

Kinetics of the Ethanethiol and Diethyl Disulfide Interconversion in Wine-like Solutions

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The interconversion of diethyl disulfide and ethanethiol in the presence of sulfite was studied. The reaction was followed by gas chromatography of headspace samples using a flame photometric detector. Two kinetic models, based on the first-order dependence of sulfite ion and disulfide concentrations, were developed and tested for the reaction at pH 3.5, 6.0, and 7.2 at 20 °C and at pH 6.0 at 30 °C. Although the rate of reaction is slow at wine pH, model predictions indicate that the reduction of diethyl disulfide to ethanethiol over time can be of sensory importance in wine. Suprathreshold amounts of ethanethiol can be formed from the reaction between 30 mg/L of free SO₂ and 50 µg/L of diethyl disulfide at a wine pH of 3.5 in approximately 700 days at 20 °C. In contrast at pH 7.2, the production of detectable amounts of ethanethiol would occur within 1 h at this temperature.

Volatile sulfur compounds have an influence, generally negative, on the perceived quality of wine. Very low concentrations of some sulfur compounds such as dimethyl sulfide have been reported to have a beneficial effect on the aroma of some wines (du Plessis and Loubser, 1974; Simpson, 1979), but the presence of thiols or disulfides in wine is generally considered to be a defect. Terms commonly used to describe the aromas of volatile sulfur compounds in wine range from asparagus, cooked corn, molasses to cooked cabbage, onion, garlic, rubber, and skunk. For example, dimethyl sulfide is described in a neutral white wine as asparagus, cooked corn, and molasses, whereas ethanethiol is higher in intensity of onion and rubber aromas (Goniak and Noble, 1987). Occurrence of trace quantities of the mono- and disulfides and thiols is problematic because of the extremely low aroma thresholds of these compounds. For example, in white wine the aroma thresholds of diethyl disulfide and ethanethiol are 4.3 and 1.1 µg/L, respectively (Goniak and Noble, 1987).

Very little is known about the formation and chemical interaction of the thiols found in wine. However, sulfur-containing amino acids have been implicated as precursors of thiols by several researchers. Considerable amounts of ethanethiol and hydrogen sulfide and traces of methanethiol were produced during yeast fermentation in a synthetic media containing cysteine, methionine, S-methylmethionine, and sulfate (Niefind and Spath, 1973). In wine, ethanethiol was not reported to arise from reduction of cysteine (Rankine, 1963). Formation of methanethiol from methionine in model solutions and beers has been reported to be pH-dependent, with the reaction fastest at pH 3.0 (Wainwright et al., 1972).

Yeast and bacteria can also contribute detectable amounts of volatile sulfur components including ethanethiol, dimethyl sulfide (Niefind and Spath, 1971), methanethiol and dimethyl disulfide (Drews et al., 1969), and dimethyl sulfide, methanethiol, and dimethyl disulfide (De Mora et al., 1986).

Other sources of ethanethiol have been suggested to be the reaction between hydrogen sulfide and acetaldehyde (Tanner, 1969). The formation of ethanethiol by

this reaction in a winelike medium has been shown not to occur (Bobet, 1987).

Thiols are susceptible to oxidation, with the major oxidation product being disulfides. Even mild oxidizing agents such as dehydroascorbate oxidize thiols to disulfides (Borsook et al., 1937). The reaction can occur in the absence of oxygen but is catalyzed by the presence of oxygen, peroxide, and radiation. This oxidation reaction can be reversed by sulfite ions, which react with disulfide groups to give thiols and organic thiosulfates (Milligan and Swan, 1962). Under acid conditions disulfides and sulfide can form thiol and elemental sulfur, apparently by the breakdown of the disulfane intermediate (Roy and Traudinger, 1970).

Of particular interest to winemakers is the reduction of disulfides to thiols by the reaction with sulfite under storage conditions especially after the wine has been bottled. In this study, the interactions of diethyl disulfide and ethanethiol in winelike media were investigated to develop a kinetic model for the prediction of long-term product formation.

APPARATUS

Headspace Analysis. All analyses were performed on a Hewlett-Packard gas chromatograph (Model 85840A) with a capillary inlet system (HP 18835B) operated in the splitless mode throughout the run. The injector plumbing was modified so that the septum purge, as well as the inlet purge, was closed during injection. A 30 m × 0.32 mm (i.d.) glass capillary column coated with 1-µm film thickness of DB-5 (95% dimethylpolysiloxane-5% diphenylpolysiloxane) (J&W Scientific) was used for all analyses. The Tracor flame photometric detector (HP 18805B) was operated in the S mode. GC operating conditions were as follows: injection temperature, 30 °C; detector temperature, 200 °C; column head pressure, 34.2 kg/m²; carrier gas (helium) flow, 1.0 mL/min; makeup gas (nitrogen) flow, 90 mL/min; hydrogen flow, 70 mL/min; oxygen flow, 10 mL/min; air flow, 40 mL/min.

