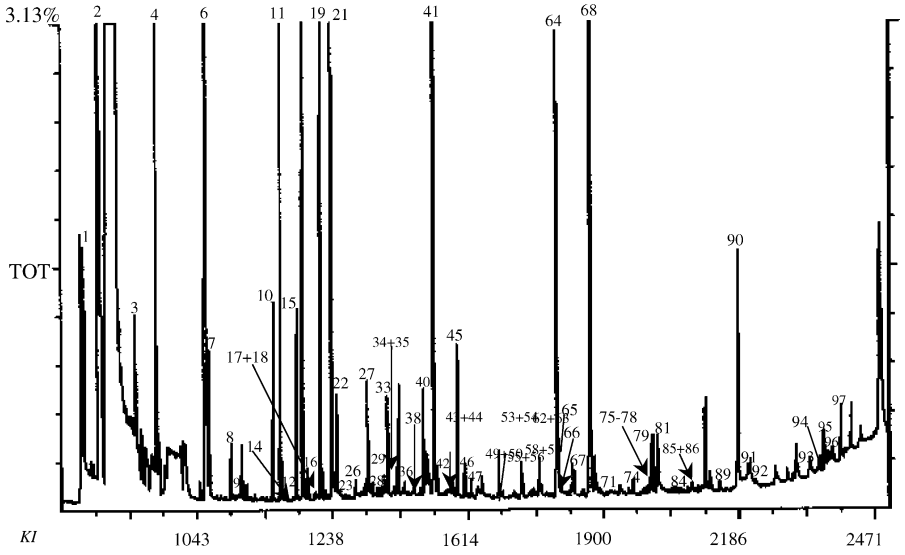

Fig. 1. Typical total ion current (TIC) chromatogram of a *Malvazia* wine sample obtained by HS-SPME/GC–MS using a 85 μm PA fibre. Identified analytes are listed in Table 1 (KI: Kováts Indice).

alcohols, *fatty acids*, *ethyl esters* and *carbonyl compounds*. Principal component analysis from data matrix was then performed in each one of the different groups in order to find the main sources of variability and to establish the relation between varieties and volatile compounds. Although the best procedure is to analyse all variables at the same time, we choose to subdivide the compounds into several groups of variables for the consistency of the results, maintaining the number of cases equal or higher than the number of variables.

3.2. Higher alcohols

When higher alcohols were analysed by PCA, a clear differentiation among varieties was found. No apparent differentiation was observed when considering the harvesting year.

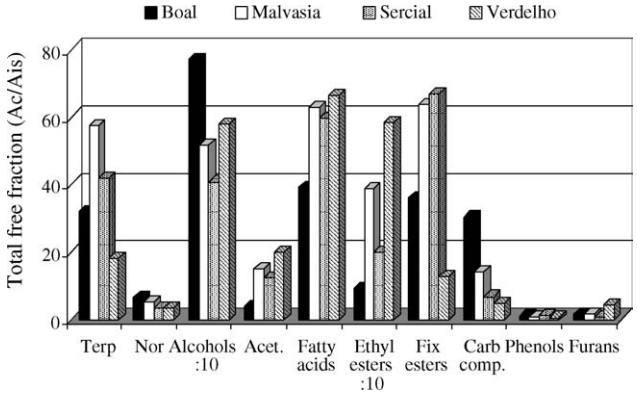

Fig. 2. Total free fraction (Ac/Ais: compound area/internal standard area) for the chemical groups studied (Terp: monoterpenes; Nor: C_{13} norisoprenoids; Alcohols: higher alcohols; Acet.: acetates; Carb comp.: carbonyl compounds).

