Effect of Polyphenol, Sulfur Dioxide, and Metal Ions on Acetaldehyde Variation during Wine Oxidation

Xiaoqian Cui[1], Shiyao Li[2], Jiaqi Wang[1], Qinglong Wang[1], Lingmin Dai[1,*], Heqiang Chang[1], Guomin Han[1,*]

[1] School of Bioengineering, Qilu University of Technology (Shandong Academy of Sciences), Jinan Shandong 250353, PR China

[2] Yantai University, Yantai 264005, PR China

[*]Corresponding author: Lingmin Dai (E-mail: dailingmin@qlu.edu.cn), Guomin Han: (E-mail: gmhan@qlu.edu.cn)

Abstract. This work seeks to investigate the effects of SO2 and polyphenol on acetaldehyde content in wine oxidation catalyzed by metal ions and to describe the changes in chemical components related to this process. Wine, containing different levels of polyphenol, was added with SO2 and copper iron ions respectively. O2 and acetaldehyde were monitored periodically; SO2, color, and polyphenol were determined after each micro-oxygenation (MOx) treatment cycle. The content of SO2 in bottling is an important factor in the acetaldehyde compound profile, the accumulation of acetaldehyde and oxygen consumption rate (OCR) in sulfur-containing wines are both higher than those in non-sulfur wines. Copper and iron ions did not show an obvious catalytic impact as expected.

Keywords: Wine, acetaldehyde, oxygenation, polyphenol, sulfur dioxide, metal ions.

1. Introduction

Wine oxidation has been considered a central role in wine chemistry for many years[1]. Several studies have described the influence of appropriate micro-oxygenation (MOx) on organoleptic characteristics of red wines, including modulation of wine color[2] and mouthfeel[3]. In this context, researches are required concerning the complex influences on wine composition and a deeper understanding of wine oxidation[4].

The substance of oxidation was initially described as a reaction between oxygen and a catechol phenolic under the catalysis of iron and copper ions to yield H2O2 and quinones[5], further generating hydroxyl radicals (OH•) to participate in the Fenton reaction, and then oxidize alcohols to aldehydes. These aldehydes formed from corresponding precursor alcohols by peroxidation[6], or can be similarly formed via Strecker degradation of the corresponding precursor amino acid[7].

Furthermore, SO2 not only accelerated the accumulation of acetaldehyde, but also was indeed strongly correlated with the consumption of oxygen. On the one hand, SO2 can recycle quinones to o-diphenols or directly form adducts with quinones, on the other hand, reacts with hydrogen peroxide[8,9]. In addition, the role of SO2 has to be considered that will react quickly with quinones[10] and relatively quickly with hydrogen peroxide[11], so it can be argued that, the ability of a wine to form these compounds can modulate loss of O2.

The phenolic compounds are among the most important components of wines and are directly related to their color, flavor, and oxidative level[12]. As an active substance, acetaldehyde has several chemical reactions with the phenolic substances of the wine during the aging process. One of the most important is the formation of ethyl-bridged compounds with anthocyanin[13], and the production of ethyl-linked oligomers with flavanols, which are further combined with acetaldehyde, anthocyanin, and flavanols to form a pyran ring or other polymer structure[14]. Overall, wines with higher polyphenols had a stronger ability to intercept radicals, and the cumulative amount of acetaldehyde will be limited.

In the present study, the trend of acetaldehyde production in different wines has been measured under conditions carefully controlled, proving an initial evaluation of SO2, polyphenol, and metal ions in wine.

2. Materials and Method

2.1 Wine Samples and Oxidation Process

During the 2018 vintage, a Yantai Rushan Cabernet Franc wine which had just finished the alcoholic fermentation was obtained and conducted as an experimental initial high-phenol wines.

