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Heterocyclic acetals in Madeira wines

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Abstract The maturation of Madeira wines usually involves exposure to relatively high temperatures which affect the aroma and flavour composition leading to the formation of the typical and characteristic bouquet of these wines. The formation of heterocyclic acetals (1,3-dioxanes and 1,3-dioxolanes) was investigated in order to determine levels and for possible use as indicators of wine age. The results show a linear correlation of the investigated acetals with wine age but suggest that the acetalization reaction is not particularly affected by the drastic oxidative conditions observed during maturation.

Keywords Acetals · 1,3-Dioxanes · 1,3-Dioxolanes · Wine-ageing · Madeira wine · GC–MS

Introduction

The vinification and ageing processes used for Madeira wines are unique. Following the typical sugar content of the grape varieties used and the residual sweetness admitted for the particular brand, the fermentation process is stopped by the addition of alcohol in order to obtain an ethanol content of 18–19% v/v. The new wine is then placed in large coated vats and the temperature is slowly increased—at about 5 °C per day—and maintained at 45–50 °C for three months. After controlled cooling, the wine is moved to conventional lodges to undergo wood ageing and flavour development. The heating process (known as estufagem) is used in the preparation of Madeira wines for the last two centuries and is associated with the characteristic toasted aroma.

Other than the general change in the bouquet, these oxidative conditions lead to increase of aldehydes, mainly acetaldehyde, and acetals [1]. Due to the higher content of the acetaldehyde, the reaction of acetalization between acetaldehyde and glycerol, one of the major wine components, is highly favoured at wine pH leading to the formation of four heterocyclic acetals: *cis*- and *trans*-5-hydroxy-2-methyl-1,3-dioxane (*cis*-dioxane and *trans*-dioxane) and *cis*- and *trans*-4-hydroxymethyl-2-methyl-1,3-dioxolane (*cis*-dioxolane and *trans*-dioxolane). These compounds were identified in different types of wines [2, 3, 4, 5] and their evolution in Port wines was described in detail [2].

The objectives of this work were to determine typical values of these isomers in current Madeira wines, to evaluate the influence of the "heating process" and ageing on the concentrations of these compounds and to determine whether these substances can be used as indicators of wine age.

Materials and methods

The fifty-five samples of Madeira wines used in this study were prepared from four different varieties (Bual, Malvazia, Sercial and Verdelho), aged from 1 to 25 years old and matured in oak barrels. The samples were supplied by the Instituto do Vinho da Madeira and Madeira Wine Company and correspond to generic wines submitted to the traditional heating process. Non-heated young wines were also included in order to evaluate any possible influence.

The samples (50 mL) were spiked with 0.422 μg mL^{-1} of octan-3-ol (Sigma Aldrich) as internal standard, by addition of 50 μL of an aqueous alcoholic solution at 422 mg L^{-1}. The acetals were extracted twice with 5 mL dichloromethane (LabScan). The two organic phases obtained were blended and dried over anhydrous sodium sulfate (Merck). This organic phase (2 mL) was then concentrated fivefold, under a nitrogen stream, according to the method proposed for Port wines [2].

Gas Chromatography/Mass spectrometry

Extracts were analysed using a Varian STAR 3400Cx gas chromatograph equipped with a Varian Saturn 3 mass selective detec-

