Author: A. C. Clark, P. D. Prenzler and G. R. Scollary
Author Address: aclark@csu.edu.au, pprenzler@csu.edu.au
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Impact of the condition of storage of tartaric acid solutions on the production and stability of glyoxylic acid

ANDREW C CLARK*, PAUL D PRENZLER and GEOFFREY R SCOLLARY

National Wine and Grape Industry Centre,

Charles Sturt University,

Locked Bag 588

Wagga Wagga 2678, Australia

* Corresponding author

Dr Andrew C Clark

National Wine and Grape Industry Centre

Charles Sturt University

Locked Bag 588

Wagga Wagga, 2678

Australia

Tel: +612-6933 4181

Fax: +612-6933 4068

Email: aclark@csu.edu.au

Abridged Title:

Production of glyoxylic acid from tartaric acid

Abstract

The production and stability of glyoxylic acid was followed during the storage of tartaric acid solutions under various conditions. The solutions were prepared both with and without ethanol. Quantification of glyoxylic acid and other oxidation products, including hydrogen peroxide and formic acid, were performed using ion exclusion chromatography. Glyoxylic acid was only detected in tartaric acid samples that had been stored outdoors and sunlight was identified as the critical component of outdoor storage that allowed its formation. The hydrogen peroxide and glyoxylic acid generated under these conditions were of limited stability due to their reaction with each other to produce formic acid. The concentration of the glyoxylic acid was greatly increased when ethanol was omitted from the sample matrix. Copper(II) enhanced the stability of glyoxylic acid but slowed its production. The reaction pathway responsible for the sunlight-induced production and subsequent stability of glyoxylic acid is discussed.

Abbreviations: **PDA, photodiode array detector; MS, mass spectrometry; ET, 12% aqueous ethanol solution with 0.011 M potassium hydrogen tartrate and 0.008 M tartaric acid; T, ethanol-free ET; C, 0.6 mg/L copper(II); IEC, ion exclusion chromatography; SWV, square wave voltammetery; ICP-OES, inductively coupled plasma – optical emission spectroscopy.**

keywords: glyoxylic acid, tartaric acid, copper, iron, oxidation, hydrogen peroxide, Fenton chemistry, photodegradation, wine

1. Introduction

Tartaric acid is one of the most concentrated naturally occurring organic acids in grapes and wine and it is as a by-product of wine production that tartaric acid is prepared on an industrial scale. Tartaric acid is relatively uncommon in other fruits, however it is found in small amounts in pears and mandarins. Tartaric acid is also used in the production of jams, sweets, jelly, tinned fruit and vegetables, coca powder and frozen dairy produce; mainly as an acidity adjuster but also in the form of an emulsifier. In regard to acid adjustment, tartaric acid is one of the strongest naturally occurring acids in fruit and is the strongest acid in grapes and wine (pK_{a1} = 2.90) (Ough & Amerine, 1988; Azab, Ahmed & Mahmoud, 1997). It is well known in the wine industry that tartaric acid is relatively microbiologically stable compared to the other naturally occurring organic acids, such as malic and citric acids.

Recently the oxidative degradation of tartaric acid has been linked to the production of pigments in model wine media (Es-Safi, Le Guernevé, Fulcrand, Cheynier & Moutounet, 1999). It has been suggested that tartaric acid oxidises to form glyoxylic acid that reacts with (+)-catechin (Fulcrand, Cheynier, Oszmianski & Moutounet, 1997), a common polyphenolic compound present in wine, to afford xanthylium cation pigments (Es-Safi, Le Guernevé, Larbarbe, Fulcrand, Cheynier & Moutounet, 1999). These pigments absorb in the visible region at 440 nm and consequently appear yellow. The production of such pigments in white wine may contribute to the 'oxidative browning' spoilage phenomenon of the wine. The presence of either iron(II) or copper(II) in the model wine media is known to accelerate the production of the xanthylium cation pigments (Oszmianski, Cheynier & Moutounet, 1996; Clark & Scollary, 2002). The role of these metal ions is postulated as enhancing

oxidative degradation of tartaric acid (Fulcrand et al., 1997), while copper(II) is also known to accelerate the reaction between (+)-catechin and glyoxylic acid (Clark, Prenzler & Scollary, 2003).

