

Influence of Some Technological Parameters on the Formation of Dimethyl Sulfide, 2-Mercaptoethanol, Methionol, and Dimethyl Sulfone in Port Wines

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Volatile sulfur compounds of 15 young port wines and 12 old port wines were determined. As there is a great difference in the pool of sulfur compounds between the two groups of wines, an experimental protocol was performed to determine which technological parameter (dissolved O₂, free SO₂ levels, pH, and time/temperature) was related with the formation/consumption of these compounds. Four sulfur compounds were selected for this purpose: dimethyl sulfide, 2-mercaptoethanol, dimethyl sulfone, and methionol. The synergistic effects of increasing temperature and O₂ at lower pH had the largest impact. Dimethyl sulfide was formed during the experimental period in the presence of O₂. Dimethyl sulfone had the same behavior. Methionol decreased significantly in the presence of O₂, but no methional was formed. 2-Mercaptoethanol, considered to be an important "off-flavor" in dry wines, also decreased during the experimental period (54 days) in the presence of O₂, and the respective disulfide was formed. These results corroborate the fact that old port wine (barrel aged) never develops "off-flavors" associated with the presence of methionol (cauliflower), 2-mercaptoethanol (rubber/burnt), or methional (cooked potato). In fact, temperature and oxygen are the major factors in the consumption of these molecules. However, some notes of "quince" and "metallic" can appear during port wine aging, and these can be associated with the presence of dimethyl sulfide.

KEYWORDS: Port wines; aging; volatile sulfur compounds; dimethyl sulfide; 2-mercaptoethanol; methionol; dimethyl sulfone

INTRODUCTION

Volatile sulfur compounds constitute a group of chemical compounds that often exhibit odors qualified as being unpleasant. In recent years, the composition of sulfur compounds in wines has become a subject of many studies concerned with their identification and origin, as well as their character and impact on wine quality. These compounds can be divided according to their volatility into two categories: the "highly volatile" (boiling point below 90 °C) and the "low volatile" compounds (boiling point above 90 °C) (1–3). Normally those belonging to the first group, particularly hydrogen sulfide, ethanethiol, and methanethiol, when present at concentrations higher than their perception threshold have a major negative impact on the wine aroma. Nevertheless, there are molecules among the first group such as dimethyl sulfide (DMS) that have been considered to contribute positively to the bouquet of wines (4). There are numerous studies involving DMS; however, there appears to be little consensus as to its origin in wine or its influence on the global aroma (5). Some authors described DMS as "corn", "molasses", or "asparagus" (3), others as "quince" or "truffle", when the concentration in wines is near the olfactory

perception threshold; at higher concentrations the wine gains aromas related with metallic characteristic (2). It has been shown that DMS concentration increases during wine aging and contributes to the "aging bouquet" (4, 6). The origin of this compound is well-known in beer, being formed from a precursor, *S*-methylmethionine (5). Nevertheless, the origin of DMS in wine is not clear, as the precursor *S*-methylmethionine is not detectable in musts. Some studies show that yeast can produce DMS from dimethyl sulfoxide (DMSO) by DMSO reductase activity (8, 9). Other studies (2) contradict this hypothesis as DMSO was not found in musts from which DMS-containing wines were produced. The pathway of DMS formation in young wines is yet to be fully elucidated.

Most of the less volatile sulfur compound, 3-(methylthio)-1-propanol (methionol), is present in dry wines at concentrations up to 5 mg/L. When this compound occurs at concentrations above its threshold value (1–2 mg/L), it contributes a "cauliflower" aroma (2, 10). Most of the other less volatile sulfur compounds identified in wine are usually found at levels below their threshold values; these include 2-mercaptoethanol, "poultry-like" aroma; 2-methyltetrahydrothiophen-3-one, "metallic" or "natural gas" odor; 2-methylthioethanol, "French bean"; ethyl 3-(methylthio)propionate, "metallic" or "sulfur" aroma; acetic