For the experiment, divided low-phenol wines into 4 clean anoxic bottles (550 mL) stuck with PSt3 oxygen sensors, under the protection of nitrogen. The four bottles of wine were treated in the following four ways: one bottle only injected the potassium pyrosulfite (50 mg/L) through the syringe; the second one only added copper (0.30 mg/L) and iron (2.50 mg/L) ions also used syringe; and the third bottle both added the same amount of potassium pyrosulfite and copper and iron ions; the last one added nothing: untreated low-phenol wines (L), low-phenol with copper and iron ions (LI), low-phenol with sulfur dioxide (LS), low-phenol with sulfur dioxide, copper and iron ions (LIS). Simultaneously, the high-phenol wines were treated the same. Eight experimental wines were prepared in duplicate.

The headspace (HS) of the liquid level in the anoxic bottles left around 10 mL of space for oxygen supply. During the aging process, the content of dissolved oxygen (DO) in wines was monitored by a Nomasense oxygen analyzer from Nomacorc S.A. (Thimister-Clermont, Belgium) every 24 hours. One oxygen exposure cycle was considered completed once DO dropped to 0.1 mg/L, or a week later. Then a small number of samples were taken out from the bottles and prepared for chemical analysis. In addition, a bottle of sulfur-containing wine was taken for the determination of free SO_2 and bound SO_2 using the aspiration SO_2 method. The remaining samples were continuously injected with oxygen about 2 mg/L through the syringe, meaning to enter the next oxygen exposure cycle. Such MOx treatment has been carried out four times in total. All wines were stored in an incubator at a constant temperature (25℃) at dark.

2.2 Acetaldehyde Analysis

For analyzing acetaldehyde, a recently improved method[15] was used.

2.3 Statistical Analysis

The effects of SO_2, total phenol, copper, and iron ions on all wines were analyzed by a three-factor ANOVA analysis using SPSS software. All the figures are made in Sigma Plot. All the data were tested in triplicate, and the significant difference analysis was also carried out by SPSS software.

3. Results and Discussion

3.1 Initial Chemicals Determining Or Affecting Oxygen Consumption Rate

Table 1. Initial and average oxygen consumption rates and data about the oxygen and sulfur dioxide consumed.

	OICR (mg/L/day)	OACR (mg/L/day)	TCS (mg/L)	SAR (1+2) (mg/L/day)	SAR3 (mg/L/day)	SAR4 (mg/L/day)
L	1.43	0.59	-	-	-	-
H	1.12	0.61	-	-	-	-
LI	1.51	0.78	-	-	-	-
HI	1.27	0.63	-	-	-	-
LS	1.75	0.72	24.0	2.51	0.69	0.32
HS	1.76	0.71	43.2	2.74	2.86	0.80
LIS	2.52	0.88	44.8	2.74	2.97	0.96
HIS	2.03	0.85	43.2	4.57	1.60	0.00

Wines distributed in independent anoxic bottles containing oxygen sensors were oxidized along four times consecutive oxygen exposure cycles. Both OICR and OACR from different oxygen sensors were recorded and calculated as shown in Table 1.

OICR: O_2 Initial Consumption Rate (the oxygen doses of the first day in the first air-saturation cycle). OACR: O_2 Average Consumption Rate (the average value of the four consecutive air-saturation cycles). TCO: Total Consumed O_2 (total consumed O_2 of the air-saturation cycles). TCS: Total Consumed SO_2 (total consumed SO_2 air-saturation cycles). SAR (1+2): SO_2 average consumption rate in the first and second air-saturation cycles. SAR3 and SAR4:SO_2 average consumption rate in the third and fourth air-saturation cycles, respectively. SO_2:O_2: Molar mass ratio of SO_2 to O_2.

SO_2 exhibited a promoted effect against the consumption of O_2, and the data suggested a small enhancement in O_2 consumption levels as a result of SO_2 treatment, perhaps by recycling quinones to o-diphenols or forming adducts with quinones, or reacting with hydrogen peroxide directly to promote the oxidation process forward, thus increasing oxygen consumption[9]. The OACR of LIS and HIS wines had separately reached 0.88 and 0.85 mg/L/day, respectively, higher than the LI and HI wines. The data was consistent with the experimental results as we expected. To investigate the oxygen consumption rate (OCR) of the role of SO_2 alone, the comparison of the wines that only contained SO_2 (LS and HS) with initial wines (L and H) that treated nothing will provide an estimation of the fraction of SO_2 also promoted the OCR without the catalysis of copper and iron ions. The ability to consume O_2 in this case was weaker than the OCR under metal catalysis.