The oxidation of tartaric acid has been the focus of considerable historical research. The most famous study by Fenton in 1894 involves the presence of metal ions, especially iron(II), and an oxidant, typically hydrogen peroxide but also hypochlorous acid. Other studies have shown oxidation products generated from tartaric acid in solutions containing added iron(III) and/or iron(II) and dissolved oxygen (Benrath, 1917; Wieland & Franke, 1928; Baraud, 1954). Furthermore, the light-induced redox reactions of iron(III) tartrate have been utilised in early photographic procedures (Ware, 1999) and in detectors for organic acids (Pérez-Ruiz, Martínez-Lozano, Tomás & Sanz, 1998).

The oxidative degradation of tartaric acid is thus known to occur in the presence of added iron but the conditions conducive to both its oxidative degradation and production of glyoxylic acid in the absence of added metal ions are not well understood. This is despite the fact that tartaric acid may be exposed to a variety of conditions during its storage and use in the processing of foods. For instance, the wine industry has more recently moved to maintain stock of tartaric acid in aqueous solutions, rather than as a solid, as it is in the aqueous form that tartaric acid is more efficiently added to wine for acid adjustment.

This paper describes experiments that follow the production and stability of glyoxylic acid during the storage of tartaric acid under a variety of conditions. The most extreme storage

condition consisted of the out-door storage of tartaric acid solutions in order to potentially accelerate production of glyoxylic acid. This form of storage is known to generate glyoxylic acid and other oxidation products from tartaric acid solutions (Clark & Scollary, 2003). However, as this past study was only semi-quantitative, a more detailed study was required to allow further insights into the sequence of reactions for glyoxylic acid production and its associated stability in these media. The tartaric acid was prepared in both aqueous and 12 % aqueous ethanol solutions to assess the influence of ethanol on the concentrations of glyoxylic acid and allow insights into wine-like conditions. Similarly, the influence of copper(II) was investigated as copper(II) sulfate is often added to white wines.

2. Materials and methods

2.1. Chemicals

All glassware and plastic-ware were soaked for at least 16 hours in 10 % nitric acid (BDH, AnalaR) and then rinsed with copious amounts of Grade 1 water (ISO 3696). Solutions and dilutions were prepared using Grade 1 water. Chemicals were obtained from Sigma (potassium hydrogen tartrate (> 99 %), L(+)-tartaric acid (> 99.5 %), glyoxylic acid (98 %)), BDH (copper(II) sulfate pentahydrate (AnalaR), acetaldehyde (>99.5%)), Ajax (ethanol (AR), oxalic acid (AR), iron(II) sulfate heptahydrate (AR)), Chem-Supply (30 % hydrogen peroxide (AR)), ABCR GmbH & Co (Tartronic acid (98 %)) and APS (formic acid (AR)).

2.2. Tartaric acid solutions

The tartaric acid solution (T) was prepared by addition of 4.16 g (0.011 M) of potassium hydrogen tartrate and 2.40 g (0.008 M) of tartaric acid to 2 L of water and the solution was then stirred overnight, at room temperature in the dark. The 12 % (v/v) aqueous ethanol tartaric acid solution (ET) was prepared in a similar manner except that the final 2 L solution also contained 240 mL of ethanol. The pH of these solutions was 3.2 ± 0.1. In samples that contained copper(II), it was added in the form of copper(II) sulfate pentahydrate at a concentration of 0.6 mg/L (9.4 μM) copper(II).

Samples (1 L) were placed in 1 L reagent bottles, with a head space of around 100 mL, and stored either out-doors or in-doors. The samples stored out-doors were positioned in an east to west linear arrangement with an order that was randomised daily. The samples stored indoors were all stored in darkness either at room temperature or in a 45 $^{\circ}$C water bath. The main experiment was conducted over 10 days during the Australian summer at the National Wine and Grape Industry Centre in Wagga Wagga, NSW. The additional 32 day and 4 day experiments were conducted at the same location and also in Australian summer conditions. The weather data was obtained from the Australian Bureau of Meteorology. Throughout the experiment samples, unless stated otherwise, were opened daily and stirred for 5 minutes and on analysis days an aliquot of sample was taken for LC-DAD measurement. The 95 % confidence limit for the quantification of hydrogen peroxide, glyoxylic acid and formic acid was set at 20 % of the mean as this was found to be the maximum error observed.