Applying PCA to the normalized relative amounts of the 11 analytical variables (higher alcohols) and 36 objects (wines), two factors were extracted explaining 81.2% of the total variance of initial data set. The observation of the loading scores suggests that two variables, having coefficients magnitude <0.8 – propan-1-ol and 3-methylbutan-1-ol – are insufficient to adequately describe the samples according to variety, and were removed from the matrix. The new set (data matrix 36 × 9) account for 87.9% of the total variance in the data. The first principal component (PC1) explains 62.2% of the variance in the initial data set and the second principal component (PC2) explain 25.7%. The eigenvalues, percentage of variance and the cumulative percentage explained by the two first principal components are showed in Table 3.

The projections of the samples along the directions identified by the first two PC's, is reported in Fig. 3a where the first principal component (PC1) of wine samples is plotted against the second principal component (PC2). The separation of the different categories of wine samples from this PC1–PC2 scatter point plot is obvious. This figure shows that wines from *Malvazia*, *Sercial* and *Verdelho* varieties were separated by the second principal component, while wines from *Boal* variety are most influenced by the variables related with the first PC.

The coefficient that defines the weight of the original variable in the PC's can be investigated to understand which chemical compounds are responsible for the ranking of wines. Benzyl alcohol (0.98), 2-phenylethanol (0.95), butan-1-ol (0.92) and (*E*)-hex-3-en-1-ol (0.90) were highly loaded on the first PC, while (*Z*)-hex-3-en-1-ol (0.92) and (*E*)-hex-2-en-1-ol (0.91) were loaded on the second PC explaining most of the variability. Hexan-1-ol (0.81; 0.53) is important on both PC1 and PC2 components. Fig. 3 shows the corresponding loadings plot that establishes the relative importance of each

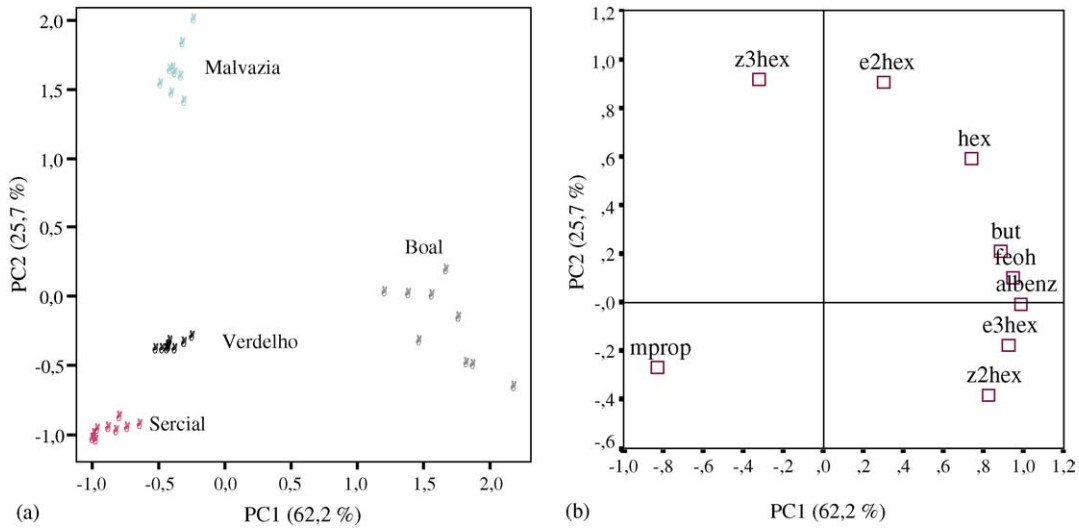

Fig. 3. PC1 vs. PC2 scatter plot of the main sources of variability between the Madeira wines (a) distinction between the samples (scores) and (b) relation between the nine higher alcohols (loadings).

variable and it is therefore useful for the study of relations among the higher alcohols and between variables and wines.