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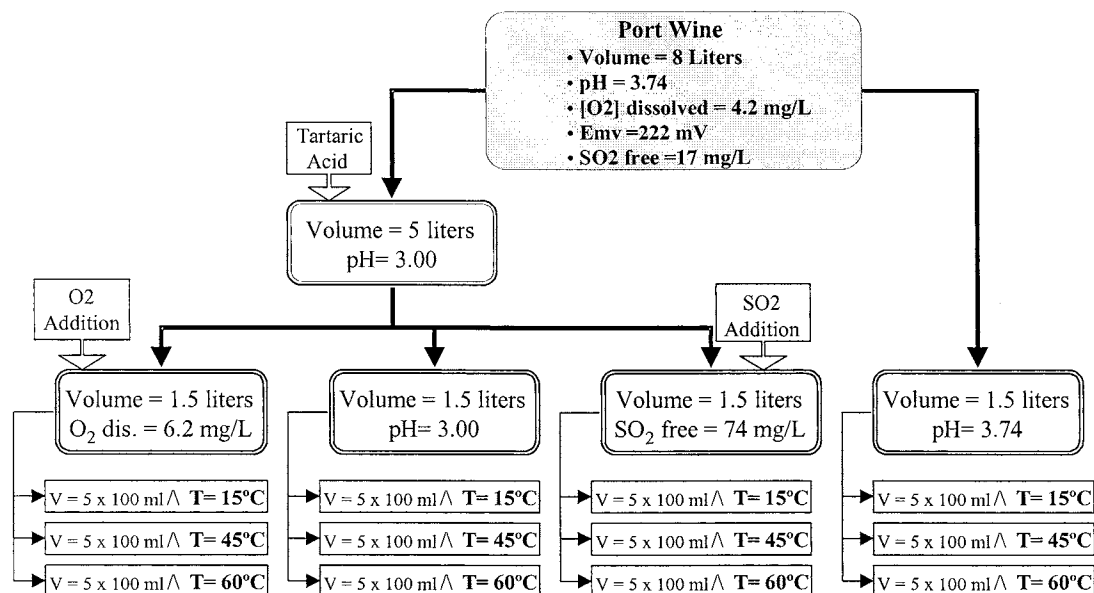


Figure 1. Schematic representation of the preparation of the port wine samples.

acid 3-(methylthio)propyl ester, "cooked potatoes"; and 4-(methylthio)butanol, "chive-garlic" aroma (2, 10, 11). With exceptions of 3-(methylthio)-1-propanol, 3-(methylthio)-1-propanol, 4-(methylthio)-1-butanol, and 2-mercaptoethanol (1, 10, 12–14), the mechanisms of production of the sulfur compounds discussed here are still not fully described.

Port is a naturally sweet wine produced by interrupting alcoholic fermentation by the addition of grape spirit. Although, by definition, Port is produced in the Douro valley of northern Portugal exclusively from grapes grown in the region, similar production methods are applied in many other parts of the world. In addition to the enzymatic formation, chemical reactions occurring during Port wine aging can contribute to the changes observed on sulfur compound levels. In fact, volatile sulfur compounds analysis performed in our laboratory on young and old port wines showed that young port wines have a pool of sulfur compounds similar to that of dry wines. Conversely, old port wines contain very small quantities of these compounds. It is important to note that Port wine suffers long periods of aging (>4 years), for either bottle-aged ("vintage category") or barrel-aged ("tawny" category) wines. Changes in the composition of sulfur compounds during Port wine aging can be related to parameters such as dissolved O₂, pH, and temperature. For this reason an experimental protocol was established to analyze the effect of some parameters that are believed to be important in the aromatic modification of Port wines, namely, temperature, oxygen concentration, pH, and SO₂ concentration.