According to the observed consumption of SO_2 during the oxidation, three patterns were proposed: (1) At the beginning of oxidation, O_2 will quickly take in the first oxygen saturation cycle with little consumption of the normally available SO_2, and the pattern is named "pre-SO_2". The average OICR at this stage was 1.67 mg/L/day more than 2.32 times the mean of OACR (0.72 mg/L/day), indicating that different oxidation mechanisms may exist at the initial and other stages of the experiment[16].(2) When the amount of available SO_2 in wines was gradually increased, the fastly consumed rate in which SO_2 acted both reduced back quinones and removed H_2O_2. Generally speaking, the SO_2 average consumption rate (SAR) of this model was the fastest and named the "normal" pattern (Table 1. SAR 3). (3) Once the Fenton reaction began to form free radicals, the content of H_2O_2 having the ability to react with SO_2 and capable of generating free radicals reduced[17], and the corresponding SAR (Table 1 SAR 4)was lower. This pattern is called "radical forming". Understanding how SO_2 is consumed was essential to detect the existence of different phases or mechanisms in the oxidation process[16].

The practical importance of metals for polyphenol oxidation in wines was unclear. The OACR of the wines treated with metals (0.30 mg/L for copper and 2.50 mg/L for iron) was 0.88 and 0.85 mg/L/day for LIS and HIS, respectively, both were higher than LS and HS wines. This indicated that such fast oxygen uptake was initially attributed to the catalysis of Fe (III) and Cu (II) that was mentioned in the Fenton reaction. However, just adding metals was still surprisingly useful. It had been observed that the OACR of LI and HI wines was higher than initial wines but lower than LIS and HIS wines and indicated that the metal ions will promote oxygen consumption when used alone.

Additionally, the experiment indicated that some polyphenols are negatively related to oxygen uptake. For example, LIS wine has a higher OACR than HIS wine, and LI was also higher than HI. This unexpected result may indicate that some of the complexes formed by phenols with copper and iron ions may somehow disrupt the catalytic cycle[16]. For initial wines (L and H) without any treatment, the effect of total phenol on the OCR of wines was weak in the absence of SO_2 and metal ions.

A marked difference in SO_2 consumption due to the content of total phenol was evident shown in Fig 1. Wines treated with polyphenols seemed to be more able to promote the consumption of SO_2. The bond of SO_2 in HS wine (Fig.1 B) had decreased to zero on the 21st day of the treatment, and the HIS wine (Fig.1 D) only took 14 days. Meanwhile, wines containing low-phenol had not fallen to zero in the bound of SO_2 even after four times oxygen cycles. These results demonstrated that

polyphenols were likely to promote the SO_2 consumption rate by reacting with H_2O_2 and quinone as a result of SO_2 redox. In addition, the essential catalytic effect of copper and iron ions in this process cannot but ignored.