The wines from varieties *Sercial* and *Verdelho* are located in the third quadrant (negative PC1 and PC2). Only 2-methylpropan-1-ol (−0.86) is in accordance with this requisite. These wines show low values of (*Z*)-hex-3-en-1-ol and (*E*)-hex-2-en-1-ol and very low values of hexan-1-ol and all other variables influencing positively the first PC. *Malvazia* wines are located in the second quadrant (negative PC1 and positive PC2). (*Z*)-Hex-3-en-1-ol (−0.32; 0.92) and (*E*)-hex-2-en-1-ol (0.77; 0.49) are strongly associated with this quadrant. *Boal* wines are essentially represented in the fourth quadrant (positive PC1 and negative PC2). Benzyl alcohol (0.98; −0.12), 2-phenylethanol (0.95; −0.02), butan-1-ol (0.92; 0.09), (*E*)-hex-3-en-1-ol (0.92; −0.18) and (*Z*)-hex-2-en-1-ol (0.83; −0.38) are the variables related with these wines (Fig. 3b). They are characterized by low values of 2-methylpropan-1-ol (−0.86; −0.20).

3.3. Fatty acids

The 13 variables from the initial data matrix of fatty acids (Table 2) explained 66.8% of the variance. The redundant variables not contributing to the explanation of total variance (coefficients magnitude <0.8) were removed from the data set. PCA showed a clear separation of wines according to varieties when a data matrix (36 × 6) with the fatty acids – propionic, hexanoic, octanoic, 2-hydroxybenzenepropionic, 2-ethyl-hexanoic and benzoic – is used. The first two principal components accounted for 85.8% of the total variance of

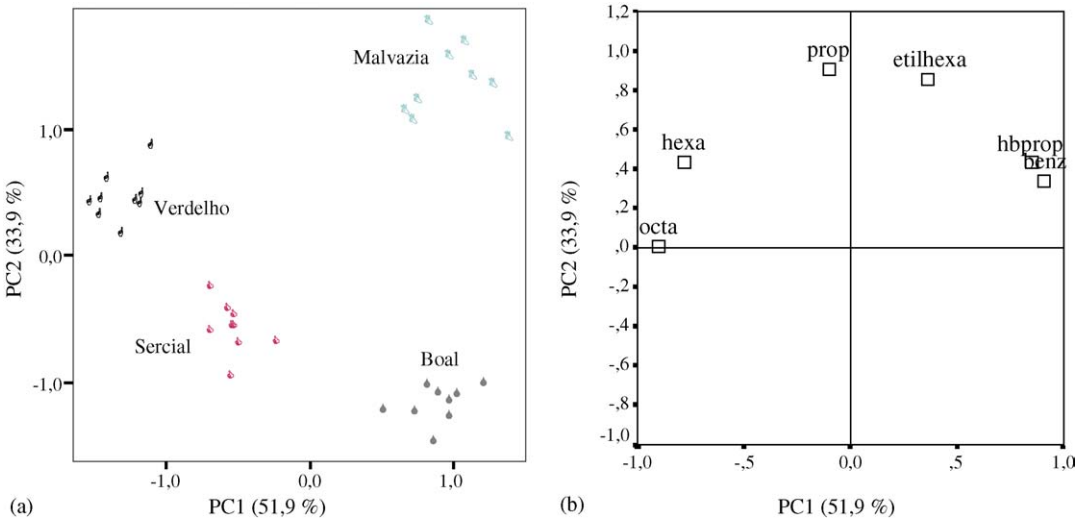

Fig. 4. PC1 vs. PC2 scatter plot of the main sources of variability between the Madeira wines (a) distinction between the samples (scores) and (b) relation between the six fatty acids (loadings).

