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Varietal flavour compounds of four grape varieties producing Madeira wines

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Abstract

Boal, Malvasia, Sercial and Verdelho are the main white grape varieties used in Madeira wine production. To estimate the free fraction of varietal aroma compounds of these varieties, 39 samples of musts were analysed to determine their content of monoterpenols and C_{13} norisoprenoids (terpenoids), using dynamic headspace solid-phase microextraction coupled with gas chromatography–mass spectrometry. The *r*-values for linearity studies of the analytical method used, varied between 0.977 (nerolidol) and 0.999 (linalool). The repeatability for each compound varied between 2.5% (citronellol) and 11.8% (β-ionone).

The mean values from three vintages (1998, 1999 and 2000) confirmed that these musts have differentiated contents of terpenoids. In opposition to Verdelho musts, Malvasia showed the highest free terpenoids content. In order to establish relations between the compounds and the varieties under investigation, principal component analysis and linear discriminant analysis were applied to the data, revealing a good separation and classification power between the four groups as a function of varietal origin.

Keywords: Boal variety; Malvasia variety; Sercial variety; Verdelho variety; Must; Free terpenoid compounds; Headspace solid-phase microextraction; Gas chromatography–mass spectrometry; Principal component analysis; Linear discriminant analysis

1. Introduction

The aroma is one of the most important factors in determining wine character and quality. Several studies recognized a relation between the wine and the grape and musts volatile compounds, namely terpenoids [1–3].

Despite their minor contribution to the general aroma of the wine, it has emerged from various studies that the terpenoid compounds form the axis for the sensory expression of the wine bouquet which is typical of its variety and that they can therefore be used analytically for varietal characterisation since they are not significantly affected by the fermentation stage [4]. Numerous monoterpenes have been identified in grape musts and wines. Several authors [5–7] have shown that terpenoids play a significant role in the varietal flavour of wines due to their interesting flowery odours, as well as the odour of fruits, seeds and roots. Due to these aromas it is thought that free monoterpenes are produced to attract insects and other potential seed carriers.

Monoterpenes, secondary plant constituents, are formed by biosynthesis, and C13 norisoprenoids result from biodegradation of diterpenes and carotenoids [8]. In general, the levels of free and bound monoterpenol fractions increase with maturation of the berry [9].

Once the winemaking process starts, all form of monoterpenes undergo various types of reactions. In the grape, monoterpene reactions and rearrangements are enzymatically induced [10]. In must and wine, pathways for modification of monoterpenes involve acid and enzyme catalysed hydrolysis, isomerization and cyclisation. Catalysed hydrolysis reactions cleave the sugar moiety from the base monoterpene, forming either an odourless polyol or an aromatic free monoterpene. Polyols directly form free monoterpene through acidic hydrolysis [11].

Aging and storage provides time for the slow transformation of the free and bound monoterpenes in the wine

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[12]. It is generally believed that hydrolysis of glycosides and polyols into aromatic monoterpenes is faster than free monoterpene isomerization and rearrangement. Over time bound terpenes are slowly converted to aromatic terpenes, which slowly rearrange into new compounds. Some of these products can be less aromatic, depending on the concentration of the compound [13].

The monoterpene content is influenced by several factors [14]. Light is a major factor in terpene development, even more than temperature. Colder sites had a greater accumulation of terpenes than warmer sites. The microbiological flora of the vineyard can also alter the terpene content. *Botrytis cinerea* is known to oxidize various monoterpenes into a variety of other monoterpenes and related compounds. Heating clarified juice to a high temperature followed by gradual cooling promotes the rapid hydrolysis of the precursors giving rise to the terpene content. The other factors that need to be considered are fermenting and storage temperature, pH, and wine composition [15]. All these contribute to influencing the pathways of monoterpene evolution.

The present study analyses the varietal composition (free monoterpenols and C_{13} norisoprenoids) of musts of Boal, Malvasia, Sercial and Verdelho varieties, obtained over three consecutive vintages (1998, 1999 and 2000) with the objective of finding typical profiles of free varietal compounds. As the grapes of the four varieties were from the same vineyard, it is assumed that they have common characteristics of soil, climate and treatment. Therefore the differences found in varietal composition of the musts should only be due to the variety used. These profiles were compared to establish differentiation criteria as a function of the varieties from which the musts are made.

Multivariate techniques of data analysis—principal component analysis (PCA) and linear discriminant analysis (LDA)—were employed for these comparisons.