2.3. Ion exclusion chromatography with photodiode array and/or mass spectrometry detection

Ion exclusion chromatography (IEC) with photodiode array (PDA) detection was conducted using a Waters 2690 Separation Module, run by Millennium[32] software, that was connected to a Waters 2996 photodiode array detector. The chromatography was performed on two 300 x 7.8 mm Aminex HPX-87H organic acid analysis cation exchange columns (Bio-Rad Laboratories), connected in series, with a guard column of the same stationary phase. The IEC analyses were carried out with a sample injection of 10 µL and flow rate of 0.5 mL/min with an isocratic elution of 0.085 % phosphoric acid in water. Detection of organic acids was performed at 210 nm, hydrogen peroxide was detected at 250 nm and acetaldehyde at 275 nm.

IEC with both PDA and mass spectrometry (MS) detection was conducted using a SpectraSYSTEM LC, run by Xcalibar software, using a P4000 sample pump that was connected to UV6000LP PDA detector and Finnigan AQA quadrapole MS with an electrospray source. The same column and flow rate was used as described in the IEC-PDA section but the isocratic elution was performed with 0.5 % acetic acid in water. MS was conducted in the negative ion mode, with an ion spray voltage of –4 kV and an orifice voltage of –30 V.

2.4. Inductively coupled plasma – optical emission spectroscopy

The analysis of iron and copper contamination in the ET and T solutions was performed by inductively coupled plasma optical emission spectroscopy (ICP-OES). Prior to analysis, the ET and T solutions were acid digested and concentrated six-fold in the following manner: 150 mL of either ET or T was mixed with 15 mL concentrated nitric acid, then boiled for 1 hour and the final solution made up to 25 mL with water. Both ET and T samples, and a blank, were prepared in quadruplicate for analysis.

ICP-OES studies were performed on a Varian Liberty Series II spectrometer with a glass concentric spray chamber nebuliser (Meinhard) and axial torch. Samples were introduced via tubing (0.76 mm i.d.) with a peristaltic pump at a rate of eight revolutions per minute (rpm). The plasma power was 1.5 kW and readings were taken in triplicate with a dwell time of 1s. Quantifications were performed using calibration graphs.

2.5. Gamma irradiation and Fenton chemistry

Gamma irradiations were performed at the Physics Division of the Australian Nuclear and Science Technology Organisation located in Menai, NSW. Samples (20 mL) and a stirring bead were placed in scintillation vials and positioned on a stirring mantel within the cavity of a cylinder that was then lowered into the source. The cobalt-60 source was enclosed in a cylindrical shield of lead. Nitrous oxide and molecular oxygen were bubbled through the samples during irradiation to increase hydroxyl radical yield and to provide the oxidative conditions conducive to glyoxylic acid formation. The dosage of the gamma irradiator was

calibrated using the Fricke Dosimeter technique (O'Donnell & Sangster, 1970) and found to be 5.9 $\pm$ 0.5 Gy/min. Based on this dose rate, the 7 hour irradiations that were performed were calculated to produce a molar ratio of hydroxyl radical to tartaric acid of 1:12.5. After irradiation the ET and T samples were immediately analysed by IEC with PDA and MS detection.

Fenton Chemistry was performed on a tartaric acid solution (100 mL) containing 0.15 mM iron(II) sulfate heptahydrate and 1.50 mM of hydrogen peroxide. This concentration of hydrogen peroxide was expected to generate a molar ratio of strong oxidant (presumably the hydroxyl radical) to tartaric acid of 1: 13. The hydrogen peroxide was added in three aliquots of 0.50 mM over six hours to provide the final concentration of 1.50 mM. This hydrogen peroxide addition scheme allowed the tartaric acid degradation to proceed in a manner more consistent with the gamma irradiation experiment, that is, with the total hydroxyl radical concentration being generated over 6-7 hours rather than in one instant.

3. Results

3.1. Tartaric acid solutions exposed to various storage conditions

A range of tartaric acid solutions (Table 1) were prepared and exposed to a variety of storage conditions, including Australian outdoor summer conditions (December), for a period of 10

190 days. The chromatograms generated from a 12% aqueous ethanol tartaric acid solution (ET) and an aqueous tartaric acid solution (T) after 10 days of outdoor storage are shown in Figure 1. The peaks in the chromatogram were assigned as hydrogen peroxide (peak 2), oxalic acid (peak 5), tartaric acid (peak 1), glyoxylic acid (peak 7), ethyl tartrate (peak 3) and formic acid (peak 4) respectively. All of these assignments were made with reference to
195 absorption spectra, comparison with the retention time of standards and LC-MS data. The ability to quantify hydrogen peroxide by its peak in the chromatogram was confirmed by comparison studies with square wave voltammetry (Bradshaw, Prenzler & Scollary, 2002).