Table 1
Volatile compounds identified in *Malvazia* wines

Terpenes	
20	*trans*-Linalool oxide
23	*cis*-Linalool oxide
26	Unknown 1 (93 + 121 + 136)
33	Linalool
38	2,6-Dymethylocta-1,7-dien-3,6-diol
47	α-Terpineol
55	(+)-δ-Cadinene
57	Citronellol
59	Unknown 2 (93 + 121 + 136)
66	Geraniol
86	Nerolidol
91	Farnesol
C_{13} norisoprenoids	
288	Vitispirane I
29	Vitispirane II
48	Unknown 3 (177 + 192)
52	TDN
63	β-Damascenone
Higher alcohols	
3	Butan-1-ol
5	4-Methylpentan-2-ol
6	2-Methylbutan-1-ol
11	Hexan-1-ol
12	(*E*)-Hex-3-en-1-ol
15	(*Z*)-Hex-3-en-1-ol
16	(*E*)-Hex-2-en-1-ol
17	(*Z*)-Hex-2-en-1-ol
32	Butan-1,3-diol
34	Nonan-1-ol
50	3-Methyltio-propan-1-ol
67	Benzyl alcohol
68	2-Phenylethanol
84	2-Phenoxyethanol
Acetates	
4	3-Methylbutyl acetate
8	Hexyl acetate
62	2-Phenylethyl acetate
Fatty acids	
21	Acetic acid
31	Propionic acid
35	Dimethylmalonic acid
40	Butanoic acid
44	3-Methylbutanoic acid
49	2-Hydroxybenzenepropionic acid
65	Hexanoic acid
71	2-Ethyl-hexanoic acid
81	Octanoic acid
83	Nonanoic acid
91	Decanoic acid
93	Benzoic acid
95	Dodecanoic acid
99	Tetradecanoic acid
Ethyl esters	
2	Ethyl acetate
7	Ethyl hexanoate
19	Ethyl octanoate
30	Ethyl nonanoate
41	Ethyl decanoate
42	Ethyl benzoate
46	Ethyl *cis*-9-decenoate
58	Ethyl benzeneacetate
64	Ethyl dodecanoate
79	Ethyl tetradecanoate
90	Ethyl hexadecanoate
97	Methyl-7,10-octadecadienoate
Ethyl esters of fix acids	
9	Ethyl 2-oxopropanoate
10	Ethyl lactate
18	Ethyl 2-hydroxy-3-methylbutanoate
25	Ethyl 2-hydroxypropanoate
45	Diethyl succinate
76	Isopropyl miristate
78	Ethyl 3-hydroxyhexanoate
Carbonyl compounds	
1	Acetaldehyde
27	Benzaldehyde
94	1-(2-Methylphenyil)ethanone
Phenols	
56	Methyl salicilate
75	4-Ethyl-2-methoxyphenol
77	(1,1-Dimethylethyl)-2-methoxyphenol
88	Eugenol
Furans	
22	Furfural
87	5-(Acetoxymethyl)furfural
96	5-(Hydroxymethyl)furfural
69	pantolactone
53	γ-Butyrolactone
Others	
43	Non-1-ene
51	Non-3-ine
73	1,3-Dymethylnaphtalene
89	1,6-Dimethyl-4-(1-methylethyl)-naphtalene

the initial data set. Table 3 present the eigenvalues, cumulative percentage and total variance explained by the two first principal components. The first component explains 51.9% of the variability in the initial data set and the second component explains 33.9%.

In Fig. 4a, the first principal component (PC1) is plotted against the second principal component (PC2). The separation among different categories of wine samples from this PC1–PC2 scatter point plot is obvious. Fig. 4b shows the corresponding loadings plot that establishes the relative importance of each variable and it is therefore useful for the study of relations among the acids compounds and relations between fatty acids and wines.

The variables with the highest contribution to the first component, explaining 51.9% of total variance of data set, are benzoic acid (0.91), octanoic acid (−0.89), 2-hydroxybenzenepropionic acid (0.85) and, in minor extent, hexanoic acid (−0.78). The second principal component (33.9% of total variability) is strongly correlated with propionic acid (0.91) and 2-ethyl-hexanoic acid (0.85) (Fig. 4b).