PROCEDURES

Headspace. Headspace samples of 10 mL were injected with a 20-mL Hamilton gas-tight syringe. The injection was made uniformly over a 1-min period. The first 20 cm of the column was submerged in liquid nitrogen during the injection. The cold trap was removed 2 min after the injection was completed and the oven held at 30 °C for 4 min and then programmed to 200 °C at a rate of 1 °C/min.

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Standard Curves. Standard curves were determined for each compound and expressed as the concentration in the liquid phase as a mean of at least three replicates. The standard curves for thiophene (internal marker for leak detection) and diethyl disulfide were fitted to an exponential function (eq 1; $n = 1.5$ and

$$A = aC \exp(n) \quad (1)$$

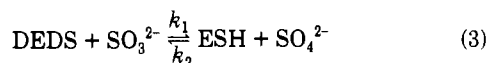
2.0, respectively), where $C = S$ compound concentration in the liquid phase, $A =$ peak area corresponding to the vapor phase, $a =$ proportionality constant from the exponential equation for a given compound, and $n =$ exponential proportionality constant for a given compound. Ethanethiol was quantified with a parabolic equation (eq 2), where A_0 , A_1 , and A_2 are proportionality constants. These are alternative descriptions of the nonlinear detector response at different parts of the saturation curve.

$$A = A_0 + A_1C + A_2C^2 \quad (2)$$

Sample Preparation. Reactions were conducted in 100-mL glass vials closed with 20-mm-diameter mininert valves (Alltech). A 50-mL sample was added to each vial and the container then wrapped with aluminum foil to prevent photooxidation.

All of the reactions were carried out in duplicate. At pH 3.5 and 7.2 (± 0.05) and at 20 °C, the initial reagent concentrations were 10 mg/L (55 μ M/L) of diethyl disulfide (Aldrich) and 100 mg/L (1.5 mM/L) of SO_2 prepared in the appropriate buffer (potassium bitartrate, pH 3.5; KH_2PO_4 , pH 6.0; $\text{KH}_2\text{PO}_4/\text{Na}_2\text{HPO}_4$, pH 7.2). Thiophene (Aldrich) was added at 1 mg/L as an internal marker at the time of sample preparation. The reaction was also carried out at pH 6.0 at 20 and 30 °C as described above, except that a starting concentration of 20 mg/L (110 μ M/L) of diethyl disulfide was used.

Computer Modeling. The proposed forward and reverse reactions (eq 3) were used as the starting point for the reaction of diethyl disulfide (DEDS) with sulfite (SO_3^{2-}).



The differential expression used for the rate of ethanethiol (ESH) formation (eq 4) considers the formation to be first order

$$d[\text{ESH}]/dt = k_1[\text{DEDS}][\text{SO}_3^{2-}] - k_2[\text{ESH}][\text{SO}_4^{2-}] \quad (4)$$

with respect to diethyl disulfide and sulfite ion and the reverse reaction to be first order with respect to thiol and sulfate ion (SO_4^{2-}), on the assumption that the rate-limiting step was the formation of the thiosulfate ester (the Bunte salt) and not its hydrolysis.

A second model of this system allowing for the formation of an intermediate complex was also developed and tested (Bobet, 1987). The rate equation was integrated with respect to time with Euler's integration formula with a time step of 0.1 h (Gear, 1971).

The program for this model calculated the free sulfite concentration according to the ionization constants of sulfurous acid and the pH of the medium. The values of k_1 and k_2 were chosen iteratively, which together with the initial concentrations of disulfide and sulfite, rate of formation of disulfide (eq 4), and the mass balance of the thiol and disulfide forms from eq 3 were used to predict the concentration of disulfide and thiol with respect to time. The values of k_1 and k_2 were altered to minimize the mean root sum of squares deviation between observed and predicted concentrations.