In contrast to the samples stored outdoors, only ethyl tartrate (peak 3, Figure 1a) was
200 formed for the samples stored in darkness and this was formed only in the ethanol-containing samples stored at 45 °C. No hydrogen peroxide or glyoxylic acid was observed. Ethyl tartrate is formed in the temperature dependent reactions between ethanol and tartaric acid.

205 The peak corresponding to tartaric acid in the 210 chromatograms (Figure 1, peak 1) only decreased in height for those samples stored outdoors. The decrease in tartaric acid was greatest for the T sample (Figure 2) and corresponded to a 9 % loss of the total tartaric acid in the sample. The decrease in tartaric acid for the ET and the copper-containing samples, ETC and TC, corresponded to 4, 1 and 3 %, respectively, of their original amount. The
210 comparison of tartaric acid losses for ET and T suggested an inhibitory influence of ethanol, but when copper(II) was present (*viz* ETC and TC) this inhibitory influence of ethanol was not significant ($P = 0.05$). Alternatively, the inhibitory influence of copper(II) on the loss of

tartaric acid, when comparing ET with ETC or T with TC (Figure 2), was significant regardless of the presence of ethanol.

The time-course for the formation of hydrogen peroxide, glyoxylic acid, and formic acid for the ET and T samples that were stored outdoors are shown in Figures 3a-c. In the case of ET, the hydrogen peroxide concentration (Figure 3a) reached two maxima at days 2 and 9, whereas for T, the hydrogen peroxide concentration remained effectively constant after day 3. The concentration of hydrogen peroxide in these ET and T samples was relatively similar to each other apart from the days in which the ET samples reached their maximum concentrations.

For ET, the glyoxylic acid concentration (Figure 3b) had a similar profile to that for the hydrogen peroxide concentrations (Figure 3a). In contrast, the glyoxylic acid concentration for T increased somewhat linearly with an overall rate of 100 ± 20 μM/day (Figure 3b, inset) and reached a level almost five times higher than the maximum observed in ET. The fluctuations in the glyoxylic acid and hydrogen peroxide concentrations for ET demonstrate the importance of following the tartaric acid degradation products over a period of time. The formic acid concentrations observed in ET and T were similar (Figure 3c) and increased in an approximate linear manner throughout the outdoor storage period at overall rates of 34 ± 7 and 50 ± 10 μM, respectively.

Peak 6 (Figure 1) was prominent in the T sample after the our-door storage period but only present at trace levels in ET. Interestingly, the area of peak 6 increased (data not shown)

with a similar profile to the increase in glyoxylic acid concentration (Figure 3b). This peak was assigned as tartronic acid (Scheme 2) based on the identical retention time observed for a tartronic acid standard and also based on the identical mass spectrum obtained for peak 6 and the tartronic acid standard. However, the lack of peak symmetry for peak 6 in the 210 nm chromatogram made the assignment tentative and suggested that peak 6 may be the result of at least one other co-eluting compound. The lack of peak symmetry for peak 6 was more evident prior to day 10 (Figure 1b).

Assessment of the change in the concentration of oxalic acid could only be tentative due to the poor resolution of this peak with that of hydrogen peroxide at 210 nm (Figure 1b). A general increase in the concentration of oxalic acid in T could be observed during the exposure period and negligible levels were found in any other samples.

The only other major peak that had a significant variation in peak area throughout the exposure period was a peak in the 275 nm chromatogram at a retention time of 42 minutes. This peak had an absorbance spectrum and retention time consistent with acetaldehyde and had a general increase in peak area over the ten-day period (data not shown). Acetaldehyde was not quantified due to its volatility and the expected losses incurred during sampling.

3.2. Tartaric acid solutions with added copper(II) exposed to various storage conditions

Copper(II) is generally present in beverages containing tartaric acid due to contamination during production or processing, but in wines it may be added as copper(II) sulfate to remove sulfidic off-odours in the wine. In the latter case, residual copper(II) will remain in the wine, potentially complexing with other wine compounds. For this reason, and the lack of literature on the degradation of tartaric acid by copper(II), the influence of copper(II) on the generation of glyoxylic acid and other degradation products from tartaric acid was investigated.

The ETC and TC samples were based on the samples, ET and T, but with 0.6 mg/L of added copper(II). This level of copper(II) is within the range of that found in wine (Green, Clark & Scollary, 1997; Wiese & Schwedt, 1997). The ETC and TC samples were exposed to identical storage conditions as the ET and T samples (Table 1).

