JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Kinetics of Oxidative Degradation of White Wines and How They Are Affected by Selected Technological Parameters

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The negative effects of oxygen on white wine quality and the various factors which influence it (including temperature, dissolved oxygen, pH, and free SO_2) are well documented both at the sensory and compositional levels. What is less defined is the quantitative relationship between these parameters and the kinetics of the development of the negative effects of oxidation. The experiment presented here attempts to generate data which can be used to predictively model the oxidative degradation of white wines. Bottled wines were submitted to extreme conditions (45 °C temperature, O_2 saturation) during 3 months witth samples taken every 15 days for both sensorial and chemical analysis (GC−O/FPD/MS, 420 nm). The synergistic effects of increasing temperature and O_2 at lower pH are evident, both on the decrease in levels of terpene alcohols and norisoprenoids (which impart floral aromas), and on the development of off-flavors such as "honey-like", "boiled-potato", and "farm-feed" associated with the presence of phenylacetaldehyde, methional, and 1,1,6-trimethyl-1,2-dihydronaphthalene.

KEYWORDS: **White wine degradation; off-flavors; methional; phenylacetaldehyde; 1,1,6-trimethyl-1,2-dihydronaphthalene; linalool oxides**

INTRODUCTION

The oxidative degradation of white wines rapidly leads to a loss of their sensorial qualities. From an aromatic point of view, this phenomenon leads to a loss of characteristic aromas of young wines, namely the floral and fruity aromas, and subsequently leads to the formation of new aromas characteristic of older wines or atypical aromas associated with the deterioration of the product. From a chromatic point of view, there is the development of a brownish color (nonenzymatic browning). It is recognized that the aromatic deterioration occurs prior to the chromatic changes (*1*−*3*).

Oxidative degradation is mainly due to oxidative reactions promoted by oxygen itself, which is quite different from the scenario encountered in aging, in which the changes in composition are due to various types of chemical reactions. Wines differ in their vulnerability with respect to oxidative degradation; some degrade in a matter of weeks, whereas others resist the process much longer. The incidence of this rapid-onset degradation seems to be higher in wines produced in warmer regions, but even within these regions some wines are more susceptible than others. From an industrial point of view, it should be noted that there is no systematic way to predict the shelf life of bottled white wines.

There is little published information about the chemical compounds associated with oxidative degradation. The presence of off-flavors has been attributed to compounds such as methional, eugenol, sotolon, and 2,3,5-trimethyl-1,3-dioxolane (*1*). On the other hand, it has been shown that cyclic acetals are strongly related to the continuous formation of ethanal, as well as of sotolon, during oxidative aging of Port wines (*4*). 1,1,6-Trimethyl-1,2-dihydronaphthalene (TDN), a substance responsible for the kerosene-like aroma typical of aged wines from the Riesling grape has been reported, and its concentration increases with accelerated oxidation (*5*).

It has been shown that oxygen consumption in wines during storage is related to the presence of certain compounds, including phenolics, and to the pH at which the wine is stored (*1, 6, 7*). In fact, compounds such as dihydrophenols can suffer autoxidation, leading to the consumption of oxygen and to the formation of hydrogen peroxide, a strong oxidizing agent. The rate of autoxidation of phenolic compounds is also pH dependent, and for some compounds is nine times higher at pH 4 than at pH 3 (*8*). Thus, the oxidative degradation of wine aroma is dependent upon several factors, including the concentration of dissolved oxygen, pH, storage temperature, concentration, and types of phenolic compounds, as well as the presence of exogenous antioxidants, such as SO_2 and ascorbic acid.

Various researchers have tried to reproduce in the laboratory the aroma degradation associated with oxidative aging. These studies focused on the effects of temperature and oxygen on the formation of off-flavors (*5, 7, 9*). Nevertheless, there are little data available concerning the effect of pH and the

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10.1021/jf0115847 CCC: $22.00
Published on Web 09/14/2002