2. Experimental

2.1. Sample musts

Vitis vinifera varieties in good sanitary conditions (Boal, Malvasia, Sercial and Verdelho grapes) from 1998 to 2000 harvests, were collected at the final stage of ripening in the Direcção Regional da Agricultura experimental yard, and the production of musts was carried out in Instituto do Vinho da Madeira (IVM). The 36 samples musts (nine of each variety) were stored at −28 °C until analysed.

2.2. Sample extraction conditions

Free monoterpenols and C_{13} norisoprenoids were extracted by headspace solid-phase microextraction (HS–SPME) after optimisation of the major parameters that influence the extraction process [16,17]. Optimal conditions of extraction were obtained using the following procedure: 2.4 ml of must was transferred to a 4 ml vial (headspace volume was 1.6 ml, so the phase ratio $1/\beta = 0.6$) [17], the ionic strength was adjusted to 30% with NaCl and the pH was maintained at 3.3–3.5 (pH of the must). The samples (50 ml) were spiked with 0.422 μg l^{-1} of 3-octanol (Sigma–Aldrich) as internal standard, by addition of 50 μl of the alcoholic solution at 422 mg l^{-1}. The vial was sealed and headspace extraction was performed for 60 min at 40 °C with 85-μm PA fiber, keeping the sample under continuous stirring. The compounds were desorbed by inserting the fiber into the gas chromatograph injector for 5 min.

2.3. Gas chromatography–mass spectrometry (GC–MS) conditions

The must extracts were analysed by 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-fused silica capillary column, connected to a Varian Saturn III mass selective detector, according to the method described by Câmara et al. [18]. Splitless injections were 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° min^{-1} and 180–220 °C, at 25° min^{-1}. Each step was preceded by a small period at constant temperature (2, 1 and 10 min, respectively). The injector temperature was 250 °C and the transfer line was held at 220 °C. The detection was performed by an Saturn III mass spectrometer in the EI mode (ionisation energy, 70 eV; source temperature, 180 °C). The acquisition was made in scanning mode (mass range 30–300 *m/z*; 1.9 spectra s^{-1}).

2.4. Quantitation

This was performed by GC–MS. Duplicate calibration graphs, at five concentrations levels, were constructed by least square linear regression using the results for a standard solution, submitted to the same procedure as that samples. The calibration graphs were linear with *r*-values between 0.977 (nerolidol) and 0.999 (linalool). The repeatability study, calculated from six analyses of a must sample, varied between 2.5% (citronellol) and 11.8% (β-ionone). The 3σ detection limit varied between 0.4 μg l^{-1} (β-damascenone) and 3.0 μg l^{-1} (linalool).

2.5. Statistical analysis

To establish the relationship between the composition and the must variety, principal component analysis (PCA) and linear discriminant analysis (LDA) were carried out using the SPSS program, version 11.0. These techniques were applied to the normalized concentrations of monoterpenols and C13 norisoprenoids.

