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Glyoxal/Glycolaldehyde: A Redox System Involved in Malolactic Fermentation of Wine

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To verify the presence of glycolaldehyde in wine resulting from reduction of glyoxal after malolactic fermentation, sterilized solutions of synthetic cultures were inoculated with a lactic bacterium of the type *Oenococcus oeni*. Fermentation was also carried out on solutions with glyoxal added. The resulting glycolaldehyde concentrations turned out to be associated with the amounts of glyoxal present, and glyoxal was seen to decrease as glycolaldehyde increased. In addition, it was observed that glyoxal principally forms from breakdown of sugars and that reduction to glycolaldehyde is mainly promoted by bacterial activity. Finally, the ability of glycolaldehyde to induce browning of (+)-catechin in a model wine system was verified and turned out to be about 10 times higher than that of ascorbic acid.

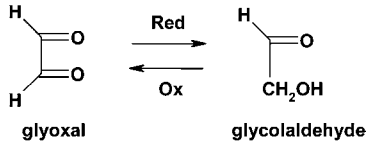
KEYWORDS: Glycolaldehyde; glyoxal; malolactic fermentation of wine; PFBOA (*O*-(2,3,4,5,6-pentafluorobenzyl)-hydroxylamine); (+)-catechin browning; GC/MS

INTRODUCTION

In malolactic fermentation (MLF) of wine, not only is L(−) malic acid transformed into L(+) lactic acid, but other classes of compounds also undergo profound changes. One of the most important changes involves carbonyl compounds, which are important in that they confer aromatic complexity on the wine (*1*). Some of these compounds are involved in redox systems due to cell metabolism, examples being glyoxal, methylglyoxal, and hydroxypropandial, the contents of which in wine increase after MLF. They may be produced by microorganisms such as *Saccharomyces cerevisiae* and *Leuconostoc oenos* or result from attack on grapes by *Botrytis cinerea* (*2*−*4*). These dicarbonyl compounds are more toxic than their reduction products, and reduction is thus advantageous for yeasts and bacteria, since it increases NAD and NADP levels (*5*, *6*).

In addition, glyoxal and glycolaldehyde are involved in food browning as a result of Maillard's reaction (*7*). It has been observed that chemical transformations in carbohydrates and amino acids induced in heated mixtures lead to freeing of glyoxal and its subsequent reduction to glycolaldehyde, which participates as an efficient radical precursor (*8*, *9*). It has also recently been proposed that dicarbonyl compounds deriving from Maillard's reaction are precursors of advanced glycation end products, which play an important role in the pathogenesis of angiopathy in diabetic patients and in the aging processes (*10*, *11*).

In a previous work, we studied changes in carbonyl compounds in two wines, Chardonnay and Cabernet Sauvignon, subjected to MLF by two *Oenococcus oeni* heterofermenting bacterial strains (*12*). Glycolaldehyde was found in all samples at a maximum concentration of 0.5 mg/L, and its contents increased with fermentation, parallel with those of glyoxal. A glyoxal/glycolaldehyde redox system is thus hypothesized to be involved in the metabolism of lactic bacteria:



To verify the presence of glycolaldehyde as a true reduction product of glyoxal, in the present work, we studied the trends of contents of the two compounds during MLF in synthetic solutions, with and without the addition of the bacterium. To examine how glycolaldehyde is bound to glyoxal levels, fermentation was also carried out in solutions with glyoxal added. The ability of glycolaldehyde to induce browning of wine polyphenols was also examined.

MATERIALS AND METHODS

Samples. MLFs were performed by the membrane resistant (MBR) Uvaferm MLD bacterial strain (Esseco spa, Trecate, Italy), with direct inoculation according to the manufacturer's guidelines.

