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Heterocyclic Acetals from Glycerol and Acetaldehyde in Port Wines: Evolution with Aging

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In Port wine, isomers of glycerol and acetaldehyde acetals have been found at total contents ranging from 9.4 to 175.3 mg/L. During oxidative aging, the concentrations of the 5-hydroxy-2-methyl-1,3-dioxane and 4-hydroxymethyl-2-methyl-1,3-dioxolane isomers increased with time showing a linear correlation ($r > 0.95$). The flavor threshold for the mixture of the four isomers was evaluated in wine at 100 mg/L. Thus, it is expected that they contribute to "old Port wine" aroma in wines older than 30 years. Experiments with model solutions and wine clearly demonstrated that SO_2 combines with acetaldehyde and blocks the acetalization reaction.

KEYWORDS: 1,3-Dioxanes; 1,3-dioxolanes; acetalization; oxidative wine-aging

INTRODUCTION

The condensation reaction between glycerol and acetaldehyde under acid conditions (at wine pH) leads to the formation of four isomers: *cis*- and *trans*-5-hydroxy-2-methyl-1,3-dioxane and *cis*- and *trans*-4-hydroxymethyl-2-methyl-1,3-dioxolane. These compounds were identified in sherry wine (*1*, *2*). In a comparative work of the volatile aroma composition of two blended red fortified wines of 20 and 100 years old from Australia, the *cis*-5-hydroxy-2-methyl-1,3-dioxane was reported in higher concentrations in the older wine (*3*).

Oxidative reactions occurring during the aging process of Port wine stored in barrels increased the contents of aldehydes and methyl ketones (*4*). Among these substances, acetaldehyde was the major aliphatic aldehyde in wine and the one that presented the most significant increasing trend with time of barrel storage. Glycerol is one of the major wine components, with concentrations in Port wine ranging from 4 to 8 g/L, and, consequently, acetal formation should be high. Thus, as expected, large amounts of the four isomers produced by this reaction were found in Port wine. Therefore, special attention was devoted to the study of these compounds, to establish their formation mechanism and their impact on Port wine aroma, and to determine whether these substances can be used as indicators of wine age.

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MATERIALS AND METHODS

Cyclic Acetal Synthesis. Synthesis was performed from glycerol and acetaldehyde by a method previously described (*5*). Chemicals were obtained from Aldrich (Lyon, France). Glycerol, (69.7 g), acetaldehyde (43.03 g), toluene (150 mL), sodium sulfate (70 g), and *p*-toluenesulfonic acid (300 mg) were mixed in a round-bottomed flask fitted with a reflux condenser cooled by water at 0 °C. The mixture was heated at the boiling point for 10 h. It was then purified by distillation and extraction of a 5-mL sample with ether (SDS, France). The organic phase was evaporated with a Rotavapor, and the final residue was injected into the GC. Acetals were identified by their retention index and mass spectrum (**Figure 1**) as compared with those previously described (*6*), and purity was calculated on the basis of flame ionization detection peak area.

Acetal Analysis. To 50 mL of wine were added 50 μL of octan-3-ol in aqueous alcoholic solution (1/1, v/v) at 432.9 mg/L as internal standard and 5 g of anhydrous sodium sulfate. The wine was extracted twice with 5 mL of CH_2Cl_2 (SDS, France). The two extracts were combined and dried over anhydrous sodium sulfate. A 2-mL portion of this organic phase was concentrated 5-fold under a nitrogen stream with a 1 L/min gas flow.

A 2-μL portion of extract was injected into the GC with an MS detector. Chromatographic conditions were the following: Hewlett-Packard HP 5890 gas chromatograph coupled with a mass spectrometer (HP 5972), electronic impact 70 eV; detection mode was selected ion monitoring (SIM) with ions of *m/Z* 83 (internal standard quantification), *m/Z* 103 (acetal quantification), and *m/Z* 117 (acetal qualification); column BP21 (SGE), 50 m × 0.25 mm i.d., 0.25 μm film; helium 5.6 Aga pressure: 55 kPa; injector temperature, 220 °C; detector temperature, 280 °C; oven temperature, 40 °C for 1 min programmed at a rate of 2 °C/min to 220 °C, the final step lasting 30 min; splitless time, 30 s; split flow, 30 mL/min.