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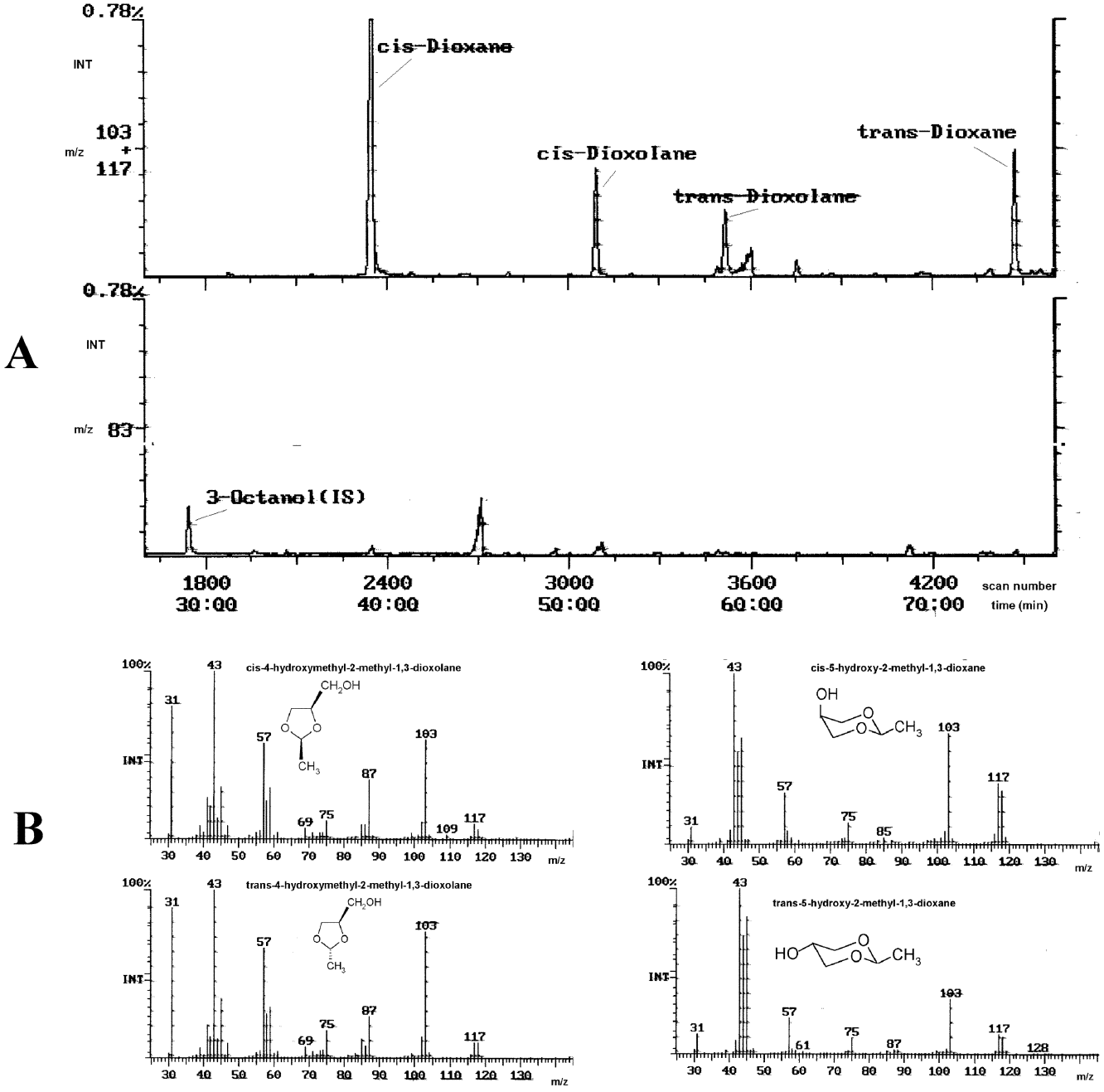

Fig. 1 **A** SIM profile of the dichloromethane extract of a 1990 Verdelho wine (acetals: ions of m/z 103+m/z 117; IS: ions of m/z 83). Column: Stabilwax (Restek), 30 m×0.25 mm i.d.×0.25 µm film thickness. **B** Mass spectra of the four acetals as obtained from the dichloromethane extract of a 1990 Verdelho wine

tor and the software version 5.2. The column used was Stabilwax (30 m×0.25 mm, 0.25 µm) fused silica (Restek). The injector port was heated to 250 °C and the carrier was helium N60 (Air Liquid) maintained at the constant flow of 1 mL min^{-1}. The initial oven temperature was set to 40 °C. The temperature was increased in three steps: 40 to 120 °C, at 1 ° min^{-1}; 120 to 180 °C, at 1.7 ° min^{-1} and 180 to 220 °C, at 25 ° min^{-1}. Each step was preceded by a small period at constant temperature of 2 min, 1 min and 10 min, respectively. All mass spectra were acquired in the electron impact (EI) scan mode and the mass range was 30–300 m/z. The transfer line and trap temperatures were set to 220 °C and 170 °C, respectively, and the maximum ionisation time was 25 000 µs.

The injection volume was 1 µL and the analysis was performed in Full Scan mode. Identification was achieved by comparison of the mass spectra obtained for the analysed samples with those present in the NIST 92 MS Library Database or indicated in the literature [2].

Quantification was performed in SIM (selected ion monitoring) mode, selecting ions of m/z=83 for the internal standard and m/z=103 and 117 for the dioxanes and dioxolanes.