From the plot of the 36 wines on the plane defined by these first two principal components, the wines *Malvazia* appear on the first quadrant. These samples are characterized by the variables associated to positive values of the

Table 2
Identification of the 42 variables used in the multivariate analysis

Variable	Identification
Higher alcohols	
prop	Propan-1-ol
mprop	2-Methylpropan-1-ol
mbut	2 + 3-Methylbutan-1-ol
but	Butan-1-ol
hex	Hexan-1-ol
e3hex	(*E*)-Hex-3-en-1-ol
z3hex	(*Z*)-Hex-3-en-1-ol
e2hex	(*E*)-Hex-2-en-1-ol
z2hex	(*Z*)-Hex-2-en-1-ol
albenz	Benzyl alcohol
feoh	2-Phenylethanol
Fatty acids	
eta	Etanoic acid
prop	Propionic acid
mbut	3-Methylbutanoic acid
but	Butanoic acid
dmm	Dymethylmalonic acid
hexa	Hexanoic acid
etilhexa	2-Ethylhexanoic acid
hbprop	2-Hydroxybenzenepropionic acid
octa	Octanoic acid
nona	Nonanoic acid
Deca	Decanoic acid
dodeca	Dodecanoic acid
Benz	Benzoic acid
Ethyl esters	
C6C2	Ethyl hexanoate
C8C2	Ethyl octanoate
C9C2	Ethyl nonaoate
C10C2	Ethyl decanoate
deceet	*cis*-9-Ethyl decenoate
benzacet	Ethyl benzeneacetate
C12C2	Ethyl dodecanoate
C14C2	Ethyl tetradecanoate
C16C2	Ethyl hexadecanoate
C18C2	Ethyl octadecanoate
sde	Diethyl succinate
Carbonyl compounds	
nona	Nonanone
benzal	Benzaldehyde
fur	Furfural
emf	5-(Ethoxymethyl)furfural
hmf	5-(Hydroximethyl)furfural
butiro	γ-Butirolactone
pantol	Pantolactone

The variables used in the SLDA are indicated in bold.

two first principal components being characterized, primarily, by benzoic acid (0.91; 0.33) and in minor extent by 2-hydroxybenzenepropionic acid (0.85; 0.43) and 2-ethylhexanoic acid (0.36; 0.85). Propionic acid (0.91; −0.10), octanoic acid (−0.89; 0.01) and hexanoic acid (−0.78; 0.43), are the characteristic variables for *Verdelho* wines (negative PC1 and positive PC2).

Sercial and *Boal* samples are represented in the third (negative PC1 and PC2) and fourth (positive PC1 and negative PC2) quadrants, respectively. The first PC explains the separation from *Sercial* and *Verdelho* wines to *Boal* and *Malvazia* wines, while the second PC separated *Sercial* and *Boal* wines from *Verdelho* and *Malvazia* wines (Fig. 4a).

3.4. Ethyl esters

Principal component analysis from the data matrix (36 × 10) built with ethyl esters, was also performed. It was observed that 91.0% of the total variance of these compounds could be explained by the first two principal components (Table 3). The first principal component, explaining 51.8% of the variance, is mainly associated with ethyl octadecanoate (0.95), ethyl tetradecanoate (0.95) ethyl benzeneacetate (0.88), while ethyl octadecanoate (0.98), *cis*-9-ethyldecenoate (0.97) and ethyl hexanoate (0.95) contribute in great extent to the second principal component, explaining 39.2% of the total variance (Fig. 5a).

From the plot of the 36 wines on the plane defined by these first two principal components, the *Malvazia* wines appear on the right side of the plane, grouped by variety, while wines of varieties *Boal*, *Sercial* and *Verdelho* are found on the left side of the plane (Fig. 5b).