RESULTS AND DISCUSSION

The concentrations of disulfide and thiol in the liquid phase over time for trials conducted at pH 3.5, 6.0, and 7.2 are shown in Figures 1–3. The apparent rate of the reaction increases as the pH is raised, with virtually no thiol formed after 5 days at pH 3.5. At higher pH val-

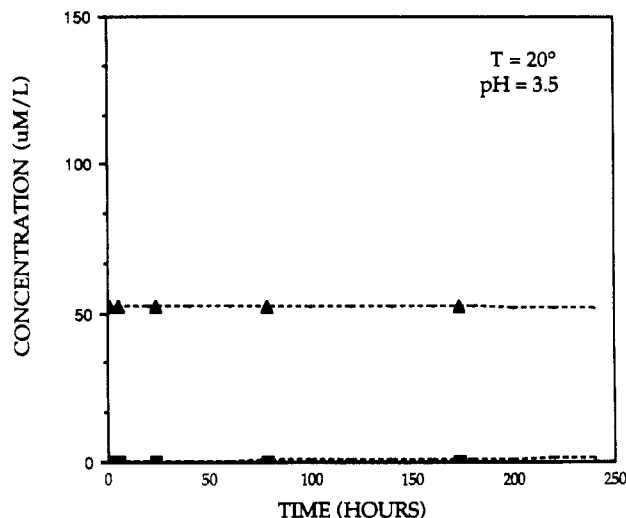


Figure 1. Reaction of diethyl disulfide with sulfite at pH 3.5: modeled (---) and experimentally measured concentrations of diethyl disulfide (\blacktriangle) and ethanethiol (\blacksquare) with time ($n = 2$).

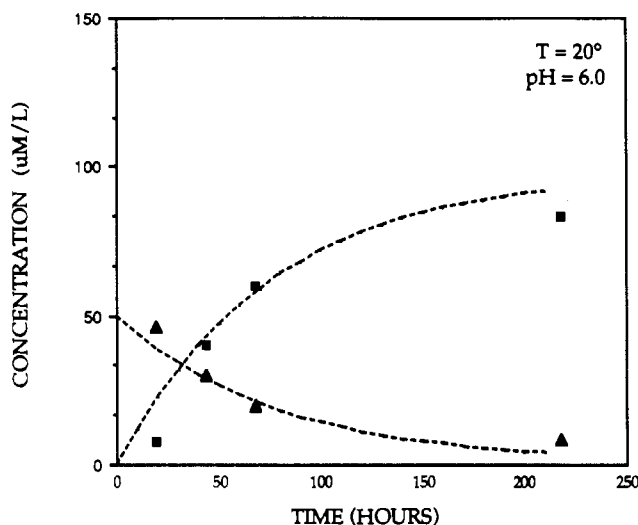


Figure 2. Reaction of diethyl disulfide with sulfite at pH 6.0: modeled (---) and experimentally measured concentrations of diethyl disulfide (\blacktriangle) and ethanethiol (\blacksquare) with time ($n = 2$).

ues, the reaction is much more rapid, nearly reaching completion after 217 h at pH 6.0 or 21 h at pH 7.2.

As expected from the relative nucleophilic strength of sulfite, the equilibrium of eq 3 under neutral or alkaline conditions is strongly in favor of disulfide and sulfite. The values for the equilibrium constants are strongly pH-dependent owing to the ionization of the reacting species (Stricks et al., 1955). The rates strongly influenced by the net charge in the vicinity of the S–S bond are slow at low pH values because of the formation of the weaker nucleophilic bisulfite ion (Oae, 1977).

At the pH values studied, the kinetic model without an intermediate complex fitted the data better than when it was included. The rate constants for all pH values at 20 °C were the same; the values for k_1 and k_2 were, respectively, $1.4 (\pm 0.1) \times 10^{-4}$ and $2.0 (\pm 0.1) \times 10^{-6}$ L/ μ M·h. Therefore, it can be concluded that only nucleophilic sulfite reacts with the disulfide and also that only the change in sulfite ion concentration affects the rate of reaction when pH is changed.

From rate constants, k_1 and k_2 , the activation energies of these reactions were estimated to be 6.23 and 6.36 kJ/mol, respectively.