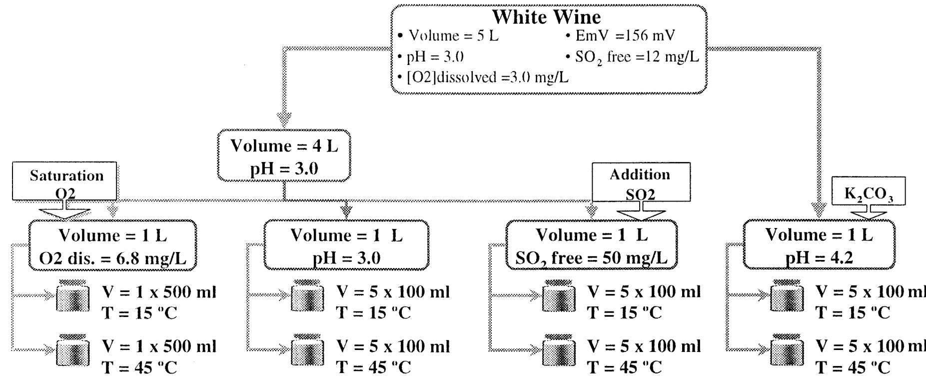

Figure 1. Schematic representation of the preparation of the wine samples.

prevention effect of adding antioxidants on the development of undesirable molecules.

In this present study, an experimental protocol was established in order to analyze the effect of parameters which are believed to be important in the aromatic degradation of white wines, namely temperature, oxygen concentration, pH, and SO_2 concentration, on the formation of these off-flavors. The objectives of the experiments were as follows: (a) to study the changes in sensorial properties and chemical composition associated with changes in the technological parameters mentioned above; (b) to determine which compounds are related to the typical aromas of oxidative degradation; and (c) to gather information about the rates of formation and consumption of these compounds.

MATERIALS AND METHODS

Wine. The wine used in this study was young, dry white wine, produced in the Alentejo region (South Portugal) from the 1999 vintage, following standard winemaking procedures, without wood contact.

Preparation of Wine Samples. Samples were prepared according to the scheme in **Figure 1**. To test the effect of pH, one of the portions was kept at pH 3, whereas another was adjusted to pH 4 by adding K_2CO_3. To test the effect of high concentrations of oxygen (oxidative environment) and the effect of an exogenous antioxidant, two portions at the initial pH were treated as follows: one was saturated with oxygen (6.8 mg/L) by air bubbling, and the other was adjusted to 50 mg/L of SO_2 in free form by adding potassium metabisulfite.

As shown in **Figure 1**, the portion saturated with oxygen was divided into two samples of 500 mL each (which were subsequently stored in 1-L sealed vessels), and the other three portions were divided into two sets of five samples of 100 mL each, stored in sealed vessels. Half of the vessels were stored at 15 °C and the other half were stored at 45 °C. The samples were analyzed at 0, 17, 32, 47, and 59 days of storage time. The samples initially saturated with oxygen were re-saturated at each sampling stage. This experiment was performed in duplicate.

Methional Precursor Selection. To three vessels containing a 12% hydro-alcoholic solution, we added three possible precursors of methional, namely: (A) methionol (0.1 mg/L); (B) methylglyoxal (0.5 mg/L) and methionine (0.2 mg/L); and (C) 4-(methylthio)-2-oxobutanoic acid (1 mg/L). These solutions were subjected to the same treatments as the wine samples.

Sensorial Analysis. The sensory panel consisted of 14 trained tasters. Tests were performed in tulip glasses containing 30 mL of wine. The descriptor selection was based on a white wine that was unanimously considered as oxidatively spoiled in several tasting sessions ("reference spoiled wine").

The procedure used to select the most important descriptors related to the spoiled aroma was based on the AFNOR NFV-09-021 (*18*). In a first set of sessions, every member of the panel was asked to freely describe the aroma of the spoiled wine. The hedonic and redundant terms, as well as the nonpertinent terms, were then disregarded, and a first group of descriptors was thus obtained. Then the panel was asked

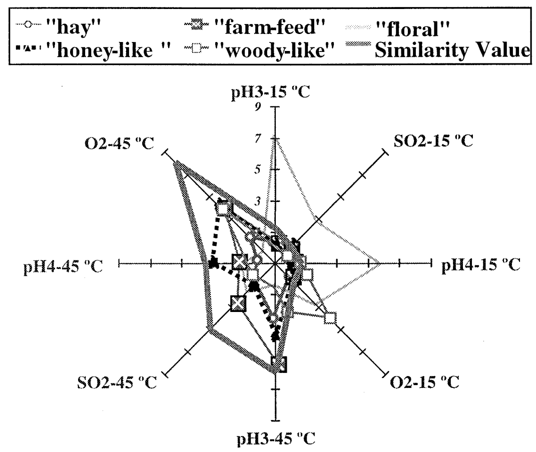

Figure 2. Web diagram of the sensorial results obtained after 59 days of storage.