Verdelho wines are characterized by the variables contributing with positive values to PC2 and negative to PC1. Thus *cis*-9-ethyldecenoate (−0.11; 0.97) and ethyl hexanoate (−0.19; 0.95), are the major contributions to the differentiation of *Verdelho* from other types of Madeira wines. *Sercial* and *Boal* wines are contained in the third quadrant (negative values for PC1 and PC2). Ethyl octadecanoate (0.95; 0.23), ethyl benzeneacetate (0.88; −0.32), ethyl hexadecanoate (0.86; 0.31) and diethyl succinate (0.84; −0.51), are strongly associated to *Malvazia* wines.

3.5. Carbonyl compounds

Applying the principal component analysis to the data matrix (36 × 7) built with the seven variables of carbonyl compounds, two principal components were extracted explaining 87.1% of the total variability. The variables strongly associated with the first component, 5-(ethoxymethyl) furfural (0.99), nonanone (0.98) and 5-(hydroxymethyl) furfural (0.96), explain 45.9% of the variability in the initial data set. The second component explained 41.2% and is mainly associated with γ-butyrolactone (0.98) and furfural (0.89). The eigenvalues, percentage of variance and cumulative percentage explained by the two first principal components for carbonyl compounds are presented in Table 3.

Fig. 6a shows the scores scatter plot on the two first principal components, representing the differences among the 36 wine samples. Fig. 6b represents the corresponding loadings plot that establishes the relative importance of each carbonyl compounds, and is therefore useful for the study of relations among the carbonyl compounds and the relations between carbonyl compounds and samples.

The second quadrant contains the *Boal* wines. These samples are characterized by the variables associated to positive values of the second principal component mainly benzalde-

Table 3
Eigenvalues, percentage of variance and cumulative percentage explained by the two principal components for each chemical group under study

Group	Principal component	Eigenvalue	Rotation sums of squared	
			Variance (%)	Cumulative (%)
Higher alcohols	1	5.657	62.231	62.231
	2	2.317	25.744	**87.975**
Fatty acids	1	3.112	51.859	51.859
	2	2.038	33.969	**85.828**
Ethyl esters	1	5.179	51.787	51.787
	2	3.921	39.214	**91.001**
Carbonyl compounds	1	3.212	45.881	45.881
	2	2.886	41.230	**87.111**

The bold values are indicative of the total percentage explained by the two first principal components.

hyde (−0.41; 0.89). The third quadrant contains the *Malvazia* and *Sercial* wines (negative PC1 and PC2). There is no carbonyl compounds associated with this quadrant.

Verdelho samples are represented in fourth quadrant (positive PC1 and negative PC2). 5-(Ethoxymethyl) furfural (0.99; 0.04), nonanone (0.98; 0.05) and 5-(hydroxymethyl) furfural (0.96; −0.05) are the variables most related with this wine variety.

3.6. Stepwise linear discriminant analysis (LDA)

After PCA, a linear discriminant analysis was applied to look at the most useful variables in the differentiation between wines and to find discrimination functions for the classification of new samples in the correct group. This parametric method is widely used for classification purposes. The classification was performed according to wine variety: *Boal*, *Malvazia*, *Sercial* and *Verdelho*. Two statistically significant discriminant functions were obtained, explaining 98.5% of the variability. The variables: (*Z*)-hex-3-en-1-ol and diethyl succinate (first root—92.9%) and 5-(ethoxymethyl)furfural, ethyl octanoate and hexanoic acid (second root—5.6%), had the highest *F*-value, so they were the most important variables for the differentiation of the wines from these four varieties.

The prediction capacity of the SLDA model was evaluated by "leave-one-out" cross validation. During this cross validation test, ungrouped cases are removed from the initial matrix of data. The classification model is rebuilt and the cases removed are classified in this new model. Table 4 summarises the results of the classification matrix of the obtained SLDA model, obtained for all the samples and separated for variety, showing an average classification of 96.4%, meaning that 7/8 of the objects were correctly classified (Table 4). All Madeira wines studied showed high percentage of correctly classified cases, almost 100% in the case of *Malvazia* and *Verdelho* wines. Then the results can be considered satisfactory and acceptable and the selected variables are useful to