MATERIALS AND METHODS

Materials. The wines used in this study were as follows. Group 1: young port wines, from one particular vintage (1999), from five different cultivars (Touriga Nacional, Touriga Francesa, Tinta Roriz, Tinto Cãe, and Tinta Barroca) from two different subregions of the Douro valley (Cima Corgo and Douro Superior) (wine-making procedures depended on the producers). Group 2: 10-, 20-, and 30-year-old tawny Port wines (these wines were blended and barrel-aged). Group 3: red Port wine from 1999 vintage (8 L), with a pH of 3.74, an amount of dissolved oxygen of 4.2 mg/L, an instantaneous potential of 222 mV, and a free SO₂ level of 17 mg/L, prepared to analyze the effect of parameters that are believed to be important in the aromatic modification of Port wines, namely, temperature, oxygen concentration, pH, and SO₂ concentration (Figure 1). To test the effect of pH, one of the portions was adjusted to pH 3 by adding tartaric acid. To test the effect of high

concentrations of oxygen (oxidative environment) and the effect of an exogenous antioxidant, a portion of wine at pH 3 was saturated with oxygen (6.2 mg/L) by air bubbling, but for the last portion of wine at pH 3, free SO₂ was adjusted to 74 mg/L by adding potassium metabisulfite. The initial wine, having a pH of 3.74, is named pH 4 in the text.

As shown in Figure 1, the four portions, each 1.5 L, were divided into sets of 100 mL and stored in sealed vessels. One part of each experiment was kept at 15 °C, another one at 45 °C, and the last one at 60 °C. The samples were analyzed at 0, 17, 32, 47, and 59 days of storage time. The samples initially saturated with oxygen were resaturated at each sampling stage. This experiment was performed in duplicate.

Analytical Determinations. Volatile sulfur compounds were determined using a Hewlett-Packard 5890 gas chromatograph, equipped with a flame photometric detector (FPD) and HP Chemstation software. Extraction was performed according to the methodology published in ref 15. Fifty milliliters of wine, with ethyl (methylthio)acetate (50 µg/L) as internal standard, was extracted twice with 5 mL of dichloromethane, for 5 min. The organic phases were concentrated to 1/10 under a nitrogen atmosphere. The extract (2 mg/L) was injected (splitless, 0.3 min) into a CP-Wax 58 (FFAP)-CB column (Chrompack) of 50 m × 0.32 mm and 0.2 µm phase thickness. The temperature program was from 40 °C (10 min) to 220 °C (40 min) at a rate of 2 °C min⁻¹. Injector and detector temperatures were 250 °C. The carrier gas used was H₂ at 1–2 mL min⁻¹. The FPD used hydrogen at 50 mL min⁻¹ and a mixture of nitrogen/oxygen (80:20) at 70 mL min⁻¹. The makeup gas was nitrogen at 70 mL min⁻¹ (15).

Gas Chromatography—Mass Spectrometry (GC-MS). Extracts were analyzed using a Varian CP-3800 gas chromatograph equipped with a Varian Saturn 2000 mass selective detector and Saturn GC-MS workstation software version 5.51. The column used was Stabilwax-DA (60 m × 0.25 mm, 0.25 µm) fused silica (Restek). The injector port was heated to 220 °C. The split vent was opened after 30 s. The carrier gas was helium C-60 (Gasin), at 1 mL min⁻¹, constant flow. The oven temperature was 40 °C (for 1 min), then increased at 2 °C min⁻¹ to 220 °C and held for 30 min. All mass spectra were acquired in the electron impact (EI) mode. The ion trap detector was set as follows: The transfer line, manifold, and trap temperatures were, respectively, 230, 45, and 170 °C. The mass range was *m/z* 33–350, with a scan rate of 6 scan s⁻¹. The emission current was 50 µA, and the electron multiplier was set in relative mode to autotune procedure. The maximum ionization time was 25000 µs, with an ionization storage level of *m/z* 35. The injection volume was 1 µL, and the analysis was performed in full scan mode. Identification was achieved by comparison of mass spectra obtained from the sample with those from pure