Results and discussion

A representative SIM chromatogram and the corresponding mass spectra obtained for the four acetals present in a

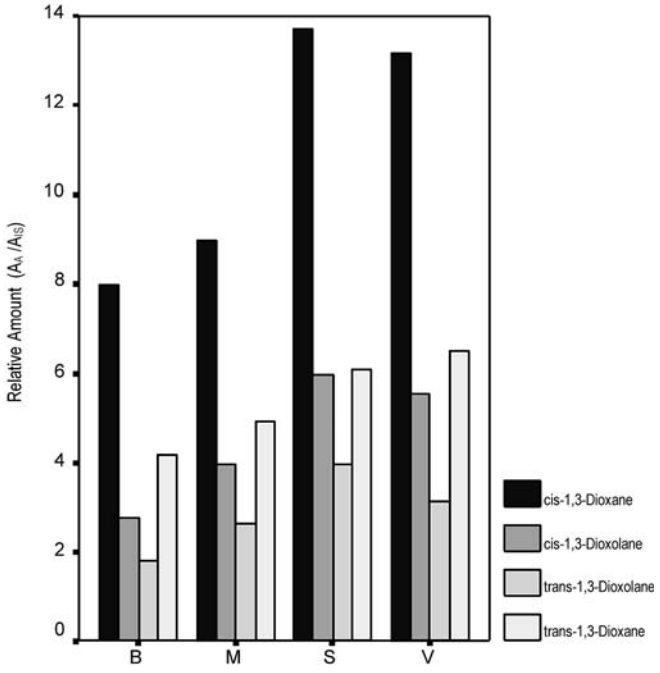

Fig. 2 Relative amounts (expressed as the area of the acetal A_A/area of the internal standard A_{IS}) of the four acetals present in the different varieties of Madeira wine under investigation: *B*, Bual; *M*, Malvazia; *S*, Sercial; *V*, Verdelho

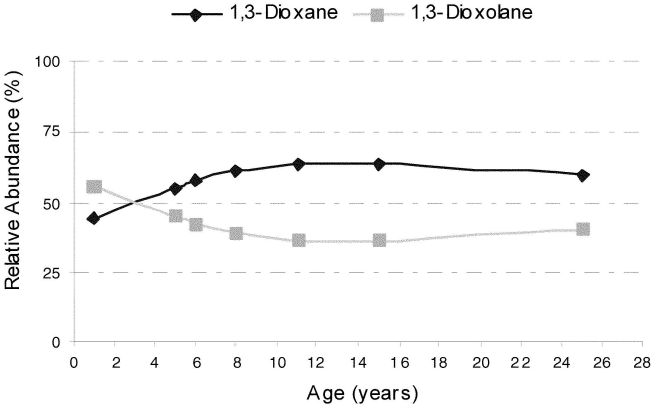

Fig. 3 Relative evolution of the forms of 1,3-dioxane and 1,3-dioxolane with the age of the Madeira wines under study

1990 Verdelho wine sample are shown in the Fig. 1. As shown, the four compounds are easily detected and quantified. The results obtained, considering only the four acetals under study, show average values of 46.5% and 22.7%, respectively, for *cis*- and *trans*-dioxane, and 19.5% and 11.3%, respectively, for *cis*- and *trans*-dioxolane. Average values are similar for the four varieties under study, the *cis*-dioxane is the isomer present in the highest quantity and the *cis* form of each isomer is always present in higher content than the *trans* form, for the four varieties of Madeira wines analysed (Fig. 2).

However, the four acetals content varies with the wine age. In young wines, up to 3–4 years old, the dioxolane