To perform the study on nonsterilized solutions with glyoxal added, lyophilized bacteria (50 mg) were hydrated with 2 mL of distilled water at 25 °C and stirred for 15 min. The bacterial suspension was added to 200 mL of PMB broth prepared according to Velazquez et al. (*13*), containing L(−) malic acid (37.3 mM) and glyoxal (0.069 mM). To perform the other study on sterilized solutions, the synthetic broth containing L(−) malic acid (37.3 mM) was sterilized by filtration with a 0.22 μm filter. All steps were performed in a sterilized environment,

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Table 1. Mean Contents of L(–) Malic and L(+) Lactic Acids and pH during MLF in Unsterilized Synthetic Solution and Unsterilized Synthetic Solution with Glyoxal Added (add)

(g/L)	1 day	1 day glyoxal add	3 days	3 days glyoxal add	7 days	7 days glyoxal add	10 days	10 days glyoxal add
malic acid	3.62	3.64	1.96	2.03	0.24	0.25		
lactic acid	1.21	1.19	2.45	2.37	2.68	2.38	0.14	0.27
pH	2.95	2.94	3.05	3.03	3.40	3.38	3.70	3.70

Table 2. Mean Contents of L(–) Malic and L(+) Lactic Acids and pH in Sterilized Synthetic Solution Not Inoculated with Lactic Bacterium and Sterilized Synthetic Solution in Fermentation (ni = Not Inoculated; i = Inoculated)

(g/L)	1 day ni	1 day i	3 days ni	3 days i	7 days ni	7 days i	10 days ni	10 days i
malic acid	4.80	2.79	4.77		4.40		4.15	
lactic acid		1.48		1.99		2.04		2.01
pH	2.98	3.20	3.08	3.48	3.03	3.40	3.06	3.44

working with sterilized glassware. Both solutions were divided into 16 portions, and all nonsterilized solutions and eight of the sterilized solutions were inoculated with bacteria. Samples were kept at 25 °C. MLF was monitored by high-performance liquid chromatography (HPLC) after 1, 3, 7, and 10 days following the disappearance of malic acid. All experiments were carried out in duplicate.

Reagents and Standards. *O*-(2,3,4,5,6-Pentafluorobenzyl)hydroxylamine hydrochloride (PFBOA), (+)-catechin, and glycolaldehyde were purchased from Sigma-Aldrich (Milan, Italy); glyoxal and *o*-chlorobenzaldehyde were purchased from Fluka (Milan, Italy).

HPLC. For HPLC, the method previously reported in the literature was applied (*14*). The system used was a Varian 9010 chromatograph (Varian Instrument, Walnut Creek, CA) equipped with a 20 μL loop, connected with a Varian 2550 UV−vis detector (wavelength 210 nm). Column: LiChrospher 100 RP-18 (250 mm × 4.6 mm i.d.; 5 μm) with a LiChrocart guard column (4 mm × 4 mm; 5 μm) (E. Merck, Darmstadt, Germany). Solvent: aqueous solution of H_3PO_4 5 × 10^{-3} M; flow rate, 0.6 mL/min. A Varian 4400 Integrator connected with WOW Chemstation software (Thermo Separation Products, Riviera Beach, FL) was used. The organic acids and pH of samples analyzed during MLF are listed in **Tables 1** and **2**.

Sample Preparation for Analysis of Carbonyl Compounds. Carbonyl compounds were determined by means of gas chromatography/mass spectrometry (GC/MS) analysis of their derived PFBOA−oximes, with methods already reported in the literature, modified for our purposes (*2*, *12*, *15*, *16*). The pH of a 25 mL sample was adjusted to 3 by the addition of HCl (4 M), and 200 μL of *o*-chlorobenzaldehyde (0.105 mM) ethanol solution was added as an internal standard (IS). The solution was treated with 12 mg of PFBOA dissolved in 2 mL of water and stirred at room temperature for 1 h. After 1 g of NaCl and 2 mg of sodium dodecyl sulfate were added, *O*-pentafluorobenzyl oximes (*O*-PFB-oximes) were extracted with ethyl ether/hexane 1:1 v/v (3 × 3 mL) by stirring for 5 min. The organic phase was dried over Na_2SO_4 and filtered. The volume was reduced to 0.5 mL under a nitrogen flow before GC/MS analysis.