In Figures 3 and 4, the experimental data and fitted

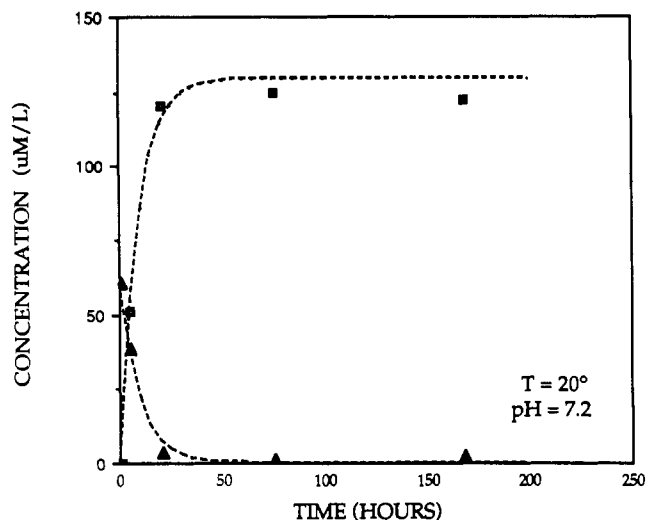


Figure 3. Reaction of diethyl disulfide with sulfite at pH 7.2: modeled (---) and experimentally measured concentrations of diethyl disulfide (▲) and ethanethiol (■) with time ($n = 2$).

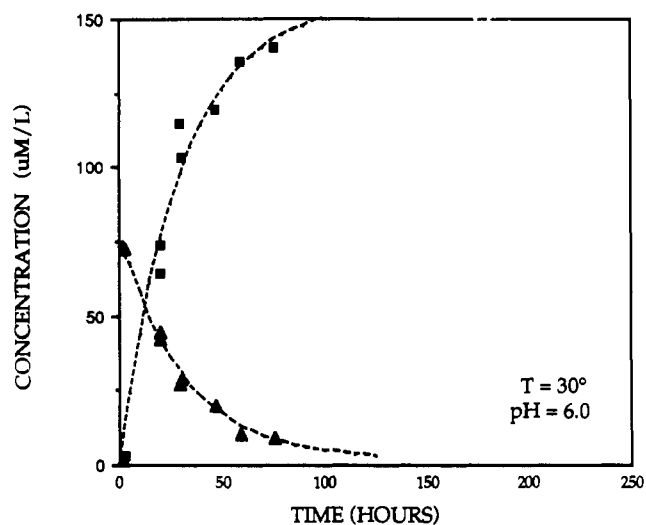


Figure 4. Reaction of diethyl disulfide with sulfite at 30 °C, pH 6.0: modeled (---) and experimentally measured concentrations of diethyl disulfide (▲) and ethanethiol (■) with time ($n = 2$).

model are plotted for the reaction at pH 6.0 at 20 and 30 °C, respectively. At 30 °C the values were $k_1 = 3.5 (\pm 0.1) \times 10^{-4}$ and $k_2 = 5.0 (\pm 0.1) \times 10^{-6}$ L/ μ M·h.

In all the experiments a molar balance showed within experimental error that the moles of thiol formed were twice the moles of disulfide that disappeared, which further indicates that no detectable intermediate complex was formed.

PREDICTION OF THIOL FORMATION

By knowing the value of the rate constants for a given temperature and considering the pH of the solution, it is possible to predict the rate of thiol formation in a wine solution, with a particular content of disulfide and sulfite.

In the integration of eq 4 for typical wine conditions with a free SO_2 concentration of 30 mg/L and a diethyl disulfide concentration of 50 μ g/L, the time necessary to get 2 μ g/L of thiol (above threshold in a neutral white wine) will be 705 days at 20 °C. Clearly, if subthreshold levels of thiol already exist in the wine, the time required to reach 2 μ g/L will be proportionately less.

This result, however, when extrapolated to wine can have some limitations resulting in a positive or negative deviation from the model's prediction. Reasons for this

can be due to competition reactions with sulfite from reducing compounds (such as phenols) or oxidation of the thiol group (presence of O_2 , etc.), which will result in a lower amount of ethanethiol, or side reactions such as reduction of diethyl disulfide due to the presence of other reducing agents (ascorbic acid for example). Finally, the ionic strength and presence of metals can also change the extent of the reaction. This can have an important consequence in wine applications since the disulfide can be the major *S*-ethyl form in the wine at the end of an oxidative process (such as wine aerated during transfer, or barrel aging), and due to its low volatility and higher threshold it would not necessarily be detectable. Under reductive conditions with the addition of sulfites as occurs in bottled wines, the wine could become "stinky" due to the regeneration of ethanethiol.

CONCLUSIONS

The rates of conversion of diethyl disulfide to ethanethiol by the action of sulfite ions in winelike solutions have been determined at 20 and 30 °C. The forward and backward rate constants of the proposed kinetic model were found to be independent of pH. Predictions of long-term reaction rates at wine pH show that suprathreshold levels of the thiol can be produced from the disulfide at room temperature within 700 days.