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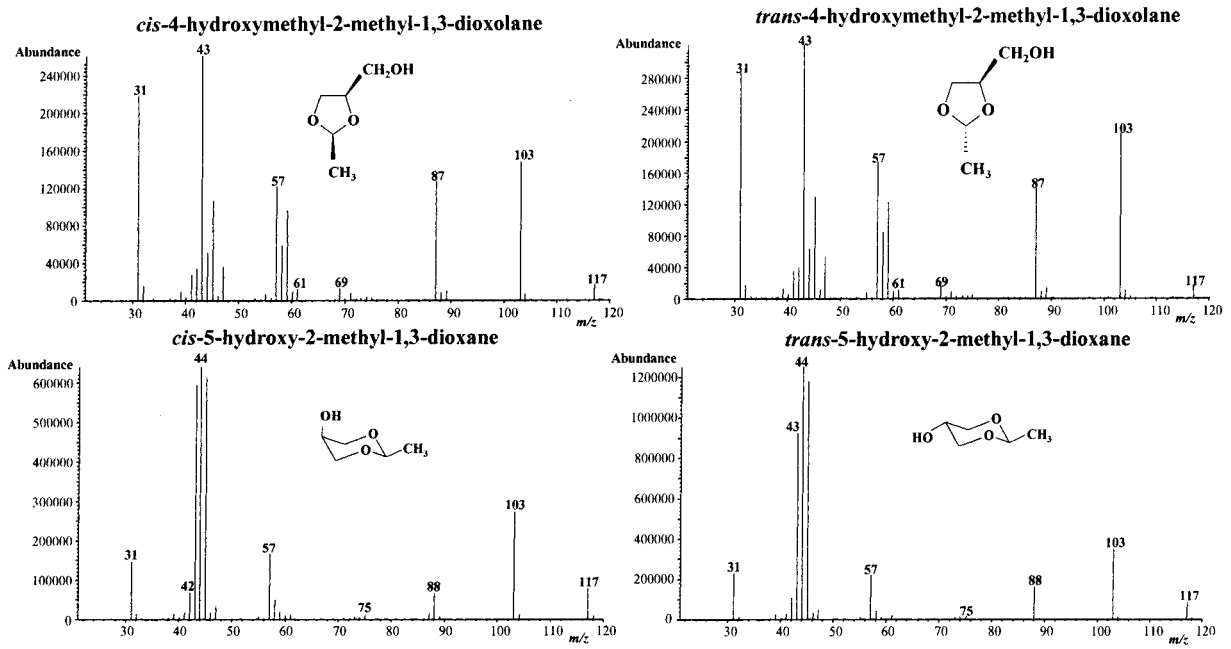

Figure 1. Mass spectra of the four acetaldehyde and glycerol acetal isomers.