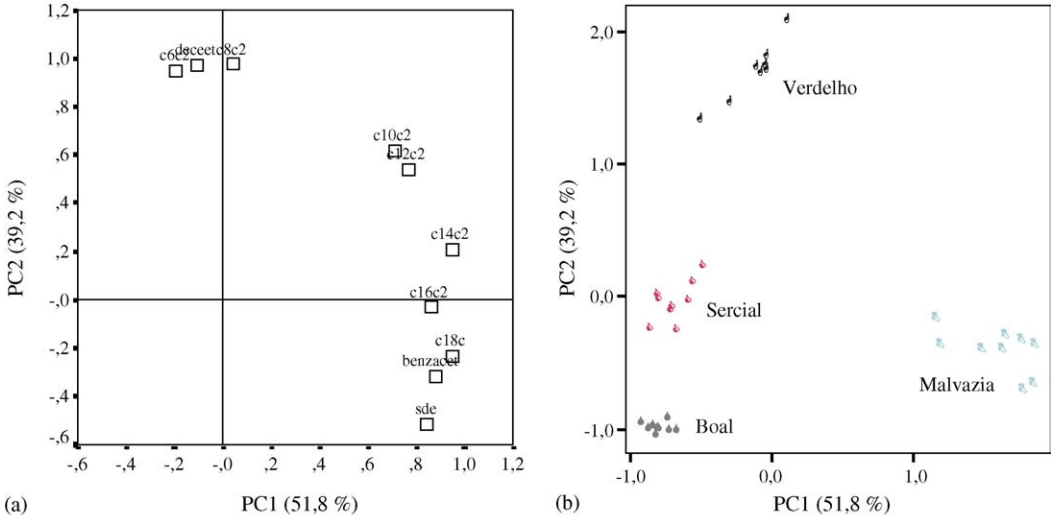

Fig. 5. PC1 vs. PC2 scatter plot of the main sources of variability between the Madeira wines (a) relation between the 10 ethyl esters (loadings); (b) distinction between the samples (scores).

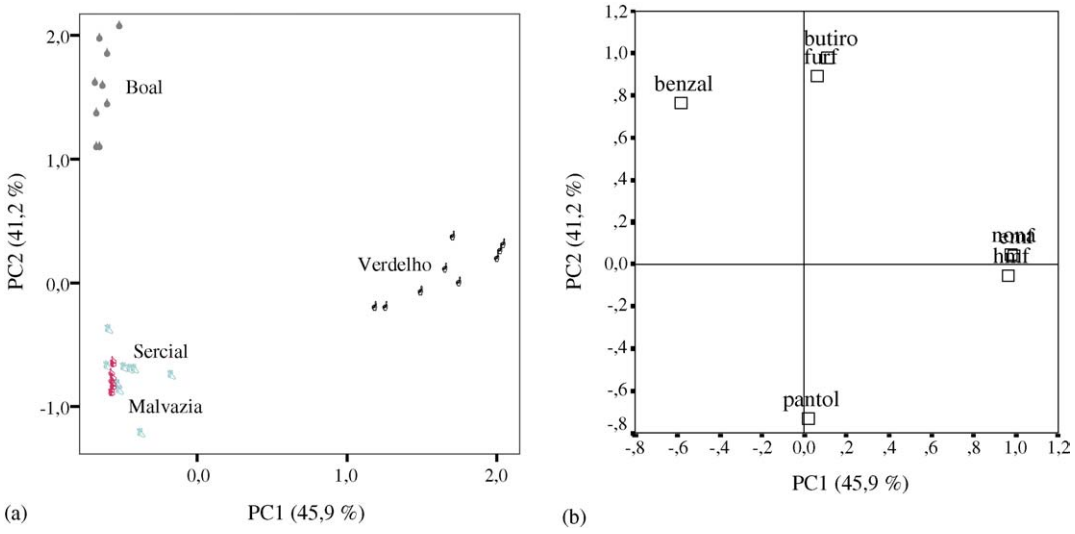

Fig. 6. PC1 vs. PC2 scatter plot of the main sources of variability between the Madeira wines (a) distinction between the samples (scores); (b) relation between the seven carbonyl compounds (loadings).

classify and differentiate these wines by their variety. Due the great importance of Madeira wines in the Madeira Island economy, this is a good result to find possible adulterations and falsifications.