to determine if the first series of descriptors were present or absent in the reference spoiled wine. Those descriptors considered as absent by 50% of the panel were eliminated, and the remaining descriptors formed a second group. The panel was then asked to rank each descriptor belonging to this group on a scale of 0 to 10. The descriptors that obtained the highest ratings were "honey-like", "farm-feed", "hay", and "woody-like".

During the experiment, the aromatic intensity of the selected descriptors was determined for each sample, on a scale of 0 to 10. At each sampling stage (0, 17, 32, 47, and 59 days of storage), the panel was also asked to rate the degree of similarity between each sample and the reference spoiled wine, on a scale of 0 (no similarity) to 10 (equal). From now on, this parameter is designated as the similarity value (SV).

Chromatographic Methods. The chromatographic quantification of compounds was performed according to methods described in the literature as follows: acetals, aldehydes, nor-isoprenoids, and furans (*4*); sulfur compounds (*10*); and terpenols, esters, and higher alcohols (*11*).

Other Analytical Measurements. Measurements of redox potential, free SO_2 concentration, and chromatic index were performed (*12*). The concentration of dissolved oxygen was measured using a WTW 340 oxygen probe.

RESULTS AND DISCUSSION

The term floral was added to the set of descriptors selected as described in the previous section, as this descriptor is associated with the "freshness" of some non-oxidized white wines. The samples stored at 45 °C were rated with higher similarity values than those stored at 15 °C, as shown in **Figure 2**, which summarizes the results of the sensorial analyses after 59 days of storage.

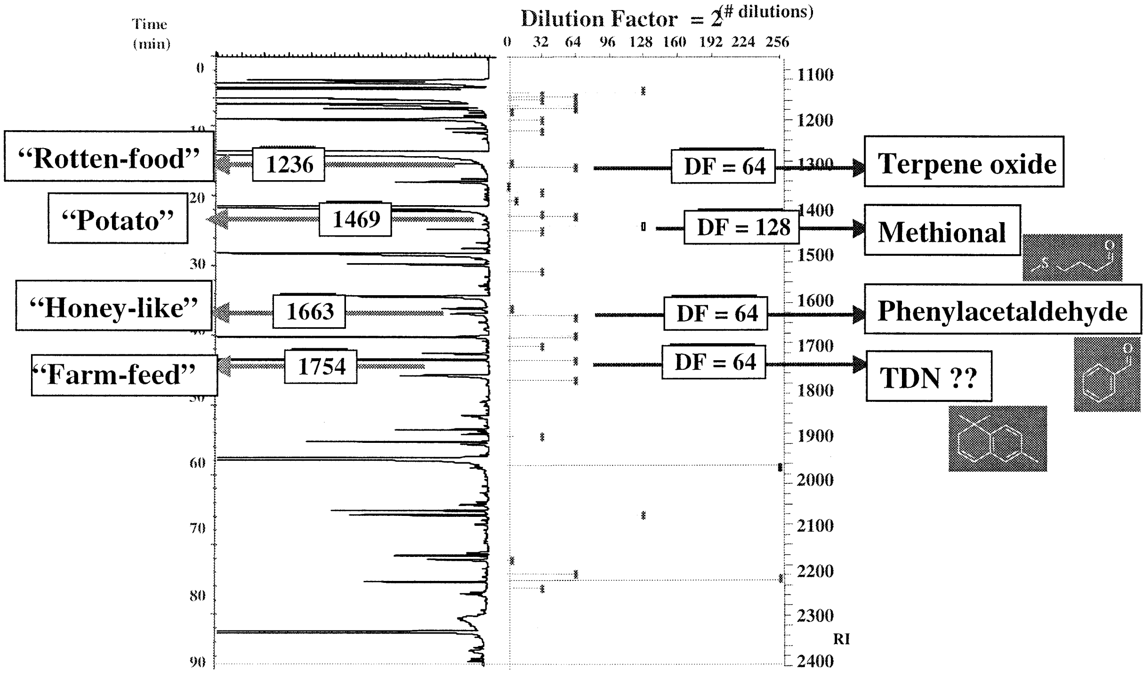

Figure 3. Olfactometric analysis and aromogram of a dichloromethane extract of an oxidative spoiled white wine.