Conditions for *O*-PFB-Oxime Determination by GC/MS Analysis. Analyses were performed with a HP 5890 gas chromatograph, coupled with a HP 5971A mass spectrometer and equipped with an HP Innowax fused silica capillary column (30 m × 0.25 mm i.d.; df = 0.25 μm). Injector temperature, 250 °C; splitless injection; volume injected, 0.5 μL; carrier gas, He; programmed oven temperature, 5 min at 60 °C, 3 °C/min to 210 °C, 5 min at 210 °C. The mass spectrometer was selected in SIM mode (selected ion monitoring) and signals *m*/*z* 181 (base peak of PFBOA derivatives), 225 (glycolaldehyde), 300 (IS), and 448 (glyoxal) were recorded. Compounds were quantified on the basis of *m*/*z* 181; concentrations of glycolaldehyde and glyoxal in samples were calculated on the calibration curves of the sum of signals of their *syn*, *anti*, *syn* + *anti*, and *anti* + *anti* PFBOA monoximes and dioximes ($y = 23.72\times$, $R^2 = 0.982$ for glycolaldehyde; $y = 45.51\times$, $R^2 = 0.991$ for glyoxal). Retention times of *o*-chlorobenzaldehyde and glycolaldehyde *O*-PFB-monoximes and of glyoxal *O*-PFB-dioximes are listed in **Table 3**.

Table 3. Retention Times on GC Column of *syn* and *anti* *O*-PFB-oximes of Glycolaldehyde and *o*-Chlorobenzaldehyde and *syn* + *anti* and *anti* + *anti* *O*-PFB-dioximes of Glyoxal

compound	PFBOA-oximes RT (min)
glycolaldehyde	28.32; 29.18
o-chlorobenzaldehyde (IS)	31.66; 32.16
glyoxal	32.84; 33.02

Browning Experiments. The model wine base was prepared by dissolving 5 g of tartaric acid and 22 mL of NaOH (1 M) solution in 1 L of 12% (v/v) aqueous ethanol, with a resulting pH of 3.2. (+)-Catechin (100 mg/L; 3.4 × 10^{-3} M) and glycolaldehyde (10 mg/L; 1.7 × 10^{-4} M) were dissolved by stirring in a volume of model wine base. The solutions of wine base model, alone and with glycolaldehyde added, were transferred into completely filled 25 mL flasks and kept in the dark at 45 °C. Experiments were repeated three times, without headspace, to limit the important role of oxygen in catechin oxidation; the solutions were in any case aerated during spectrophotometric measurements.

Absorbance Measurements. Absorbance measurements at 440 nm using a Uvikon 930 UV−vis spectrometer and quartz cells (10 mm) were recorded nine times over a period of 14 days.

RESULTS AND DISCUSSION

Glyoxal-Added Synthetic Solutions. The data on malic and lactic acid concentrations of glyoxal added and nonadded unsterilized solutions at four different times of MLF are reported in **Table 1**, together with pH values, which increase as a consequence of the transformation of malic acid into lactic acid.

Figure 1a, displaying trends of glycolaldehyde concentrations in solutions without (▲) and with (●) glyoxal added, shows that high glyoxal levels induce greater concentrations of glycolaldehyde, confirming that the latter is a product of the probable reduction of glyoxal. This result is more evident in the early phases of fermentation, when the concentration of glycolaldehyde is from three to six times higher than that in nonadded solutions. After 1 day of fermentation, the mean concentration of glycolaldehyde was 359 μg/L, as opposed to a mean of 59 μg/L in solutions without glyoxal added; 3 days later, the difference was considerable, 437 μg/L (+78 μg/L) vs 136 μg/L (+77 μg/L) in nonadded solutions. In solutions with glyoxal added, the levels of both compounds rapidly decreased at the end of fermentation, probably due to the breakdown mechanisms described by Hofmann et al. (*8*). Instead, in tests carried out without glyoxal, the levels of both compounds steadily increased, whereas those of glycolaldehyde definitely fell at the end of fermentation (**Figure 1b**).