During this period no hydrogen peroxide was detected, by either IEC or SWV, in any sample containing copper(II) and only a trace amount of formic acid was found in ETC on the last day of the experiment. Alternatively, glyoxylic acid was detected in the ETC and TC samples that were stored outdoors (Figure 4) but at much lower levels than the maximum concentrations observed for the equivalent samples without copper(II) (Figure 3b). The ETC and TC samples had overall rates of glyoxylic acid formation that were not significantly different, 5 ± 1 and 4 ± 1 μM/day respectively, but much lower than that observed for T (100 ± 20 μM/day).

To establish whether the glyoxylic acid concentration was fluctuating or increasing steadily in the samples containing copper(II) (Figure 4), the experiment was repeated over an extended period. The results confirmed both the stability of glyoxylic acid in ETC, with a rate of formation of 4 ± 1 μM/day, and the instability in ET (Figure 5).

As was observed for the T and ET samples, storage of ETC and TC samples in darkness at either room temperature or 45°C did not result in the detection of hydrogen peroxide, glyoxylic acid or formic acid (Table 1). Oxalic acid (peak 5) and peak 6 (Figure 1) were detected at trace levels in the TC sample exposed to outdoor conditions while only a trace amount of oxalic acid was detected in the ETC samples. The peak in the 275 nm chromatogram (retention time: 42 minutes) assigned as acetaldehyde was not detected at measurable levels in any ETC or TC samples.

3.3. The influence of limited aeration on tartaric acid solutions stored outdoors

To assess the influence of molecular oxygen on the production of glyoxylic acid and other tartaric acid degradation products, additional ETC samples were prepared: one set opened daily to the atmosphere (constant aeration) and the other sealed and only opened at the end of the experiment (limited aeration). ETC was chosen for this experiment as this sample was known to have a steady increase in the concentration of glyoxylic acid during its outdoor storage (Figure 4). None of the samples contained any headspace and both were initially degassed with helium. The duration of this experiment was extended to 34 days in order to exaggerate any influence of molecular oxygen. After 34 days the ETC samples with constant and limited aeration were found to have significantly different ($P = 0.05$) levels of glyoxylic acid, 150 ± 30 μM and 90 ± 20 μM of glyoxylic acid, respectively.

3.4. The influence of other factors on tartaric acid solutions stored outdoors

Further experiments were conducted to assess if the production of hydrogen peroxide, glyoxylic acid or formic acid was of microbiological nature or due to an interaction of the sample with the reaction vessel interface. Tartaric acid solutions without ethanol (T) were selected for these experiments as these solutions had the least protection from microbial activity and would also produce the highest glyoxylic acid concentration (Figure 3b). Therefore, T samples were prepared in the following ways: in normal conditions, under biologically sterile conditions and in a reaction vessel containing increased surface area, via the addition of glass shards. The results in Table 2 show the hydrogen peroxide, glyoxylic acid and formic acid concentrations after four days of outdoor exposure. There were no significant differences (P = 0.05) in the levels of glyoxylic acid between any of the samples, and similarly for hydrogen peroxide and formic acid.

The ET and T samples were acid-digested and analysed by ICP-AES to determine the level of trace copper and iron contamination in these samples. Copper was not detected (LOD = 2 µg/L or 0.03 µM) but trace levels of iron were detected at around 10 $\pm$ 5 µg/L (or 0.18 µM) in both ET and T samples. The confidence limits of the iron quantification are relatively large due to the low levels of iron being determined but the result does indicate the order of magnitude of the iron contamination.

No correlations were found in relating changes in the concentration of either tartaric acid or its degradation products with weather parameters measured during the outdoor storage experiments.

3.5. Oxidation of tartaric acids by gamma irradiation and Fenton chemistry

The oxidation of T was carried out by both gamma irradiation and via Fenton chemistry, that is, with addition of hydrogen peroxide and iron(II), to assess if similar products were formed as in the samples stored outdoors. Both of these types of oxidation procedures are known to proceed via the production of the highly oxidising hydroxyl radical species (O'Donnell & Sangster, 1970; Wardman & Candeias, 1996), although Fenton chemistry may also proceed through other oxidising intermediates (Goldstein, Meyerstein & Czapski, 1993; Masarwa, Rachmilovich-Calis, Meyerstein & Meyerstein, 2005). The chromatograms from the T samples after treatment (Figures 1b, 6a and 6b) demonstrate that despite the different oxidation methods similar products were generated albeit at different concentrations.