Within the set stored at 45 °C, the samples treated with oxygen had the highest similarity value (SV = 8.3), whereas those at pH 4 were considered to be the least spoiled (SV = 3.6). It was also observed that the treatment with SO_2 decreased the aroma degradation (SV = 5.1), when compared to the effect of oxygen treatment. It is also important to note that the similarity value at pH 3 (SV = 6.0) is almost twice that at pH 4.

These results suggest that the degradation of the wine aroma is mainly due to compounds formed by oxidative reactions. However, the faster degree of deterioration observed at pH 3 than at pH 4 indicates that molecules associated with the aroma spoilage are formed by mechanisms which are also pH-dependent.

The floral descriptor obtained the highest ratings for all samples stored at 15 °C. It should be noted that the degradation at pH 4 (floral = 5.8) is higher than that obtained at pH 3 (floral = 7.3), and that saturation of the samples with oxygen almost led to the disappearance of this aroma (floral = 2.7). At 45 °C, these ratings decreased markedly for all treatments.

Those off-flavors which were better related to the similarity value attributed by the panel were farm-feed ($r = 0.9023$), honey-like ($r = 0.8710$), and hay ($r = 0.6619$). The descriptor woody-like had no significant correlation with the similarity value ($r = 0.5730$), and, as expected, the correlation between the floral descriptor and the similarity rating is strong, but negative ($r = -0.7352$).

At 45 °C, the highest rating for the descriptors farm-feed and hay were obtained at pH 3 (5.1 and 2.7, respectively), whereas the highest ratings for honey-like and woody-like were obtained for the oxygen treatment.

Identification of Off-Flavors. To identify the compounds responsible for the off-flavors, previously mentioned, a GC-sniffing analysis was performed. Different organic solvents were used to obtain extracts of the spoiled wine reference. Similarity tests were performed on the aroma of the extracts and that of wine (*13*). Dichloromethane was chosen as the organic solvent, as the extract with this solvent best represented the initial aroma of the wine.

GC-sniffing (GC−O) analysis showe four important odorant zones where the aromas were related to some of the selected descriptors (**Figure 3**). The aroma and the correspondent Kovats Index (KI) calculated for each zone (*14*) were rotten-food (KI = 1236), boiled potatoes (KI = 1469), honey-like (KI = 1663), and farm-feed (KI = 1754). The relative importance of each of these zones was evaluated by aroma extract dilution analysis (AEDA) (*15*). Among the four odor zones considered, the highest dilution factor (DF) observed was 128 for the boiled potatoes aroma. The others three zones obtained the same value (i.e., DF = 64).

The compounds from the same extract, identified by GC−MS for these indices, were 2,2-dimethyl-5-(1-methylpropenyl)-tetrahydrofuran, methional, phenylacetaldehyde, and TDN. Adding the two pure compounds available, i.e., methional and phenylacetaldehyde, to a nonspoiled wine, the impact of these could be evaluated by the sensory panel. Samples supplemented with phenylacetaldehyde (50 μg/L) had a significant increase in honey values. The addition of 30 μg/L of methional increased the farm-feed values. As standards of TDN and 2,2-dimethyl-5-(1-methylpropenyl)tetrahydrofuran were not commercially available, their effects in wine after addition could not be confirmed. Considering the four initial descriptors, it can be assumed that honey-like is related to the presence of phenylacetaldehyde in levels higher than 25 μg/L (odor threshold). Farm feed seems to be related to the presence of more than one compound, although methional is important in the perception of this aroma. For the other two descriptors, hay (probably dimethylsulfide) and woody-like, it is not possible to associate a specific molecule with the aroma on the basis of the data presented here.