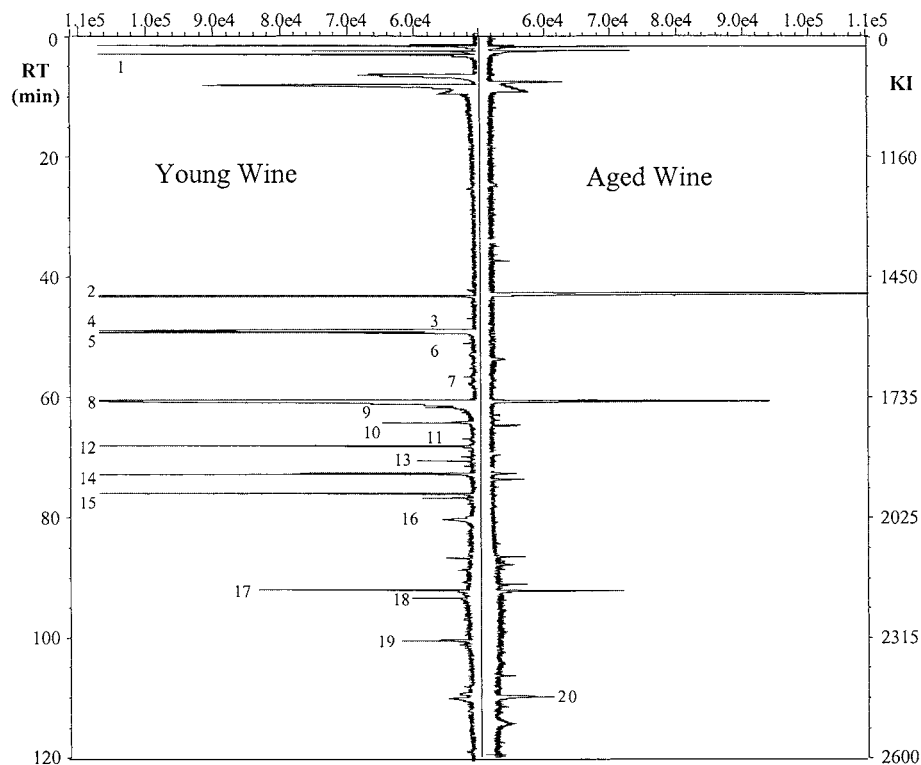


Figure 2. Chromatogram of a DCM extract of a Touriga Nacional from Douro Superior (1999 vintage) (left) and of a 20-year-old tawny port wine (right): 1, dimethyl sulfide; 2, internal standard, ethyl (methylthio)acetate; 3, 2-mercaptoethanol; 4, 2-(methylthio)ethanol; 5, 2-methyltetrahydrothiophen-3-one; 6, ethyl 3-(methylthio)propionate; 7, acetic acid 3-(methylthio)propyl ester; 8, 3-(methylthio)-1-propanol; 9, *cis*-2-methyltetrahydrothiophen-3-ol; 10, 3-(ethylthio)-1-propanol; 11, *trans*-methyltetrahydrothiophen-3-ol; 12, 4-(methylthio)-1-butanol; 13, 3-(methylthio)hexanol; 14, dimethyl sulfone; 15, benzothiazole; 16, unknown; 17, 3-(methylthio)propionic acid; 18, unknown; 19, *N*-[3-(methylthio)propyl]acetamide; 20, bis(2-hydroxydiethyl) disulfide.

standards injected in the same conditions and by comparing the Kovats indices and the mass spectra present in the NIST 98 MS library database or in the literature.

Other Analytical Measurements. Measurements of redox potential, free SO₂ concentration, and chromatic index were performed (16). The concentration of dissolved oxygen was measured using a WTW 340 oxygen probe.

The Kovats index is calculated according to the literature (17).

Statistical Treatments. An analysis of variance (ANOVA), using Excel software Windows 98 v. 7.0, was applied to the experimental data; the results were considered to be significant if the associated *p* value was <0.05.

Standards. The following molecules were purchased from Sigma-Aldrich: 3-(methylthio)propanol (505-10-2) (98% purity); dimethyl sulfide (75-18-3) (99%); dimethyl sulfone (67-71-0) (98%); 2-mercaptoethanol (60-24-2) (98%); and ethyl (methylthio)acetate (4455-13-4) (98%).