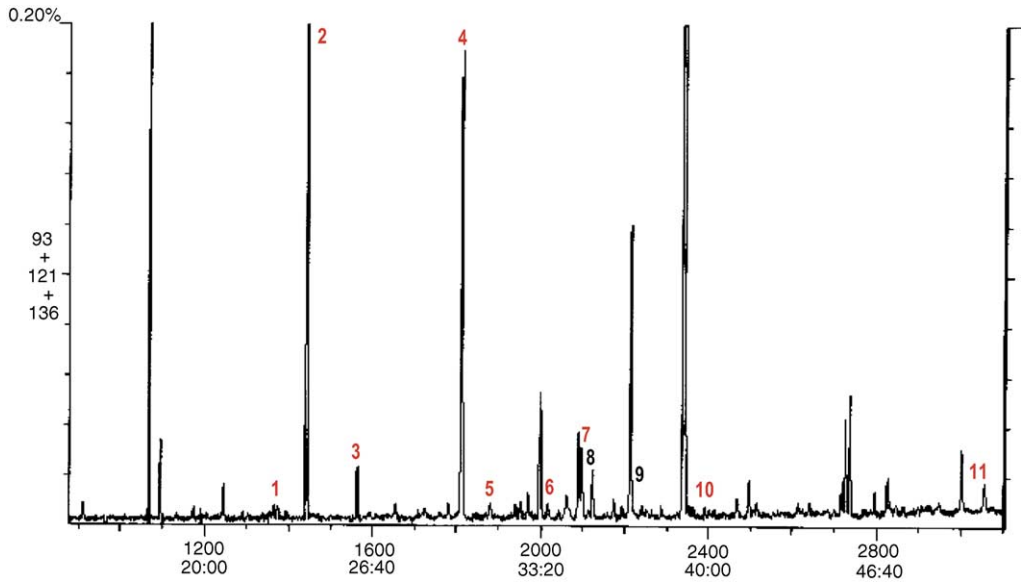

Fig. 1. Typical chromatogram (SIM, $m/z = 93+121+136$) of some terpenoids obtained from HS–SPME/GC–MS analysis of a 1999 Verdelho must sample (1, (*E*,*E*)-farnesal; 2, linalool; 3, 4-terpineol; 4, α-terpineol; 5, neral; 6, citronellol; 7, nerol; 8, β-damascenone; 9, geraniol; 10, β-ionone; 11, farnesol).

3. Results and discussion

The dynamic headspace SPME–GC–MS method was found to be fully suitable for the analysis of free monoterpenols and C_{13} norisoprenoids (terpenoids) in musts, due to its selectivity and sensitivity. Detection limits are in the few $\mu g\,l^{-1}$ range; repeatability as calculated on six successive extractions, is about 4.8% for all analytes considered. Fig. 1 shows a typical chromatogram (selected ion monitoring—SIM) obtained from HS–SPME–GC–MS analysis of a must sample.

The mean values for the free terpenoids determined in Boal, Malvasia, Sercial and Verdelho musts over the three vintages studied are presented in Fig. 2.

The results obtained show that farnesol, linalool and α-terpineol are markedly the most abundant monoterpenols in Boal, Malvasia, Sercial and Verdelho musts, but these compounds are present at levels lower than their perception threshold [19–21]. Farnesol is the main monoterpenol present in the Boal must variety. In the Malvasia musts farnesol, linalool and α-terpineol, are the predominant monoterpenols. Sercial must presents levels of linalool, α-terpineol, β-ionone and farnesol higher than the other terpenoids. Linalool and farnesol are the most abundant in Verdelho musts.

The content of terpenoids remained relatively constant throughout the three vintages studied (1998, 1999 and 2000). The total content of free monoterpenols and norisoprenoids was 133.9, 141.9 and 120.1 $\mu g\,l^{-1}$ for the 1998, 1999 and 2000 vintages, respectively. Fig. 3 show the content of free monoterpenols and norisoprenoids in Malvasia musts for the vintages considered.

The composition of total terpenoids free fraction, calculated for all three consecutive vintages, was different for the four varieties studied (Table 1). All of the musts varieties showed a generally low free monoterpene content.

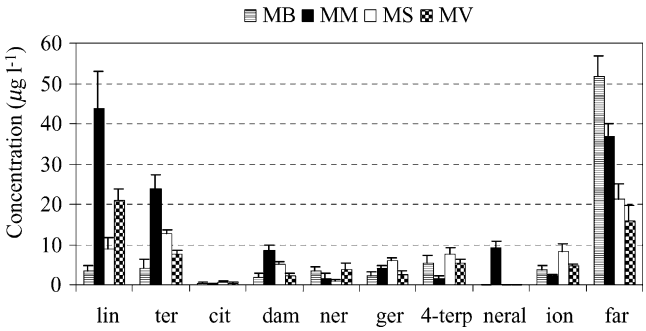

Fig. 2. Mean concentrations ($\mu g\,l^{-1}$) for free monoterpenols and norisoprenoids of Boal (MB), Malvasia (MM), Sercial (MS) and Verdelho (MV) musts (lin, linalool; ter, α-terpineol; cit, citronellol; dam, β-damascenone; ner, nerol; ger, geraniol; 4-terp, 4-terpineol; neral, neral; ion, β-ionone; far, farnesol).

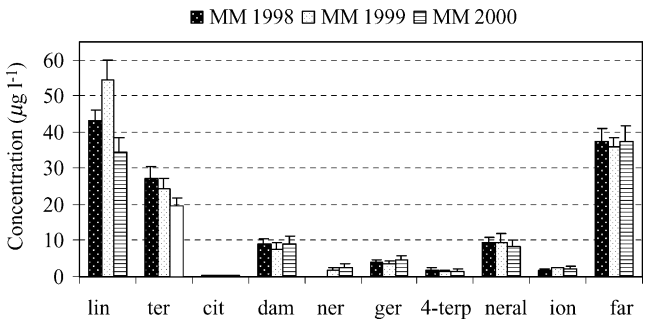

Fig. 3. Contents of monoterpenols and C_{13} norisoprenoids for three vintages of Malvasia musts.