Analysis of the Rate of Formation or Consumption of Some Volatile Compounds as a Function of Temperature, Oxygen Concentration, SO_2, and pH. Sensory analysis showed that pH, temperature, oxygen content, and SO_2 content had different impacts on the degradation of wine aroma. Therefore, the next step was to relate the wine aroma to these technological parameters by determining their effect on the rate of formation or consumption of various volatile compounds. The choice of

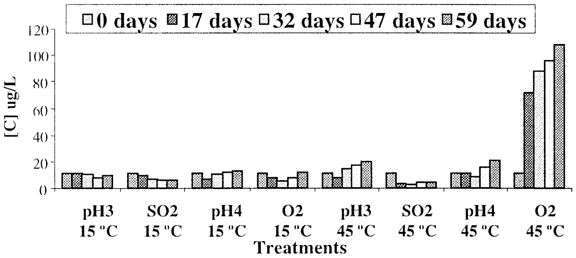

Figure 4. Concentration of phenylacetaldehyde in the samples for each treatment.

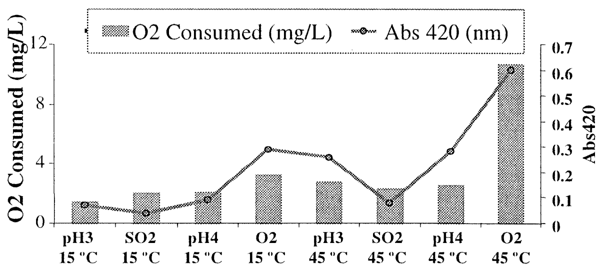

Figure 5. Oxygen consumed (mg/L) and absorbance at 420 nm after 59 days of storage.

these compounds to quantify was based on the following reasoning: (a) to quantify compounds belonging to various chemical groups related to oxidative aging; and (b) to quantify compounds which are known to have an impact on the aroma of wines but whose formation pathways are not clear, to determine how they are affected by the parameters used in this study.

Compounds Greatly Affected by the O_2/SO_2 Content. The quantification of phenylacetaldehyde showed that the level of this compound depends on the temperature and oxygen concentration. In fact, there is only a significant increase in the amount of this compound in the samples treated with oxygen. This fact is in agreement with the mechanism of aldehyde formation (Strecker reaction). The amounts found in this study after 59 days were close to 100 μg/L. This value is above the sensory threshold value in wines, 25 μg/L, determined during this study. Therefore, it can be concluded that the honey-like aroma can be explained by the formation of phenylacetaldehyde (OAV = 4).

As shown in **Figure 4** the levels found for phenylacetaldehyde were strictly related to the presence of O_2. In the same way the absorbance at 420 nm is higher in these samples (**Figure 5**).

The quantification of methylthiopropanal (methional), was also a subject of considerable interest, as this compound is considered to play an important role in the characteristic aroma of spoiled wines and this is supported by the sensory data presented above. The odor threshold of this compound in wine has been determined as 0.5 μg/L (*3*). Methional was not found in wine whose aroma was not degraded, and it has not been related to nonprecocious wine aging.

As shown in **Figure 6**, the formation of methional is clearly related to the presence of oxygen in wine. In fact, results showed that the rate of formation observed for methional is nine times higher after O_2 treatment than that in the control wine. The relative rate of formation (RRF) of this substance was estimated assuming that the concentration varied linearly with time.

The amounts of methional were mainly dependent upon, in decreasing order of importance, the temperature and the amount of dissolved oxygen. In fact, none of the treatments at 15 °C led to significant changes in the concentration of this aldehyde.

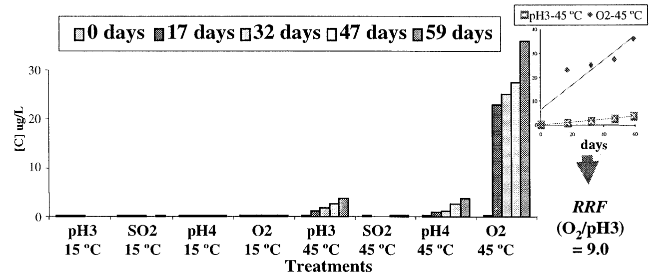

Figure 6. Methional concentration observed in samples and relative rate of formation of O_2/pH 3 (RRF at 45 °C).