RESULTS AND DISCUSSION

It is interesting to note that Port wines, which have a long period of storage, never develop off-flavors related to the presence of sulfur compounds as occurs with dry wines (14, 18–20). Even so, some port wines acquire aromas, such as quince, truffle, and metallic, that can be attributed to the presence of DMS (2). Some authors show that levels of DMS increase with bottle aging (2, 21–23). It has been shown that the presence of DMS can contribute to the fruity character of wine (4). According to other authors, in barrel-aged old wine from which the fruity character has disappeared the addition of DMS in very low levels increases notes of truffle and quince (2). To try to understand this observable fact, old tawny Port wines (10, 20, and 30 years old, wines blended and barrel-aged), young Port wines (port wines from a single year, 1999), and

wines specifically treated for this work were analyzed with regard to volatile sulfur compounds. The determination of compounds such as 2-mercaptoethanol, 2-methyltetrahydrothiophen-3-one, 2-(methylthio)ethanol, ethyl 3-(methylthio)propionate, acetic acid 3-(methylthio)propyl ester, and 4-(methylthio)butanol in many old tawny Port wines showed that they were always present in low levels (lower than their perception threshold limit). However, in young Port wines the profile and the pool of sulfur compounds are considerable as shown in **Figure 2** and **Table 1**, respectively. For these two previously mentioned reasons, first, the development of quince, truffle, and metallic aromas during Port tawny wine aging and, second, the reduction and even the disappearance of major sulfur compounds with barrel aging (**Table 1**), an experimental protocol was performed to try to explain how some parameters such as dissolved oxygen, pH, and temperature could be involved in the formation/consumption of some of these compounds.

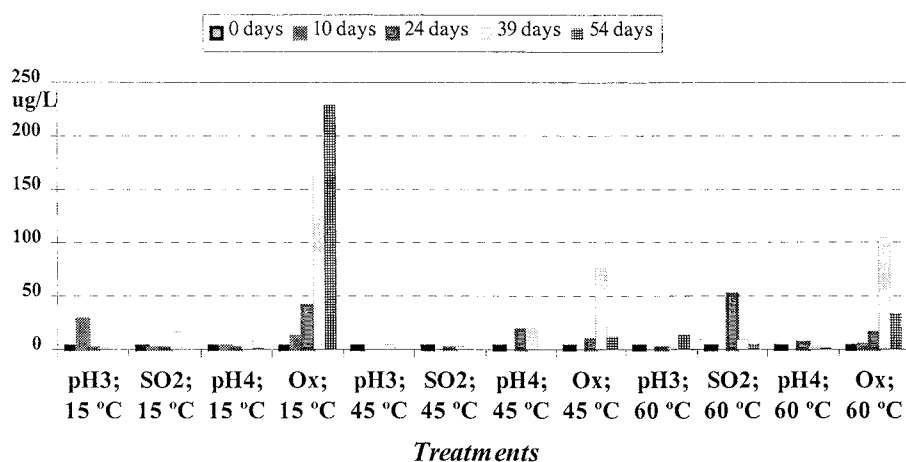
The next step was to relate the wine aroma as a function of these technological parameters by determining their effect on the rate of formation/consumption of various volatile sulfur compounds. The molecules selected were 2-mercaptoethanol and 3-(methylthio)-1-propanol (methionol), the most abundant in young wines (3), DMS, the levels of which are related with age (6), and dimethyl sulfone in order to measure its dependence on oxygen contents in wine.

These four compounds were selected as DMS is related to aging wine (2, 6, 21–23); 2-mercaptoethanol and methionol represent a group of sulfur compound molecules that, on the one hand, decrease sharply with wine aging and, on the other hand, are the most abundant highly volatile compounds present in wines. Finally, dimethyl sulfone was chosen as it is a