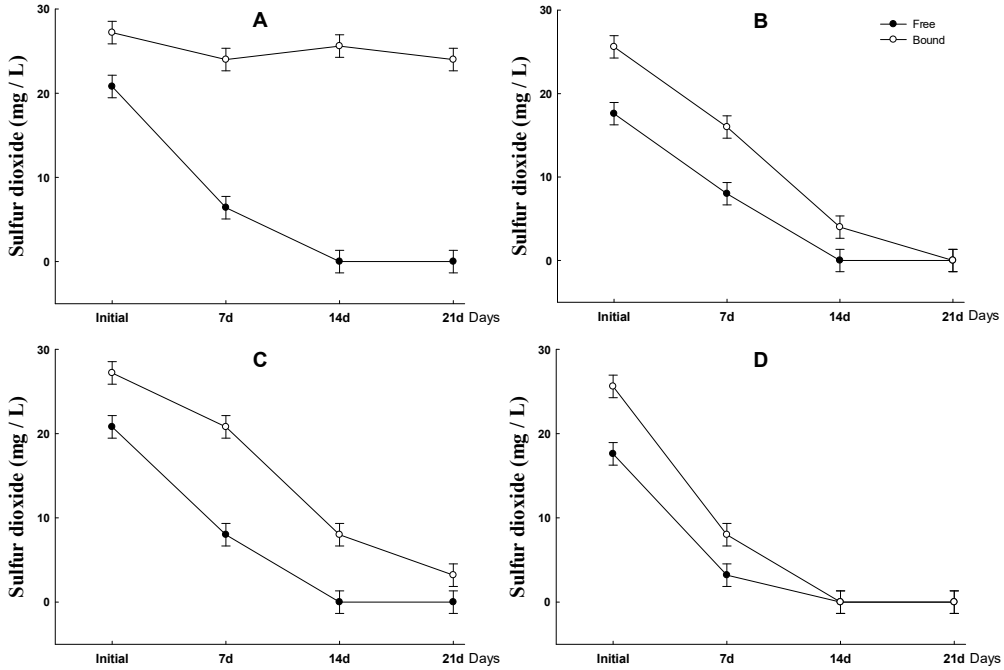

Figure 1. The evolution of bound SO2 and free SO2 in wine of the four consecutive air-saturation cycles. (A): LS wine; (B): HS wine; (C): LIS wine; (D): HIS wine. Values represent the average of three experimental replicates ± standard deviation.

3.2 Change of Acetaldehyde and the Factors Affecting its Formation

Significant differences ($P < 0.001$) between wines with and without SO_2 treatment were found for acetaldehyde. Theoretically, the oxidation product of acetaldehyde as the highest content of alcohol in wines should be accumulated. However, the content of acetaldehyde showed an unexpected significant accumulation only exhibited in sulfur-containing wines (Fig.2 curve LS, HS, LIS, HIS), and the non-sulfur wines showed almost no growth tendency. These results gave a clear view as to the crucial importance of SO_2 in ethanol oxidation.

It is well known that bisulfite reacts swiftly with H_2O_2, an intermediate formed in oxidation, as well as quinones that are formed during phenolic oxidation[18]. As observed in Figure 2, the wine (Fig.2 LIS) with the highest content of aldehyde during the experiment showed an amazing concentration of 71.80 mg/L and even the lowest one (Fig.2 LS) also reached 68.66 mg/L. The increase in acetaldehyde in sulfur-containing wines during the 14^{th} to 21^{st} days had reached the fastest compared to the entire process of accumulation. At this time, the free SO_2 of all wines was exhausted, as well as the bound SO_2 was gradually consumed except in LS wine (Fig.1). It was thought that the observed increase in free acetaldehyde was mostly caused by release from α-hydroxyalkyl sulfonates[19]. Future studies should address SO_2 oxidation mechanisms, with more care in the control of acetaldehyde and phenolic levels, to better understand their roles and interactions in consuming the products of oxidation.

The oxidation-generated acetaldehyde in non-sulfur wines (curves L, H, LI, HI), as shown in Figure 2, was maintained at 2.5 mg/L almost the same as the initial value during the experiment, meaning that the observed doses in wines can be considered to be minimal. So, it was concluded that acetaldehyde as an oxidation product of ethanol was formed but not enough accumulated during the treatment. According to this end, it can be concluded that the levels of aldehyde during wine oxidation were determined following factors: a net balance between the generation and consumption of acetaldehyde content in wines. In addition to being an ethyl bridge, the electrophile character of

aldehydes makes them reversibly bind to thiols[20], react with alcohols to form stable complexes through acetal reaction[21] or form the α-hydroxyalkylsulfonates by a reversible combination of aldehyde and SO_2[22].