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Bound Terpenoids in the Juice of the Purple Passion Fruit (*Passiflora edulis* Sims)

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Enzymatic hydrolysis of C₁₈ reversed-phase isolates from the juice of the purple passion fruit led to the identification of a wide range of terpenoids. In addition to the main component linalool and other monoterpenoids, the following C₁₃ norterpenoid aglycons were identified for the first time in passion fruit: 4-hydroxy- β -ionol; 4-oxo- β -ionol; 4-hydroxy-7,8-dihydro- β -ionol; 4-oxo-7,8-dihydro- β -ionol; 3-oxo- α -ionol; isomeric 3-oxoretro- α -ionols; 3-oxo-7,8-dihydro- α -ionol; 3-hydroxy-1,1,6-trimethyl-1,2,3,4-tetrahydronaphthalene; vomifoliol; dehydrovomifoliol.

About 150 volatiles are known as purple passion fruit constituents, with esters and terpenoids being the most abundant classes of flavor components (Whitfield and Last, 1986). Among the terpenoids numerous monoterpenes have been found as degradation products of non-volatile precursor compounds such as hydroxylated linalool derivatives (polyols) as well as monoterpene glycosides (Engel and Tressl, 1983). Similar pathways have been suggested for the generation of the carotenoid-derived C₁₃ norterpenoids, the second group of passion fruit terpenoids. But the natural precursors of these components, including the strongly odoriferous megastigma-4,6,8-trienes and isomeric edulans, key flavor components of the purple variety, remained unknown. The present work reports for the first time the occurrence of glycosidically bound C₁₃ norterpenoids in the juice of the purple passion fruit and discusses their possible role as flavor precursors.

EXPERIMENTAL SECTION

Fruits. Fresh ripe purple passion fruit (*Passiflora edulis* Sims) was available from the local market in Adelaide, South Australia.

Isolation of Glycosidic Components. The pulp of 5 kg of passion fruit (approximately 1.2 L) was centrifuged at 20000g for 1 h. The juice (720 g), possessing a pH value of 3.2, was diluted with distilled H₂O (500 mL), and the pH was adjusted to 5.0 by adding an aqueous solution of sodium hydroxide (2 N). After storage at 4 °C overnight, and further centrifugation (1 h, 20000g), the juice was peristaltically pumped down a glass column (470 × 15 mm (i.d.)) containing C₁₈ reversed-phase adsorbent. The column was washed with water (200 mL), and the retained components were subsequently eluted with methanol (250 mL). After evaporation of the solvent, the residue was

taken up in H₂O (50 mL). This solution was liquid-liquid extracted (16 h) with Freon F 11 to ensure removal of any volatiles prior to enzymatic hydrolysis.

Enzymatic Hydrolysis. In a typical experiment the extracted residue (10 mL) was diluted with 0.2 M phosphate buffer (pH 5) (30 mL). Almond glucosidase (50 mg; Serva) and *n*-octyl β -D-glucopyranoside (100 μ g; internal standard) were added, and the incubation was carried out for 48 h at 37 °C. The liberated aglycons were liquid-liquid extracted (16 h) with dichloromethane and subjected to HRGC-MS analysis.

Capillary Gas Chromatography-Mass Spectrometry (HRGC-MS). HRGC-MS analyses were made with a Finnigan 4021 GC-MS data system. The chromatograph was equipped with a J&W DB-1701 capillary column (30 m × 0.25 mm (i.d.), *df* = 0.25 μ m); helium was used as carrier gas (2.5 mL/min). The temperature program was 1 min isothermal at 60 °C and then from 60 to 250 °C at 4 °C/min.

C₁₃ Norterpenoid Reference Compounds. Authentic samples of compounds 1-5, 9, and 10 were available from previous studies (Winterhalter and Schreier, 1988a,b). Compounds 6A/B and 7 were donated samples. Compound 8 was prepared by the method of Davis et al. (1976) and showed a ¹H NMR spectrum that was in close agreement with the published data. For MS data see Table I.

RESULTS AND DISCUSSION

Isolates of freshly prepared passion fruit juice were obtained from C₁₈ reversed-phase adsorbent by methanol elution (Williams et al., 1982). After removal of the free volatiles by freon extraction, the residue was incubated with a commercially available β -glucosidase (from sweet almond; emulsin) and the liberated aglycons were extracted with dichloromethane.

Monoterpenoids. As shown in Figure 1 the main monoterpene liberated by emulsin was linalool. Approxi-