4. Discussion

4.1. Sunlight critical in the oxidative degradation of tartaric acid

The production of glyoxylic acid was influenced by a number of parameters. First, glyoxylic acid production clearly requires tartaric acid as outlined in a previous study (Clark &

Scollary, 2003) and demonstrated in Table 1. Second, from the results (Table 1, Figure 3a) it is clear that the outdoor storage of samples is required for the production of glyoxylic acid from tartaric acid. The inability of heat to generate glyoxylic acid from tartaric acid shows that sunlight alone is the critical component of the outdoor storage conditions. Also, the increased production of glyoxylic acid occurred when samples were aerated. Consequently, these results show that the outdoor storage of tartaric acid results in a sunlight-induced oxidative degradation of tartaric acid and consequent glyoxylic acid formation.

To better understand the potentially complex chemistry of tartaric acid photodegradation, we consider various aspects of the process in the remaining sections: evidence for photocatalytic oxidation (4.2); hydrogen peroxide formation (4.3); and comparisons with Fenton chemistry (4.4). Since glyoxylic acid has been identified as a key breakdown product of tartaric acid we then consider the stability of glyoxylic acid in the presence of ethanol (4.5) and copper (4.6).

4.2. Degradation of tartaric acid by photochemistry

Further insights into the mode of glyoxylic acid formation were gained by following the production of another oxidation product. Acetaldehyde, an oxidation product of ethanol, was not detected in the 12 % aqueous ethanol sample without tartaric acid but was found in the 12 % aqueous ethanol sample with tartaric acid when both were stored outdoors (Table 2). It is unlikely that tartaric acid alone was promoting oxidation of ethanol. More likely, a contaminant in the source of tartaric acid, such as the detected 0.2 μM level of iron, was required for the initiation of the oxidation reactions.

Since iron salts are present in tartaric acid solutions as a contaminant, it is likely that the photo-oxidation of tartaric acid is promoted by iron ions (Balzani & Carassiti, 1970; Abrahamson, Rezvani & Brushmiller, 1994) and in fact this reaction has been utilised in past photography methods (Ware, 1999) and for the spectrophotometric detection of tartaric acid (Perez-Ruiz et al., 1998). Also, the presence of trace amounts of metal ions in buffers has been shown to be critical in initiating oxidation reactions (Buettner & Jurkiewicz, 1996) and more specifically photochemical oxidation reactions (Reed et al., 2003). Other modes of initiation for the oxidation reactions, either microbially or via interactions between the sample and glass bottle interface, were not found to be relevant (Table 2).

Several studies have investigated the products formed in solutions of tartaric acid and added iron(II) which were exposed to sunlight (Fenton & Jackson, 1899; Benrath, 1917; Baraud, 1954). However in these studies the concentrations of added iron(II), being greater than 50 mg/L, were much higher than the trace levels identified in T and ET (<0.2 μM). These higher levels of iron, and the fact that iron was added in the form of iron(II), would be expected to have an impact on the products generated from tartaric acid and their rate of production. Thus the results may be different from those found in this study and as far as we are aware, no work has been performed on the degradation products generated from the photochemistry of tartaric acid in the presence of trace amounts of iron.

Although they did not study tartaric acid specifically, Balzani and Carassiti (1970) proposed a general mechanism for the photochemical degradation of α-hydroxy acids in the presence of iron(III) *via* oxidative decarboxylation. The mechanism has been confirmed for iron(III) citrate and kinetic evidence has been presented for a photoactive iron(III) citrate dimer being responsible for the initial oxidation (Abrahamson et al., 1994). The oxidative decarboxylation was suggested to occur via a radical intermediate and result in the production of iron(II). A

simplified version of the proposed mechanism is presented in Scheme 1. In the case of iron(III) citrate study by Abrahamson et al. (1994), the wavelength of light used was 366nm.

Based on the generalised Scheme 1, the product aldehyde expected for photochemical degradation of tartaric acid, in the presence of iron(III), would be 2-hydroxy-3-oxo-propanoic acid, a tautomer of the α-keto acid, hydroxypyruvic acid (Scheme 2). Once formed in solution, these species would expected to be transitory due to their inherent instability coupled with the relatively harsh outdoor storage conditions of their solutions. Their interaction with hydrogen peroxide, present in the solution matrix, would induce oxidative degradation at their respective aldehyde and ketone groups (Siegel & Lanphear, 1979; Perera, Parkes, Herz, Haycock, Blake & Grootveld, 1997; Yadav & Gupta, 2000). Furthermore, α-keto acids are prone to both photolytic and thermal degradation, at relatively mild temperatures (Black, Blackburn & Johnston, 1965; Cooper, Ginos & Meister, 1983). It is most likely that as a consequence of this instability, and also the instability of subsequent intermediate species, that the majority of the detected products, namely oxalic acid, formic acid and tartronic acid, do not contain the reactive aldehyde or ketone functional groups. Glyoxylic acid, containing an aldehyde group, was detected as an accumulating product but, as will be discussed in Section *4.5*, glyoxylic acid is not stable in certain of the experimental conditions.