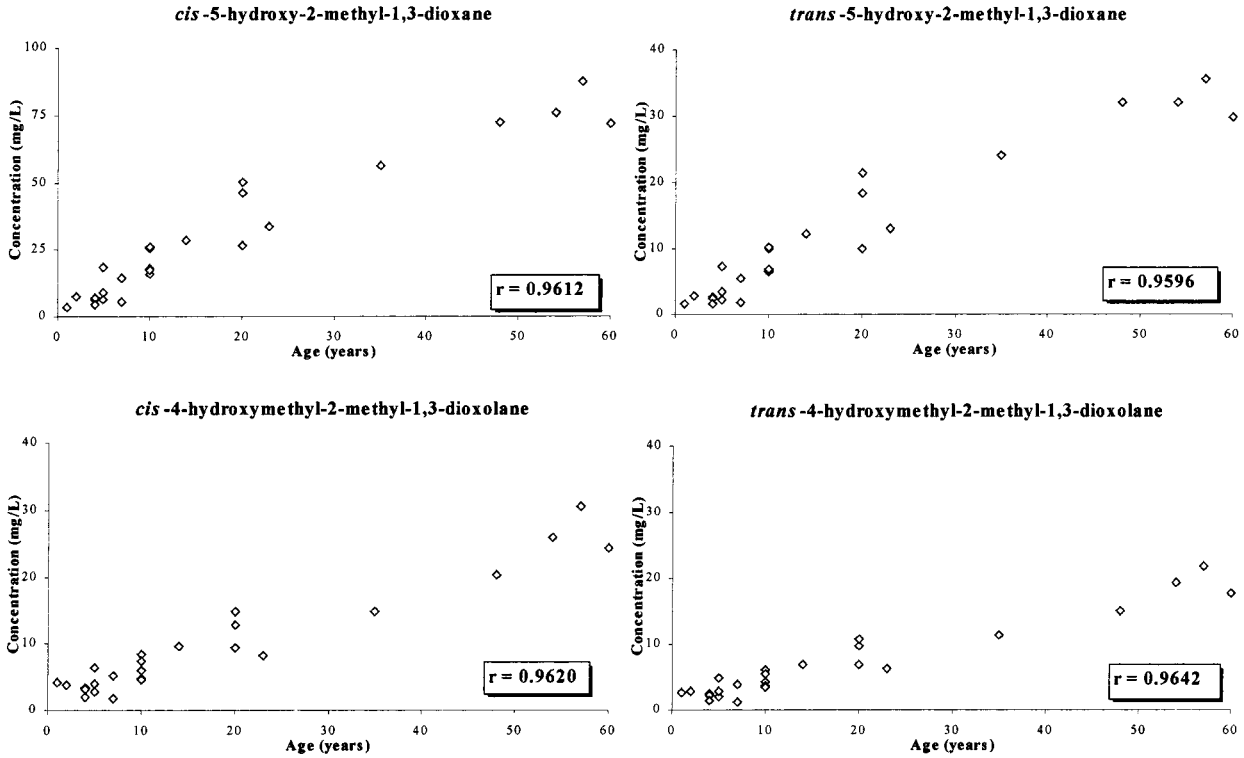

Figure 2. Evolution of glycerol and acetaldehyde cyclic acetals with age.

Precursor Analysis. The determination of acetaldehyde levels was performed by gas chromatography according to the method we previously described (*7*).

Glycerol was quantified by HPLC (reversed-phase chromatography) with refractometric detection.

Port Wine Samples. Twenty-five samples of a single harvest ranging from 1 to 60 years old and matured in oak barrels until analysis were supplied by the Instituto do Vinho do Porto.

Synthetic Solutions and Supplemented Wine Preparation. To study the acetal formation from glycerol and acetaldehyde, 600 mL of a 20% aqueous alcoholic model solution (4 g/L tartaric acid adjusted to pH 3.5 by 1 M NaOH) initially containing 100 mg/L acetaldehyde and 12 g/L glycerol were divided into 10 aliquots, placed in stoppered flasks, and kept at 40 °C. The levels of the four isomers were measured periodically between 3 and 334 h after blending.

To evaluate the influence of free SO_2 level on the formation of these acetals, two 2-L samples were prepared: the first was a 5-year-old Port wine, and the second was a 20% aqueous alcoholic model solution (4 g/L tartaric acid adjusted to pH 3.4 by 1 M NaOH). Wine was aerated until free SO_2 was not detected. Acetaldehyde and glycerol were also added to samples in order to obtain approximately 100 mg/L and 15 g/L as initial concentrations of each. The volume of each of the two samples was divided into two equal parts, one of each being supplemented with a sufficient quantity of SO_2 to obtain 50 mg/L of

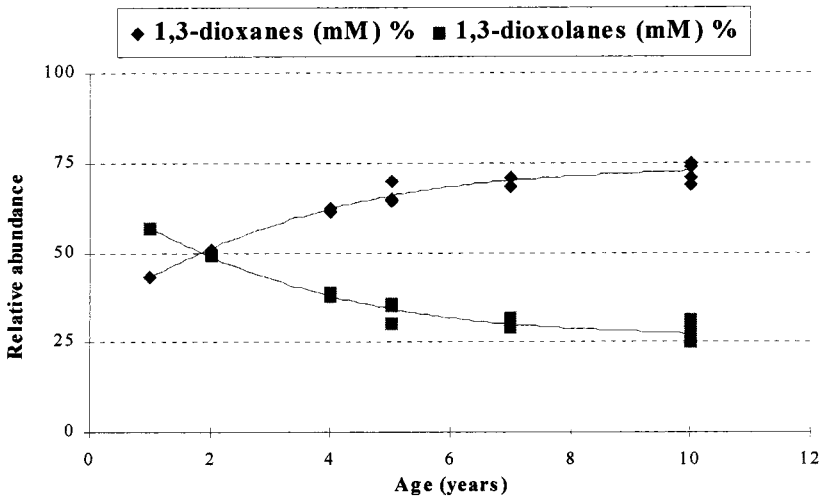

Figure 3. Relative evolution of the 1,3-dioxane and 1,3-dioxolane forms with age.