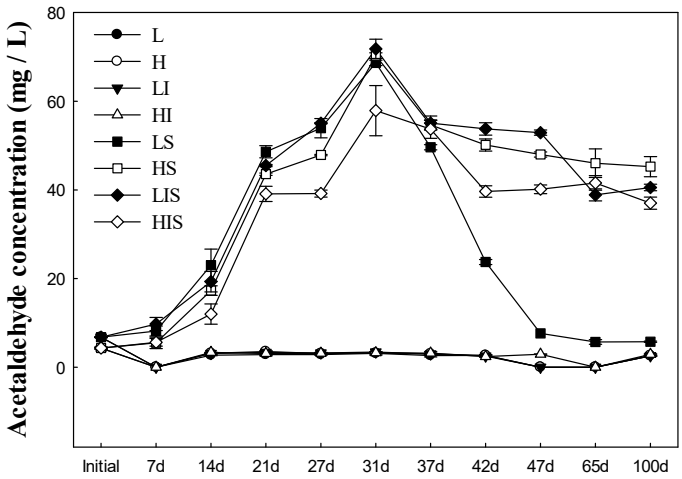

Figure 2. The evolution of acetaldehyde in wines over 100 days. Values represent the average of three experimental replicates ± standard deviation.

The importance of iron (2.5 mg/L) and copper (0.3 mg/L) in the oxidation of ethanol was then examined, and even with the introduction of trace ion ions, the oxidation rate of catechol will be significantly increased[8]. Because metal ions played an indispensable catalytic role in the formation of radical chain cycles of SO_2 autooxidation. In LS and HS wines, the production of acetaldehyde also showed a significant accumulation without copper and iron ions addition. Considering the extremely sensitive role of metals, it was not ruled out that trace metals had been introduced in the initial wine operation.

4. Conclusion

These studies have demonstrated the catalytic role of iron and copper in the oxidation of wines not only of phenols but also of SO_2 in wines. Red wines can consume oxygen at quite different oxygen consumption rates, which are dependent on wine content in metals, acetaldehyde, and SO_2 and are negatively related to phenols. The pattern of accumulation of acetaldehyde in red wine during oxidation is related to SO_2 and sulfate radical ($SO_4^{\bullet-}$) which is produced by the SO_2 auto-oxidation chain reaction. As a free radical scavenging agent, polyphenols cut off the chain reaction to inhibit the oxidation of SO_2, as a result, the acetaldehyde production of high phenol wines is lower than that of low groups.

Acknowledgments

This work was supported by National Natural Science Foundation of China (31701666); and National Natural Science Foundation of China (31901982); and Foundation of Qilu University of Technology (2023RCKY226); and Foundation of Qilu University of Technology (2023RCKY227).

References

[1] Toit, W.J.D., J. Marais, I.S. Pretorius, et al. Oxygen in Must and Wine: A review[J]. South African Journal for Enology & Viticulture, 27(1).

[2] Cano-López, M., F. Pardo-Mínguez, G. Schmauch, et al. Effect of Micro-oxygenation on Color and Anthocyanin-Related Compounds of Wines with Different Phenolic Contents[J]. Journal of Agricultural & Food Chemistry, 2008,56(14):5932-5941.

[3] Gambuti, A., A. Rinaldi, M. Ugliano, et al. Evolution of Phenolic Compounds and Astringency during Aging of Red Wine: Effect of Oxygen Exposure before and after Bottling[J]. Journal of Agricultural & Food Chemistry, 61(8):1618-1627.

[4] Carrascón, V., A. Vallverdú-Queralt, E. Meudec, et al. The kinetics of oxygen and SO 2 consumption by red wines. What do they tell about oxidation mechanisms and about changes in wine composition?[J]. Food Chemistry:S0308814617314255.

[5] Han, G., M.R. Webb, C. Richter, et al. Yeast alter micro-oxygenation of wine: oxygen consumption and aldehyde production[J]. Journal of the Science of Food & Agriculture.

[6] Juan, F.S., J. Cacho, V. Ferreira, et al. Aroma Chemical Composition of Red Wines from Different Price Categories and Its Relationship to Quality[J]. Journal of Agricultural & Food Chemistry, 60(20):5045-5056.