Sterilized Synthetic Solutions. Table 2 lists the concentrations of the two acids during tests on sterilized synthetic solutions inoculated with the *O. oeni* bacteria, as compared with unfermented solutions. In the absence of bacterial inoculum, there was neither evident decay of malic acid nor significant variations in pH. This may be reasonably taken as an indication of the sterility of the environment in which the tests were carried out. Comparing the data of **Tables 1** and **2**, after 10 days of MLF, lactic acid decomposed only in nonsterilized solutions, thus revealing that processes by other bacterial species had occurred.

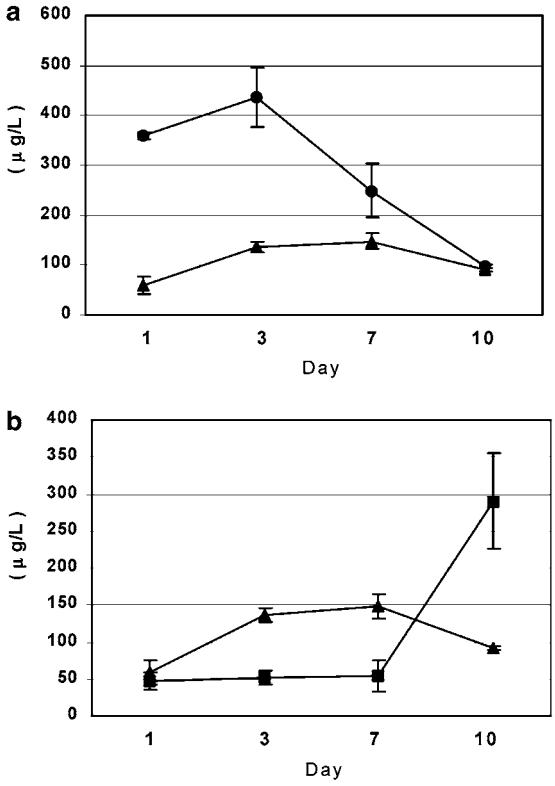

Figure 1. (a) Concentration trends (μg/L) of glycolaldehyde in four samples during MLF in unsterilized synthetic solutions with glyoxal added (●) and in the same solutions without glyoxal added (▲). Bars show differences between data on two repeated fermentations. **(b)** Concentration trends (μg/L) of glyoxal (■) and glycolaldehyde (▲) in four samples during MLF in unsterilized synthetic solutions without glyoxal added. Bars show differences between data on two repeated fermentations.

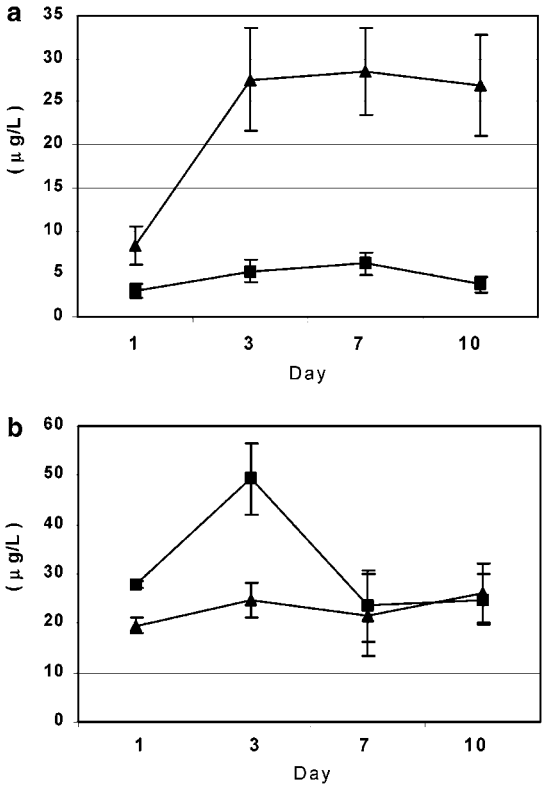

Figure 2. (a) Concentration trends (μg/L) of glycolaldehyde in four samples during MLF in sterilized synthetic solutions (▲) and in same solutions not inoculated with lactic bacterium (■). Bars show differences between data on two repeated fermentations. **(b)** Concentration trends (μg/L) of glyoxal in four samples during MLF in sterilized synthetic solutions (▲) and in the same solutions not inoculated with lactic bacterium (●). Bars show differences between data on two repeated fermentations.