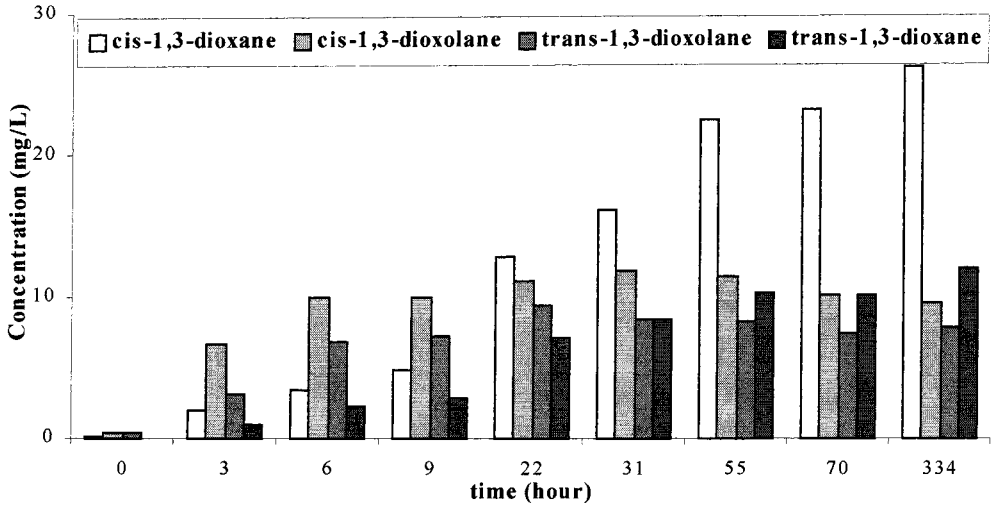

Figure 4. Glycerol and acetaldehyde acetal formation in mild conditions.

free SO_2. The wine and model solution of each set (with and without free SO_2) were placed in 150-mL screw-capped flasks and stored in the dark at 20 °C. Samples were collected and analyzed after 0, 47, 67, 111, and 140 days of storage.

Sensory Evalution. The aroma evaluation of the four acetals was performed by gas chromatography/olfactometry. Chromatographic conditions were the same as those used for quantification with GC/MS. The odors corresponding to each compound were qualified by 5 specialists. The perception threshold of the mixture of the four acetals in ratios similar to those of old port wines was established by a triangular test at five increasing concentrations in a 5-year-old Port wine. The solutions were tasted by a 10-person jury.

RESULTS AND DISCUSSION

Wine Composition. The concentrations of the four isomers increased with time, and their correlation coefficients with age were high ($r > 0.95$) as shown in **Figure 2**; *cis*-5-hydroxy-2-methyl-1,3-dioxane was the isomer present in the highest quantities. Dioxolane levels were higher than dioxane levels in young wines up to 2 years old, but gradually an inversion was observed with time between these two isomers. In fact, after 10 years of barrel storage, 75% of the total acetal contents were in the 1,3-dioxane form (**Figure 3**). This proportion remained constant thereafter and could mean that the thermodynamic equilibrium was attained. The cis form was always present at higher levels than the trans form for each isomer. The ratio between cis and trans forms remained constant independently of age; the calculated values were 2.5 ± 0.19 for the *cis*/*trans* dioxane and 1.3 ± 0.06 for the *cis*/*trans* dioxolanes.

Acetal Formation. At the first analysis ($t = 3$ h), the 4-hydroxymethyl-2-methyl-1,3-dioxolane content was higher than that of 5-hydroxy-2-methyl-1,3-dioxane (**Figure 4**). This finding is in agreement with the mechanism proposed by Gelas (*8*). Indeed, according to the reactional mechanism (**Figure 5**), two carbocations led to the formation of acetals and the carbocation [$C2^+$], which leads exclusively (2.a and 2.b intramolecular reactions) to the dioxolanes, was formed the most quickly; moreover, the cyclization of the carbocation [$C1^+$] according to 1.b leads to the same compounds too. However, from the fourth sampling ($t = 22$ h), *cis*-5-hydroxy-2-methyl-1,3-dioxane showed the highest level, and then the 5-hydroxy-2-methyl-1,3-dioxane content was greater than that of 4-hydroxymethyl-2-methyl-1,3-dioxolane. The four forms tended to an equilibrium similar to that observed in wines after aging for several years. This phenomenon may be explained by the mutual interconversion of these isomers (*9*) which leads to a thermodynamic balance. The relative abundances of the cis and trans forms was explained by Gelas and Rambaud (*10*). On one hand, an