4.3. The initial production of hydrogen peroxide

The production of hydrogen peroxide is known to occur in aerated solutions containing both transition metals, in their higher oxidation states, and reducing agents (Udenfriend, Clark, Axelrod & Brodie, 1954). In the T and ET solutions, the combination of sunlight, tartrate and

the presence of trace iron contamination, and the subsequent photochemistry, provides the conversion of iron(III) to iron(II) (Scheme 3, reaction 1). The presence of oxygen in the solutions then allows the formation of hydrogen peroxide (Scheme 3, reactions 2-4). Although the hydrogen peroxide formed may be removed by iron(II) (Scheme 3, reaction 5), the low concentrations of iron(II), and participation of iron(II) in competing reactions, may explain the accumulation of hydrogen peroxide.

4.4. Degradation of tartaric acid by Fenton chemistry

Reaction 5 (Scheme 3), termed Fenton chemistry, generates a powerful oxidant. Although hydroxyl radicals are the commonly proposed product of iron(II) and hydrogen peroxide, the formation of this radical is medium dependent, and the product may instead be a metal/hydrogen peroxide/ligand complex (Goldstein et al., 1993; Masarwa et al., 2005). This complex would be equally as strong an oxidant as the hydroxyl radical. In either case, the oxidant formed from the Fenton reagent (reaction 5, Scheme 3) would readily oxidise tartaric acid and provide another mode of tartaric acid degradation. The T and ET samples stored outdoors were known to contain both ingredients required for Fenton chemistry, trace iron and hydrogen peroxide.

The occurrence of Fenton chemistry in the T sample is supported by the observation of similar products in those samples stored outdoors (Figure 1b) as those with added Fenton reagents, namely hydrogen peroxide and iron(II) (Figure 6a). Furthermore, a technique known to generate hydroxyl radicals, gamma irradiation, also provided identical tartaric acid degradation products (Figure 6b) to sunlight and Fenton chemistry.

The reaction of iron(II) and hydrogen peroxide in the presence of tartaric acid is known to produce dihydroxymaleic acid (Fenton, 1894), the enol form of hydroxyoxaloacetic acid. The proposed reaction pathway for this oxidation is the α-hydrogen abstraction from tartaric acid (step 1, Scheme 4) to produce a radical that could then be oxidised by either molecular oxygen or iron(III) (step 2, Scheme 4) (Koppenol, 1993; Wardman & Candeias, 1996). The low concentration of iron(III) in the medium suggests that molecular oxygen may be the more likely oxidant in step 2 of Scheme 4, especially as oxygen is known to rapidly react with such radicals and result in a ketone (Gozzo, 2001).

Once dihydroxymaleic acid is formed a variety of oxidative and/or decarboxylative degradation steps could explain the products observed in T and ET (Scheme 2). Dihydroxymaleic acid is known to readily undergo decarboxylation and oxidation reactions (Baraud, 1954).

The occurrence of Fenton chemistry also explains the presence of acetaldehyde in the ET samples as iron(II) and hydrogen peroxide, in combination, can oxidise ethanol to acetaldehyde. Therefore, as the concentration of ethanol is 100-fold that of tartaric acid in the ET samples, and as the oxidant resulting from reaction 5 (Scheme 3) generally reacts in a diffusion-controlled manner (Scholes & Wilson, 1967; Buxton, Greenstock, Helman & Ross, 1988), ethanol will be oxidised in preference to tartaric acid. This is consistent with the lower amounts of tartaric acid degraded in the presence of ethanol (Figure 2). Also, ethanol is known to scavenge hydroxyl radicals (O'Donnell & Sangster, 1970) when at high concentrations and in the presence of dissolved oxygen. In contrast to Fenton chemistry

(Scheme 3, reaction 5), the photodegradation reaction (Scheme 2) would be selective for tartaric acid over ethanol, as ethanol is not able to form a photoactive complex with iron(III).