Table 1. Concentrations (Micrograms per Liter) of Some Volatile Sulfur Compounds in Young and Old Port Wines

compound	young Port wines			old Port wines		
	av of 15 wines ^{a,c}	max	min	av of 12 wines ^{b,c}	max	min
dimethyl sulfide	8.8 (6.2)	25.2	nd ^d	4.2 (14.4)	50.0	nd
2-mercaptoethanol	48 (35)	134	7	nd (3.6)	23	nd
2-(methylthio)ethanol	436 (362)	1.336	70	4 (9)	70	23
ethyl 3-(methylthio)propionate	5.6 (2.5)	9.1	nd	nd	nd	nd
3-(methylthio)-1-propanol	1055 (436)	2.144	463	174 (17)	206	149
<i>cis</i> -2-methyltetrahydrothiophen-3-ol	20 (8)	25	2	nd	nd	nd
3-(ethylthio)-1-propanol	19 (11)	45	5	nd	nd	nd
<i>trans</i> -2-methyltetrahydrothiophen-3-ol	21 (1.3)	23	5	nd	nd	nd
4-(methylthio)butanol	27 (4)	33	21	nd	nd	nd
3-(methylthio)hexanol	1.1 (0.7)	1.8	nd	nd	nd	nd
dimethyl sulfone	119 (45)	175	45	17 (31)	75	nd
benzothiazole	233 (145)	513	70	nd	nd	nd
3-(methylthio)-1-propionic acid	700 (354)	1.811	500	109 (197)	448	nd
<i>N</i> -[(3-methylthio)propyl]acetamide	189 (309)	899	10	nd	nd	nd

^a Wines from the 1999 vintage, five different cultivars, two different subregions, with different wine-making procedures. ^b Tawny wines of different ages between 10 and 30 years old. ^c Values in parentheses are standard deviations. ^d nd, not detected.

**Figure 3.** Concentration of dimethyl sulfide (DMS) in the samples for each treatment.

compound that persists and sometimes increases in Port wine aging.

Dimethyl Sulfide. Quantification of DMS showed that the level of this compound depended on the temperature and oxygen concentration. The ANOVA procedure showed significant differences of DMS concentrations among the four treatments only for samples stored at 15 °C (p value = 0.041) at the 95% level. At this temperature the amount of this compound in the samples treated with oxygen increases clearly with time (**Figure 3**).

As shown in **Figure 3** high levels found for DMS were strictly related to the presence of O₂. De Mora et al. (5) showed that during wine aging DMS can be formed from methionine and cysteine, and high storage temperatures favor this formation. According to results presented in **Figure 3**, temperatures of 45 and 60 °C were maybe too high to keep DMS in wine. High levels of DMS were produced when samples were kept at 15 °C in the presence of oxygen. If we compare oxygen treatments at 15, 45, and 60 °C there was a tendency to decreasing DMS with higher temperatures. In fact, some old tawny Port wines (barrel-aged/with oxygen presence) have levels of DMS that can attain 50 µg/L. In young Port wines the maximum level attained was 25 µg/L; nevertheless, it is important to note that usually DMS was not present (**Table 1**). Contrarily, old vintage Port wines can reach levels of DMS higher than the perception threshold (results not shown); these wines have a long period of bottle aging. Anocibar Beloqui (2), analyzing wines from the same producer and from different vintages (1984–1994)

aging at the same temperature (17 °C), showed that there was an increase in the concentration of DMS during aging. Some other authors have shown that the increase of DMS is related with the presence of DMSO (7, 9, 24). In our studies we never found DMSO [(CH₃)₂SO] in wines or musts; nevertheless, we found dimethyl sulfone [(CH₂)₂SO₂] in considerable amounts. Later we will discuss the role of this molecule in the aroma of wines.

2-Mercaptoethanol. 2-Mercaptoethanol when present in wines at levels higher than the perception threshold (100 µg/L, in a 20% hydroalcoholic solution) participated in some aroma defect (burnt/rubber) (25). According to Rapp (12) the origin of this compound in wines is cysteine. Contrarily to DMS formation 2-mercaptoethanol decreased during the experimental period (54 days) for oxygen treatment (**Figure 4**). For the pH 4 treatment, there was an increase of 2-mercaptoethanol formation until the 24th day, followed by a reduction until the end of the experiment. The formation of this compound from cysteine involved an oxidative transamination. This reaction was favored by high pH (27). Also, the velocity of oxidative reactions was higher at pH 4. These two related factors can explain the behavior of formation/consumption of 2-mercaptoethanol. Until the 24th day of the study for the two first temperatures and the 10th day for the 60 °C temperature there was a formation of 2-mercaptoethanol from cysteine. After these times, there was a degradation/conversion into other molecules such as bis(2-hydroxydiethyl) disulfide. These results can be supported by those presented in previous works (25). It should be noted that