On the other hand, at 45 °C there was a considerable increase in the amount of methional over time (except for the samples treated with SO_2). The large amounts of methional formed in the samples treated with oxygen are noteworthy: after 59 days of storage at 45 °C, the concentration was 35 μg/L. This value is 10 times higher than the amount found in the treatments at pH 3 and pH 4, for the same storage period (OAV = 7).

Several authors mention that the origin of this compound could be the direct oxidation of methionol (*9*, *10*, *19*). In this work a decrease in the concentration of methionol was observed only in the samples treated with oxygen. Another possible pathway is the Strecker reaction, where methionine is converted to methional in the presence of a dicarbonyl compound. To determine which is the dominant pathway, an experiment (described in Materials and Methods as Methional Precursor Selection) was performed.

The analysis of the three solutions (A, B, and C) after 15 days showed that there was formation of methional only in solution B (ca. 1 mg/L). It was also verified that, when this solution was treated with SO_2, the amount of methional formed was not meaningful (ca. 0.01 μg/L). Thus, it was concluded that, in this system, the Strecker mechanism is the main pathway for the formation of methional. Dissolved oxygen plays a fundamental role in the formation of methional, due to both the direct oxidation of methionol and the formation of α-dicarbonyl compounds, which are reactants in the Strecker reaction.

Although the most cited descriptor related to methional (i.e., boiled-potato) has not been identified as a descriptor of spoiled wine, the effect of this compound on the aroma should not be ignored, especially considering the high concentrations encountered, which are well above its odor threshold value.

Compounds Greatly Affected by pH 3/pH 4. The degradation rate of linalool is dependent on temperature. After storage at 15 °C there were no marked changes in the concentration of this terpene, whereas at 45 °C its concentration decreased significantly (and exponentially), being more rapid at pH 3 than at pH 4. As suggested by the exponential decrease in the amount of linalool over time, it was assumed that the reaction rate follows a first-order kinetic. Assuming this, the reaction rate constants were estimated for both experiments (the regression coefficients were 0.92 at pH 3 and 0.97 at pH 4). It was verified that the rate constant at pH 3 was 3.6 times higher than that at pH 4.

The rates of formation for linalool oxides (linalool oxide A, linalool oxide B, 2,6,6-trimethyl-2-vinyl-tetrahydropyran, 2,2-dimethyl-5-(1-methylpropenyl)tetrahydrofuran, hydroxylinalool, and *cis*-1,8-terpin) were determined for the 45 °C experiment, considering that these concentrations were a linear correlation over time (**Figure 7**)

The RRF calculated for these molecules (with exception of 2,2-dimethyl-5-(1-methylpropenyl)tetrahydrofuran) for pH 3/pH

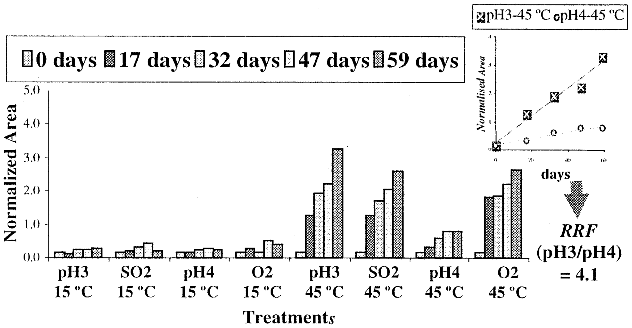

Figure 7. Linalool oxides concentration in samples and relative rate of formation at pH 3/pH 4 (RRF at 45 °C).

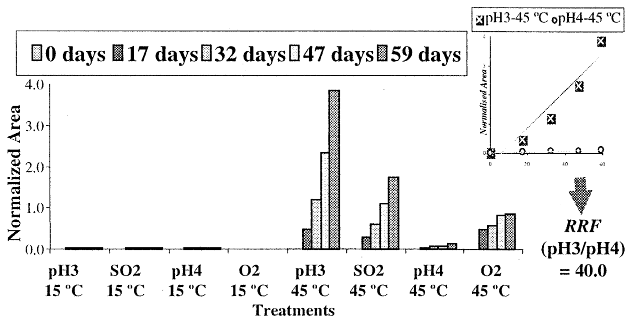

Figure 8. 2,2-Dimethyl-5-(1-methylpropenyl)tetrahydrofuran concentration in samples and relative rate of formation at pH 3/pH 4 (RRF at 45 °C).