Table 1
Total free terpenoids contents for the different musts varieties

Must variety	Concentration ($\mu g\,l^{-1}$)	R.S.D. (%)[a]
Boal	76.3	15.2
Malvasia	132.0	14.8
Sercial	71.8	6.1
Verdelho	63.3	6.5

[a] $a_n = 5$.

Malvasia is the variety with high levels of free monoterpenols and C_{13} norisoprenoids ($132.0 \pm 14.8\,\mu g\,l^{-1}$).

3.1. Principal component analysis

By application of PCA to the normalized concentrations of the nine determined analytical variables (terpenoids) and 36 objects (musts), two factors that explain 82.1% of the total variance of the initial data set were extracted. The observation of the loading scores suggests that seven variables, having coefficients of magnitude >0.8—linalool (lin), α-terpineol (ter), citronellol (cit), geraniol (ger), neral, β-damascenone (dam) and β-ionone (ion), may be enough to adequately describe the samples according to variety. These new variables explain 91.3% of the total variance.

In Fig. 4, the first principal component (PC1), of must samples is plotted against the second principal component (PC2). The separations among different categories of must samples from this PC1–PC2 scatter point plot are Clear. PCA explain 91.3% of the total variance using the first and second components.

Fig. 5 shows the corresponding loadings plot that establishes the relative importance of each variable and it

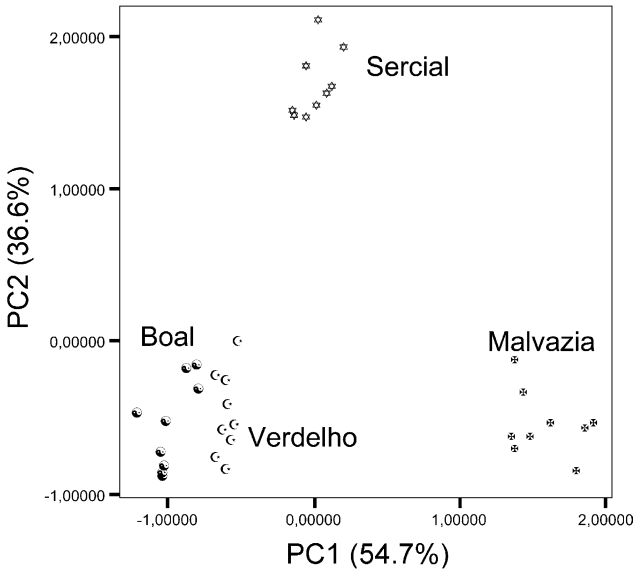

Fig. 4. Extracted principal components as a function of seven variables for 36 samples of musts.

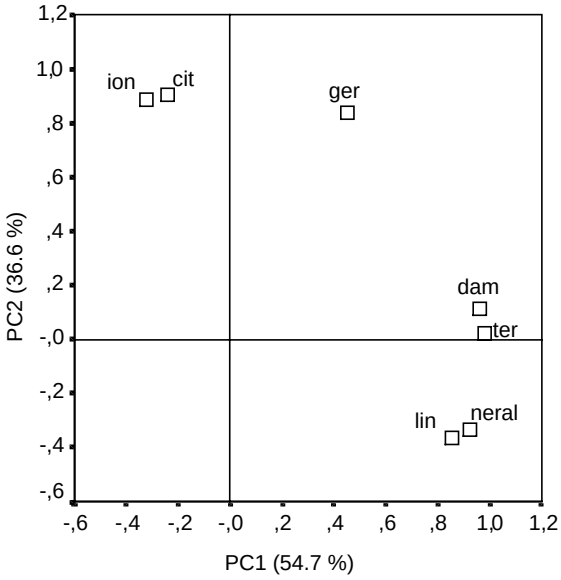

Fig. 5. Relation between the seven terpenoid compounds (loadings).

is therefore useful for the study of relations among the terpenoid compounds and relations between terpenoid compounds and samples.

The variables that contribute most to the first component, that explain 54.7% of total variance of data set, are α-terpineol, β-damascenone, neral and linalool. The second principal component (36.6% of total variability) is influenced by citronellol, β-ionone and geraniol.