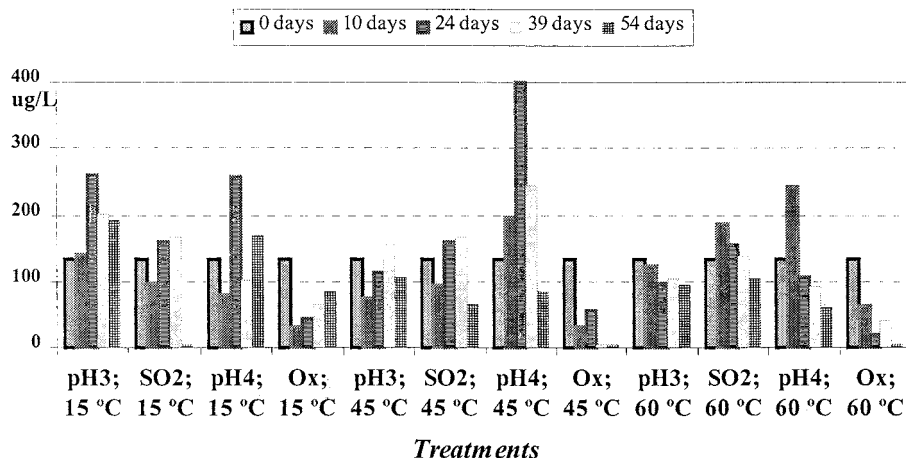


Figure 4. Concentration of 2-mercaptoethanol in the samples for each treatment.

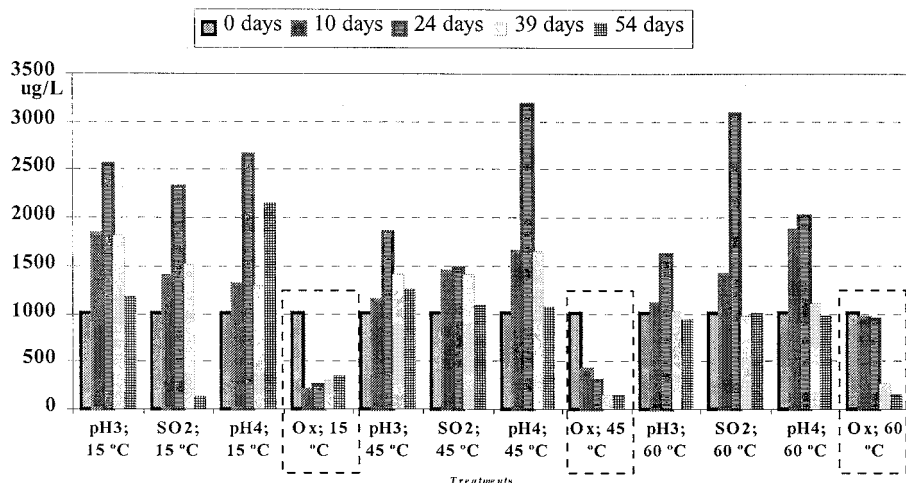


Figure 5. Concentration of methionol in the samples for each treatment.