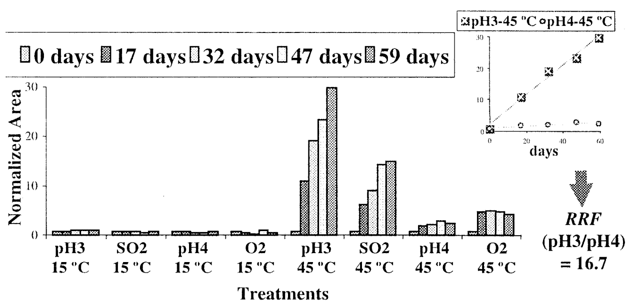

Figure 9. 1,1,6-Trimethyl-1,2-dihydronaphthalen concentration in samples and relative rate of formation at pH 3/pH 4 (RRF at 45 °C).

4 treatments was 4. This is in agreement with the result obtained for the ratio of rate constants for linalool consumption.

The RRF for 2,2-dimethyl-5-(1-methylpropenyl)tetrahydrofuran was 40, i.e., ten times higher than that of the other oxides (**Figure 8**).

The presence of this compound at KI = 1236 is noteworthy, as it corresponds to the GC/O odor zone of rotten food (**Figure 3**).

Norisoprenoid compounds such as TDN and vitispirane are also greatly affected by low pH (**Figures 9** and **10**). The RRFs calculated at 45 °C for pH 3 and pH 4 treatments were 16.7 and 4.9, respectively.

GC/O analysis showed that the aroma descriptor for a KI = 1764 is cereal (**Figure 3**). TDN, identified in aged Riesling wines, was related with the kerosene-like flavor (*5, 16*). The formation of these two norisoprenoids responsible for off-flavors in white wines is in accordance with the acid hydrolysis mechanisms of carotenoid derivative precursors proposed in the literature (*17*).

SUMMARY

The changes in chemical composition related to the process of aromatic deterioration of white wines can be considered to

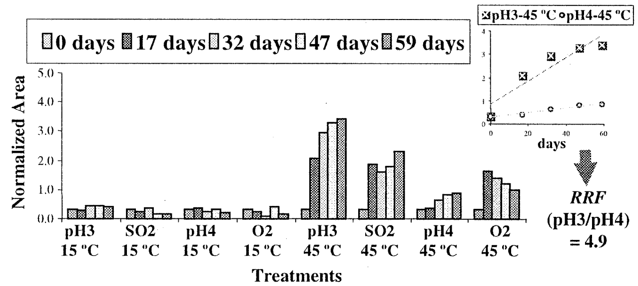

Figure 10. Vitispirane concentration in samples and relative rate of formation at pH 3/pH 4 (RRF at 45 °C).

be the combined result of two distinct effects. The first, and major, general mechanism producing new aroma active molecules is that of oxidation. The second effect is the appearance of other compounds as products of pH-dependent reactions.

Temperature and oxygen are the two main extrinsic factors which contribute to the type of white wine deterioration described here. The development of methional is greatly favored by elevated oxygen concentrations and higher temperatures. The levels observed when these two effects are combined are well above its odor threshold (OAV = 37). The precursor experiment performed allowed the inference that two mechanisms of methional production contribute to the development of this compound in such systems: direct oxidation of methionol and a Strecker reaction (between methionine and a dicarbonyl compound).

The negative aroma impact of temperature and oxygen abuse of white wines would seem to be partly due to the development of methional. Careful management of oxygen levels and storage temperatures would reduce the rate and extent of the production of this compound.

Among the other sensory descriptors selected, honey-like is related to the presence of phenylacetaldehyde, and the development of this molecule is dependent on dissolved O_2 concentration in wines.

The combined action of pH and temperature is responsible for the formation of TDN, vitispirane, and linalool oxides, which can contribute to off-flavors such as those described as farm feed and rotten food.

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Received for review December 3, 2001. Revised manuscript received June 26, 2002. Accepted June 27, 2002. António C. Silva Ferreira is in receipt of a postdoctoral grant from the Portuguese Ministry of Science and Technology (PRAXIS XXI/BPD/20181/99).

JF0115847