Figure 2a shows trends of glycolaldehyde concentrations in the sterilized solutions, respectively, without bacterial inoculation (■) and during MLF (▲); **Figure 2b** shows the same data for glyoxal. The two compounds were present even in sterilized solutions without lactic bacteria, in which glyoxal contents were also higher and those of glycolaldehyde lower, with respect to values in fermenting solutions. Although this indicates that most of the glyoxal developing in fermenting wines forms from the breakdown of sugars and that only part is due to the activity of lactic bacteria, these results highlight the fact that it is mainly the activity of bacteria, which promotes the formation of glycolaldehyde.

Last, the far lower levels of both compounds found in fermentation tests on sterilized solutions, with respect to the same tests on nonsterilized ones, confirm that in the latter case, processes activated by other bacterial species also occur.

Browning of (+)-Catechin in Glycolaldehyde-Added Wine Base Model. The effect of glycolaldehyde in wine on browning processes of polyphenolss—of particular importance in white wines—was studied by following the increases in absorbance of solutions of (+)-catechin/wine base model (A1) and (+)-catechin/wine base model with glycolaldehyde added (A2). Readings were carried out at 440 nm, the wavelength of peak absorption in the region of visible light in which increased absorbance is due to browning of catechin/wine base model. Experiments were conducted without leaving any headspace above the solutions, to limit oxidation of catechin by oxygen. Differences between the absorbance values of the two solutions are shown in **Figure 3**.

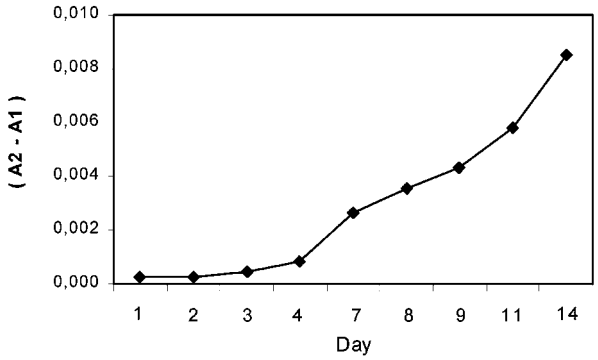

Figure 3. Effect of glycolaldehyde (1.7×10^{-4} M) on browning of a wine base model solution containing (+)-catechin (3.4×10^{-3} M). Note the difference between the absorbance at 440 nm of solution containing glycolaldehyde (A2) and that of solution with catechin alone (A1) in nine samples taken over a period of 14 days.

Glycolaldehyde solutions kept in the same experimental conditions did not show any significant increases of absorbance in the region of visible light, revealing the browning effect of glycolaldehyde on catechin. Bradshaw et al. studied the capacity of ascorbic acid to induce browning of catechin in wine base models (*17*). In that work too, as in our case, experiments were carried out without headspace, and experimental conditions of concentrations and temperatures were similar to ours. Comparing the results of the two experiments, it was found that a glycoaldehyde concentration about 10 times lower than that of ascorbic acid induces a similar degree of browning in the (+)-catechin/wine base model.

In conclusion, our experiments show that the addition of glyoxal 4 mg/L to synthetic solutions, after 1 day of fermentation, induces the formation of glycolaldehyde at a concentration of 300 μg/L, confirming that the presence of both compounds in the wine is directly correlated, probably by a redox mechanism. The formation of glycoaldehyde was also verified as promoted by lactic bacterial activity. Last, the moderate amount of glycolaldehyde that develops during MLF may play an important role in the color stability of white wines.

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