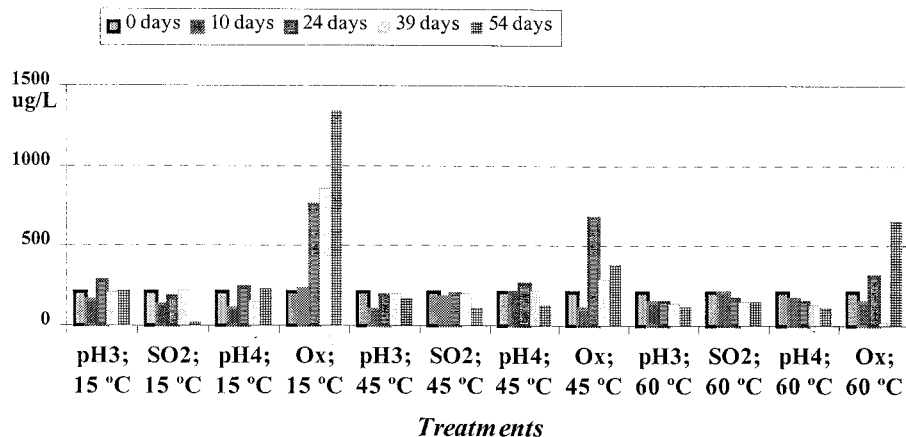


Figure 6. Concentration of dimethyl sulfone in the samples for each treatment.

samples treated with oxygen have lower levels of 2-mercaptoethanol. As this molecule is a thiol, in oxidative conditions (O_2 treatments) a disulfide, the bis(2-hydroxydiethyl) disulfide, was produced, which has no aroma impact. This may explain why in old tawny Port wines we never found aromas (burnt/rubber) related with the presence of this molecule.

Methionol. Oxygen treatment provokes an accelerated decreasing of methionol levels for the three temperatures studied (Figure 5). The decrease of its levels cannot be explained by the disulfide formation as methionol is not a thiol. Previous studies performed on dry white wines have demonstrated that methionol also decreased with the presence of oxygen (18, 26)

with concurrent formation of methional. Even so, it was also shown that the Strecker mechanism was the main pathway for the formation of methional. Results from these studies showed that methional was formed in high levels on white wine samples treated with oxygen (14, 18, 19). In Port wines submitted to the same treatments methional was not found. The decrease in methionol would lead to the formation of other compounds, not yet identified. Methional formation by Strecker reaction requires the presence of methionine and methylglyoxal. In Port wines methylglyoxal exists in levels between 0.96 and 10.3 mg/L (analysis of 38 wines with an age range between 1 and 52 years old); however, methionine is present in very low amounts in

the same Port wines to a maximum of 0.7 mg/L, and in 29 of the 38 wines analyzed methionine was not detected. The low concentrations found in Port wines could explain why, contrary to the reactions occurring in precocious aging of white wines, during Port wine aging this kind of off-flavor (cooked potato) is never detected.

Dimethyl Sulfone. The formation of this molecule is strictly dependent on the presence of oxygen (Figure 6). The ANOVA procedure showed significant differences for dimethyl sulfone concentrations among the four treatments for samples stored at 15 °C (p value = 0.0106) at the 95% level. At this temperature the amount of this compound in the samples treated with oxygen increases clearly with time. According to De Mora (1983) dimethyl sulfoxide [(CH₃)₂SO] could be the precursor of DMS. We have never found this compound in wines; however, we have identified and quantified dimethyl sulfone [(CH₃)₂SO₂]. This molecule has no important sensory impact. It is interesting to note that there is a good correlation between levels of DMS and dimethyl sulfone ($r = 0.760$), which may suggest that dimethyl sulfone could have the same precursor as DMS.

Conclusion. Some old Port wines develop aromas that can be related to the presence of dimethyl sulfide. This molecule was preferentially formed during the experimental period (54 days) at 15 °C in the presence of oxygen.

It was demonstrated that old Port wines contained fewer sulfur compounds than young Port wines, which have a pool of sulfur compounds that can participate in wine aroma.

Methionol decreased significantly during storage time and in the presence of oxygen. This fact is not related to the methionol formation, which was never found in the Port wines analyzed. This could explain why old Port wines never develop these kinds of off-flavors.

2-Mercaptoethanol decreased significantly during the 54 days of the study and in the presence of oxygen. This compound could be converted to bis(hydroxydiethyl) disulfide, which has no aroma. Levels of 2-mercaptoethanol found in old Port wines were always under its perception threshold limit.

Dimethyl sulfone was formed only in the presence of oxygen. A good correlation between DMS and DMSO ($r = 0.760$) was found, which may suggest that they have the same precursor.

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