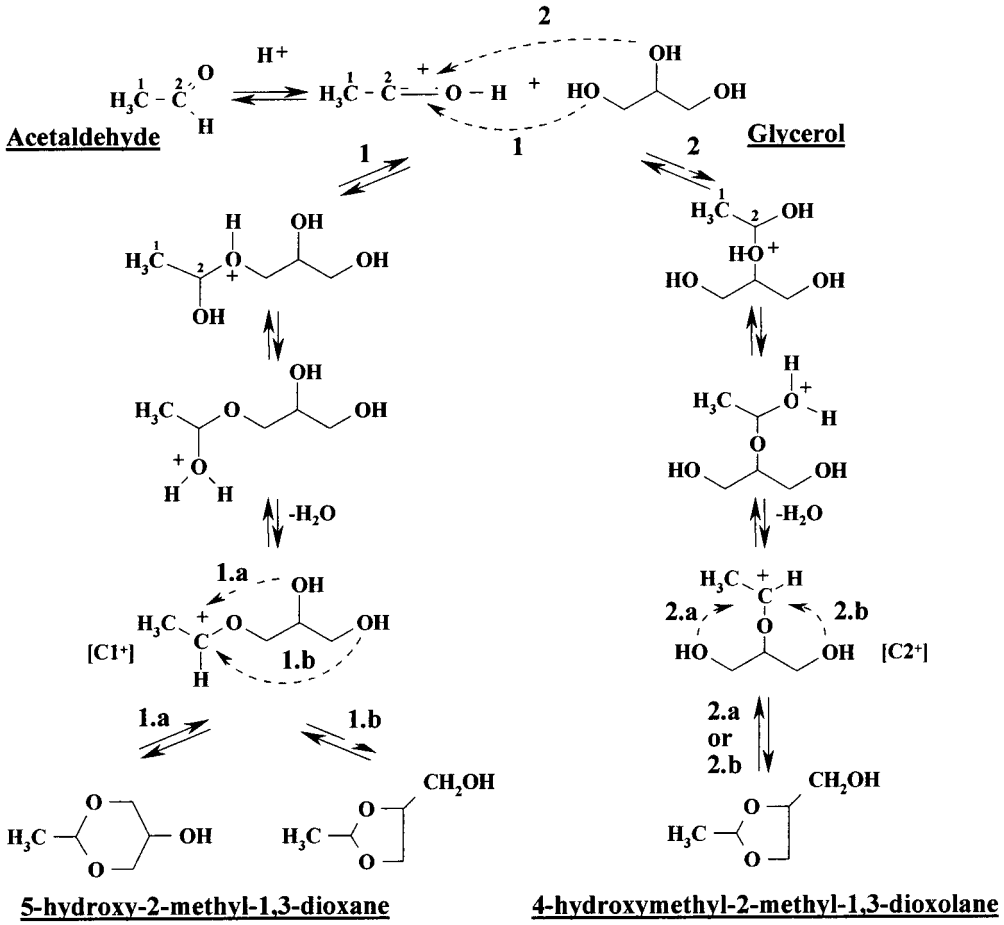

Figure 5. Formation mechanism of glycerol and of acetaldehyde acetals (*10*).