First quadrant contains Sercial musts. These samples are characterized by variables associated with positive values of the two first principal components—α-terpineol, β-damascenone and geraniol. Free terpenoids of Boal and Verdelho musts are related to the negative PC1 and PC2 side, being characterized, primarily, by neral, linalol, citronellol and β-ionone. Malvasia samples are represented in the fourth quadrant (positive PC1 and negative PC2). Neral and linalool are the variables most correlated with this must variety.

3.2. Linear discriminant analysis

After a stepwise PCA using the more discriminating variables, a linear discriminant analysis was run in order to optimise the separation of the musts under study and in order to find an operative classification role for discriminating the four must varieties that make Madeira wine. Fig. 6 shows a projection of the musts in 2-D space, explaining 98.4% of the total variance. Four groups representing each variety were clearly observed. The first two discriminant functions (roots) were effective in discriminating between must varieties.

The classification capacity of the functions obtained was evaluated introducing ungrouped samples in the initial matrix. Hundred percent of the objects (8/8) were correctly

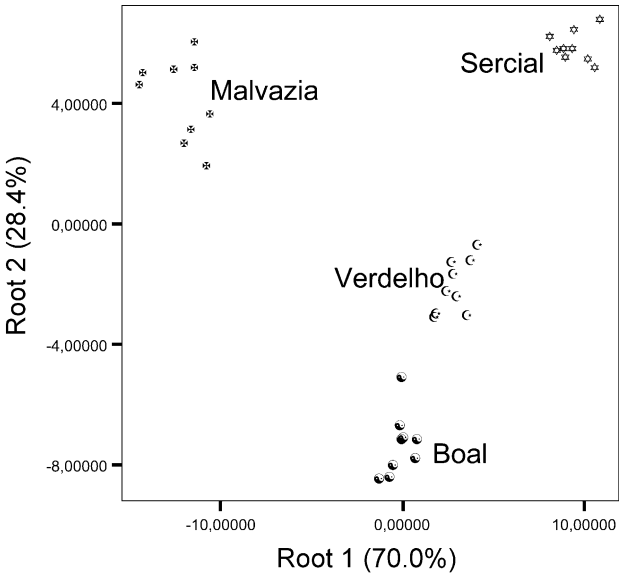

Fig. 6. Discriminant plot for the Boal, Malvasia, Sercial and Verdelho musts classification.

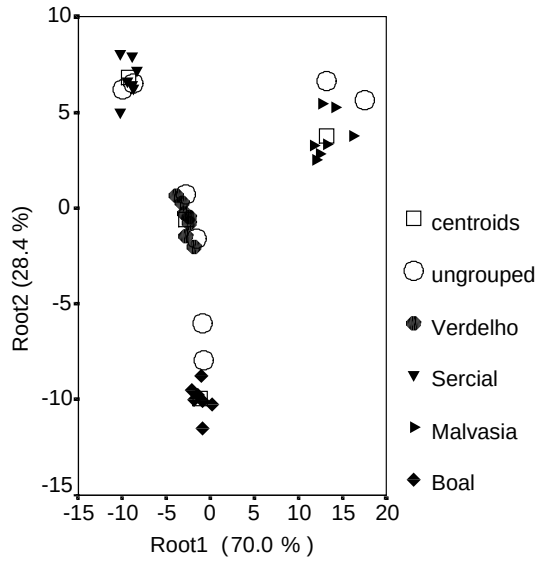

Fig. 7. Ungrouped cases incorporated in the corresponding group.

classified (Fig. 7) corroborating the good differentiation provided by the seven variables used.

4. Conclusions

The results of this work show that Boal, Malvasia, Sercial and Verdelho varieties have different profiles of terpenoids. Malvasia has a higher total amount of these compounds than other varieties. β-Damascenone, the most abundant norisoprenoid in the varieties studied, and β-ionone are present at levels higher than its perception threshold ($45\,ng\,l^{-1}$) and for this reason provide a fruity and exotic aroma to the musts of the varieties under study. The content of monoterpenols and C_{13} norisoprenoids shown by these musts remained relatively constant throughout the three vintages studied, allowing the definition of varietal profiles that are typical of each variety.

Boal, Malvasia, Sercial and Verdelho musts were independently grouped according to variety when terpenoid compounds were subjected to PCA. Boal and Verdelho must samples are characterized by neral, linalol, citronellol and β-ionone, whereas the Malvasia musts are related with neral and linalool. Sercial musts are most associated with α-terpineol, β-damascenone and geraniol.

Despite the good results obtained in this study, a larger number of musts samples should be examined in order to confirm these findings.

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