Table 4
Prediction capacity of Madeira wines discriminant model by cross validation according to wine variety

	Classification results[a,b]					Total
	Casta 1	Predicted group membership				
		VB	VM	VS	VV	
Original						
Count	VB	7	0	0	0	7
	VM	0	7	0	0	7
	VS	0	0	7	0	7
	VV	0	0	1	6	7
	Ungrouped cases	2	2	2	2	8
Percent	VB	100.0	0	0	0	100.0
	VM	0	100.0	0	0	100.0
	VS	0	0	100.0	0	100.0
	VV	0	0	14.3	85.7	100.0
	Ungrouped cases	25.0	25.0	25.0	25.0	100.0
Cross-validated[c]						
Count	VB	7	0	0	0	7
	VM	0	7	0	0	7
	VS	1	0	6	0	7
	VV	0	0	0	7	7
Percent	VB	100.0	0	0	0	100.0
	VM	0	100.0	0	0	100.0
	VS	14.3	0	85.7	0	100.0
	VV	0	0	0	100.0	100.0

VB: *Boal* wine; VM: *Malvazia* wine; VS: *Sercial* wine and VV: *Verdelho* wine.

[a] 96.4% of original grouped cases correctly classified.

[b] 96.4% of cross-validated grouped cases correctly classified.

[c] Cross validation is done only for those cases in the analysis. In cross validation, each case is classified by the functions derived from all cases other than that case.

4. Summary and conclusions

HS-SPME/GC–MS is a fast and useful method for isolation and quantification volatile compounds in wines allowing a rapid screening of aroma compounds in wines of different varieties.

Data processing through univariate analysis of variance (Anova) and multivariate analysis (PCA and SLDA) allow good differentiation, classification and prediction models for Madeira wines according to grape varieties. All chemical groups investigated achieved a good separation according to variety, but higher alcohols and ethyl esters seem to be the most important groups for the characterization of Madeira wines.

The variables most correlated with *Boal* wines are: benzyl alcohol (0.98; −0.12), (*E*)-hex-3-en-1-ol (0.92; −0.18), benzaldehyde (−0.41; 0.89) and (*Z*)-hex-2-en-1-ol (0.83; −0.38). Ethyl octadecanoate (0.95; 0.23), (*Z*)-hex-3-en-1-ol (−0.32; 0.92), benzoic acid (0.91; 0.33), ethyl benzeneacetate (0.88; −0.32) and in minor extent ethyl hexadecanoate (0.86; 0.31), 2-hydroxybenzenepropionic acid (0.85; 0.43), 2-ethyl-hexanoic acid (0.36; 0.85) and diethyl succinate (0.84; −0.51) are strongly associated to *Malvazia* wines. 2-Methylpropan-1-ol (−0.83; −0.26) is the variable most related with *Sercial* wines. *Verdelho* wines are most associated with 5-(ethoxymethyl)furfural (0.99; 0.04), nonanone (0.98; 0.05), *cis*-9-ethyldecenoate (0.11; 0.97), 5-(hydroxymethyl)furfural (0.96; −0.05), ethyl hexanoate (0.19; 0.95), propionic acid (0.91; −0.10), octanoic acid (−0.89; 0.01) 2-methylpropan-1-ol (−0.83; −0.26) and hexanoic acid (−0.78; 0.43).

Harvest years showed to have no relevant effects on the differentiation of the wines. Stepwise LDA showed to be a good classification method improving the results of the statistical analysis.

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