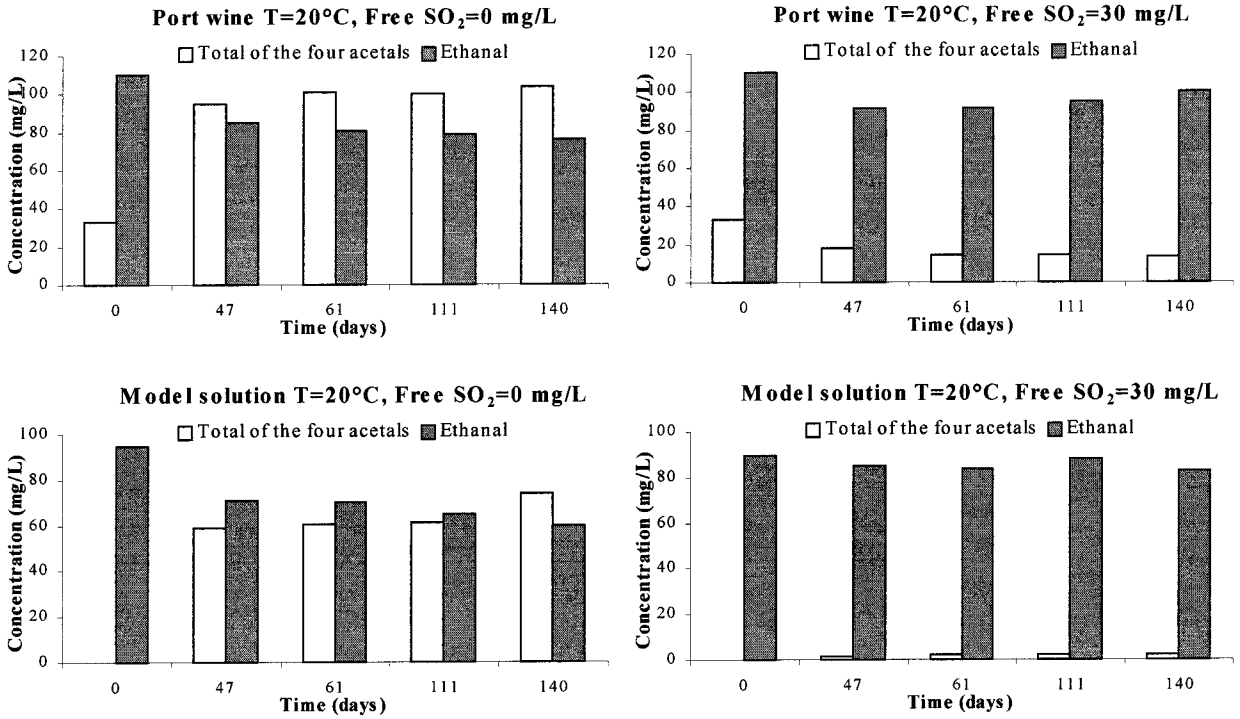

Figure 6. Acetaldehyde and cyclic acetal contents in synthetic media and supplemented wine with or without SO_2.

intramolecular hydrogen bond strongly stabilizes the *cis*-5-hydroxy-2-methyl-1,3-dioxane form, whereas for *trans*-5-hydroxy-2-methyl-1,3-dioxane the bond is only partial. In contrast, 4-hydroxymethyl-2-methyl-1,3-dioxolane diastereoisomers present an identical partial intramolecular hydrogen bonding for these two forms.

Role of SO_2. As shown in **Figure 6**, the total concentration of the four acetals was higher in the samples without free SO_2 both in wine and model solution, whereas no acetal appeared when samples were supplemented with SO_2. Moreover, when free SO_2 was absent, the total of the four acetals increased with time of storage, unlike acetaldehyde. Therefore, SO_2 clearly combined with acetaldehyde and blocked the acetalization reaction because acetaldehyde-bisulfite cannot react with glycerol to give acetals. This underlines the importance of sulfur dioxide in wine storage and the difference between oxidative and reductive aging.

Aroma Impact. Odor evaluation of the four acetals by sniffing in gas chromatography rated the *trans*-5-hydroxy-2-methyl-1,3-dioxane as having the highest intensity aroma, the odor being described as sweet and old port-like. The flavor threshold in wine was estimated for the mixture of the four isomers at a total concentration of 100 mg/L. Therefore, these compounds might contribute to the old port wine aroma in wines older than 30 years.

Moreover, high acetaldehyde quantities are consumed by the acetalization reaction, with the corresponding heterocycles accounting for about 30−40% of the "total" acetaldehyde. By this reaction, glycerol protects the wine against an excessive acetaldehyde content and thus indirectly plays an essential role in port wine aroma.

Because of the constant increase in acetaldehyde content and the absence of free SO_2 during Port wine aging in barrels, the concentrations of the acetals *cis*- and *trans*-5-hydroxy-2-methyl-1,3-dioxane and *cis*- and *trans*-4-(hydroxymethyl)-2-methyl-1,3-dioxolane increase regularly with age. Their levels are well correlated with the time of storage, and it seems that they can be used as effective age indicators of port wine kept under oxidative conditions. Moreover, they participate in the old port wine aroma detected in wines over 30 years old, and their formation is strictly depending on the free SO_2 content.

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