[7] Rizzi ,G. P. Formation of Strecker Aldehydes from Polyphenol-Derived Quinones and α-Amino Acids in a Nonenzymic Model System[J]. 54(5):1893-1897.

[8] Danilewicz, J.C. Interaction of Sulfur Dioxide, Polyphenols, and Oxygen in a Wine-Model System: Central Role of Iron and Copper[J]. American Journal of Enology & Viticulture, 2007,58(1):53-60.

[9] Waterhouse, A.L. ,V.F. Laurie. Oxidation of Wine Phenolics: A Critical Evaluation and Hypotheses[J].

[10] Nikolantonaki, M. ,A.L. Waterhouse. A Method To Quantify Quinone Reaction Rates with Wine Relevant Nucleophiles: A Key to the Understanding of Oxidative Loss of Varietal Thiols[J]. Journal of Agricultural & Food Chemistry, 60(34):8484-8491.

[11] Mcardle, J.V. ,M.R. Hoffmann. Kinetics and mechanism of the oxidation of aquated sulfur dioxide by hydrogen peroxide at low pH[J]. Cheminform, 87(26):5425-5429.

[12] Somers, T.C. The polymeric nature of wine pigments[J]. Phytochemistry, 10(9):2175-2186.

[13] Schmidtke, L.M., A.C. Clark, G.R. Scollary. Micro-Oxygenation of Red Wine: Techniques, Applications, and Outcomes[J]. Crit Rev Food Sci Nutr, 2011,51(2):115-131.

[14] Fei, H., L. Na-Na, M. Lin, et al. Anthocyanins and Their Variation in Red Wines II. Anthocyanin Derived Pigments and Their Color Evolution[J]. Molecules, 17(2):1483-1519.

[15] Han, G., H. Wang, M.R. Webb, et al. A rapid, one step preparation for measuring selected free plus SO2-bound wine carbonyls by HPLC-DAD/MS[J]. Talanta, 134:596-602.

[16] Ferreira, V., V. Carrascón, M. Bueno, et al. Oxygen consumption by red wines. Part I: consumption rates, relationship with chemical composition and role of SO2[J]. J Agric Food Chem:acs.jafc.5b02988.

[17] Carrascón, V., P. Fernandez-Zurbano, M. Bueno, et al. Oxygen consumption by red wines. Part II: Differential effects on color and chemical composition caused by oxygen taken in different sulfur dioxide-related oxidation contexts[J]. Journal of Agricultural & Food Chemistry:acs.jafc.5b02989.

[18] Simonetti, P., P. Pietta, G. Testolin. Polyphenol Content and Total Antioxidant Potential of Selected Italian Wines[J]. Journal of Agricultural & Food Chemistry, 1997,45(4):1152-1155.

[19] Bueno, M.n., V. Carrascón, V. Ferreira. Release and Formation of Oxidation-Related Aldehydes during Wine Oxidation[J]. J Agric Food Chem, 64(3):608-617.

[20] Sonni, F., E.G. Moore, A.C. Clark, et al. Impact of Glutathione on the Formation of Methylmethine- and Carboxymethine-Bridged (+)-Catechin Dimers in a Model Wine System[J]. Journal of Agricultural & Food Chemistry, 59(13):7410-7418.

[21] Perestrelo, R., A.n.S. Barros, J.S. Ca?mara, et al. In-Depth Search Focused on Furans, Lactones, Volatile Phenols, and Acetals As Potential Age Markers of Madeira Wines by Comprehensive Two-Dimensional Gas Chromatography with Time-of-Flight Mass Spectrometry Combined with Solid Phase Microextraction[J]. Journal of Agricultural & Food Chemistry, 59(7):3186-3204.

[22] de Azevedo, L.C., M.M. Reis, L.F. Motta, et al. Evaluation of the Formation and Stability of Hydroxyalkylsulfonic Acids in Wines[J]. Journal of Agricultural & Food Chemistry, 55(21):8670-8680.