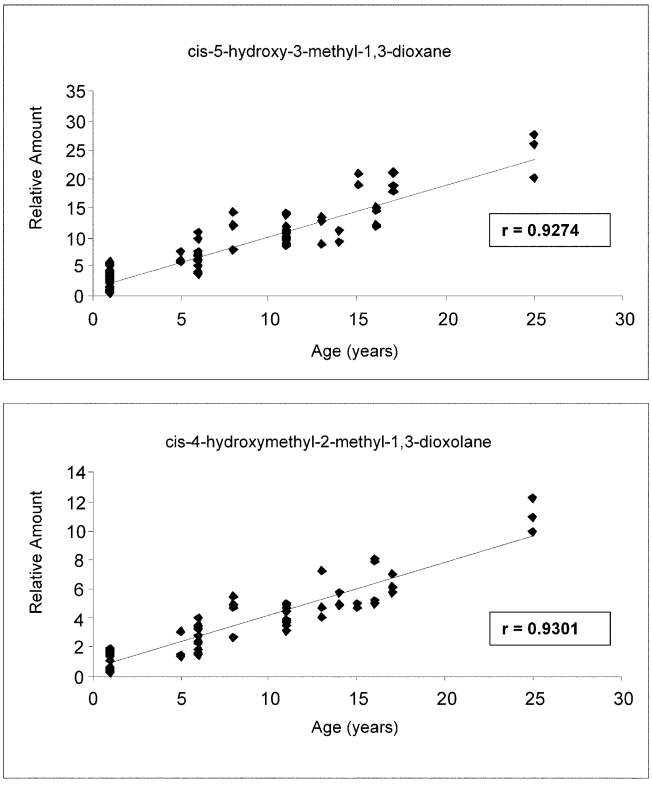

Fig. 4 Evolution of *cis*-dioxane and *cis*-dioxolane with wine age (expressed as the area of the acetal A_A/area of the internal standard A_{IS})

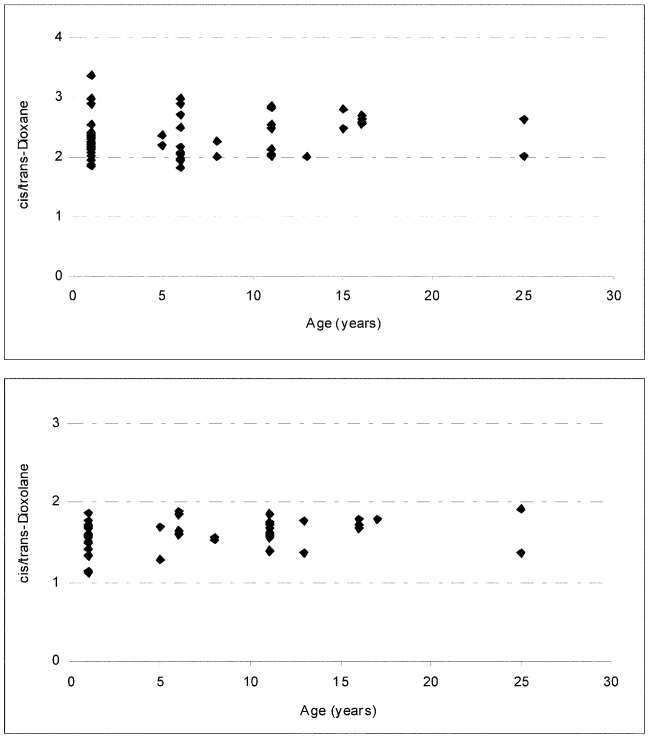

Fig. 5 Evolution of the ratio between *cis* and *trans* acetal forms with wine age

levels were higher than dioxane levels, but after this time an inversion was observed. After about 11 years of maturation, 70% of the acetal content is in the 1,3-dioxane form (Fig. 3). Thereafter, this proportion remained constant suggesting that a thermodynamic equilibrium was attained. Despite the heating process used in Madeira wines, the behaviour is similar to that obtained for Port wines indicating that this step has little effect in the acetal content of the wines.

As shown in Fig. 4 for the *cis*-dioxane and *cis*-dioxolane, the relative amounts of the isomers increase with time and show high linear correlation with age (correlation coefficients≈0.9). The *trans*-dioxane and *trans*-dioxolane show a similar behaviour indicating mutual interconversion of these isomers [6] and leading to a thermodynamic balance.

The ratio between *cis* and *trans* forms remains constant independently of the wine age (Fig. 5) and the average values were 2.35±0.31 for *cis*-/ *trans*-dioxane and 1.60±0.18 for *cis*-/ *trans*-dioxolane. These values are in good agreement with those obtained for Porto wines (2.50±0.19 for the *cis*-/ *trans*-dioxane and 1.30±0.06 for the *cis*-/ *trans*-dioxolane [2].

Conclusions

The linear correlation of the investigated acetals with the wine age allows the easy differentiation of young and old Madeira wines and can be used as an indicator of Madeira wine age. The *cis*-5-hydroxy-2-methyl-1,3-dioxane is the isomer present in the highest quantity in any of the varieties after the first 3 years (the youngest wine in the market). No effect from the heating step used in the maturation of Madeira wines was detected. The results also show that the ratio between isomers is independent of wine age and close to the values obtained for Port wines which could indicate similar behaviour.

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