The regeneration of iron(III) from iron(II) (during Fenton chemistry reaction 5, Scheme 3; and also reaction 2, Scheme 3) would mean that further photodegradation of tartaric acid could proceed. Therefore, it is likely that the oxidation of tartaric acid proceeds via a combination of photodegradation and Fenton chemistry, where trace amounts of iron can act as a catalyst. In the T solutions, both oxidative reactions would lead to tartaric acid degradation, while in ET it would be mainly the photodegradation reaction leading to tartaric acid degradation. The experimental results clearly show a decrease in both tartaric acid degradation (Figure 2) and glyoxylic acid production (Figure 3b) in the presence of ethanol. Interestingly, ethanol had little impact on the concentrations of formic acid suggesting that formic acid was mainly a consequence of the photodegradation initiated reactions (Scheme 2) rather than Fenton chemistry.

Hydrogen peroxide could be ultimately generated as a consequence of the reaction of the Fenton reagent (Scheme 3, reaction 5) with either ethanol (Scheme 5) or tartaric acid (Scheme 4). Both these reactions may generate the hydroperoxyl radical that could then disproportionate into hydrogen peroxide (reaction 4, Scheme 3). Therefore, it is not unexpected that the concentration of hydrogen peroxide in the outdoor stored ET and T samples (Figure 3a) are similar.

The production of glyoxylic acid from tartaric acid in wines not exposed to light is a subject of further study. Wines contain phenolic compounds, which are able to generate hydrogen peroxide during oxidation, and also concentrations of iron that would be over 100-fold that

found in the T and ET solutions used in this study. These conditions, favouring Fenton chemistry reactions, have been already shown to result in the production of glyoxylic acid-derived pigments in model wine solutions that contain tartaric acid (Es-Safi, Le Guernevé, Fulcrand, Cheynier & Moutounet, 1999). However, the direct measurement of the glyoxylic acid generated during phenolic compound oxidation in the presence of iron and tartaric acid has not been conducted.

4.5. Glyoxylic acid stability and the influence of ethanol

Given the possible reaction pathways for the formation of glyoxylic acid, some insights can also be gained on its stability in the T and ET media. It is evident that the stability of glyoxylic acid (Figure 3b) in ET closely parallels that of hydrogen peroxide (Figure 3a) suggesting that these species were reacting, in a manner already described by Yadav and Gupta (Yadav & Gupta, 2000), to generate formic acid (Figure 3c):

$$H_2O_2 + OHCCO_2H \longrightarrow HCO_2H + H_2O + CO_2$$

The presence of ethanol had little impact on the hydrogen peroxide (Figure 3a) and formic acid (Figure 3c) concentrations, apart from the increased variability in hydrogen peroxide concentrations, but a negative impact on the glyoxylic acid concentration (Figure 3b). It appeared that the rate of production of glyoxylic acid was lowered in the presence of ethanol to such an extent that it could be totally removed by reaction with hydrogen peroxide. As already mentioned, this lowered production is most likely due to ethanol scavenging the oxidant formed from reaction 5 (Scheme 3) before its reaction with tartaric acid. The overall

rate of combined glyoxylic acid and formic acid production was four times higher for T compared to ET (150 ± 30 and 34 ± 6 μM/day respectively).

4.6. The influence of copper(II) on glyoxylic acid production

The presence of copper(II) in the T or ET samples that were stored outdoors caused glyoxylic acid to accumulate at a slower rate than if it were absent (Figure 4). This was despite trace amounts of iron still being present in the samples with added copper(II).

The means by which copper(II) can decrease the glyoxylic acid concentrations is not certain, but copper(II) may inhibit the initiation of the oxidation reactions. This mode of copper(II) interference is supported by the observation of less oxidation products, including acetaldehyde, in samples prepared with copper(II) and a decrease in the amount of tartaric acid degraded in the experiment (Figure 2). Copper(II) may be disrupting the iron(III) tartrate interaction required for photochemical reactions due to the higher concentrations of copper(II) (0.6 mg/L; 10 μM) compared to trace iron (< 0.2 μM). Copper(II) has been observed to decrease the concentrations of iron(II) formed from the photochemical reaction of iron(III) oxalate (Pérez-Ruiz, Martínez-Lozano, Tomás & Val, 1995). Copper(II), in contrast to iron(III), has generally displayed little photoactivity with organic acids, an example being the negligible photoactivity of copper(II) citrate (Reed et al., 2003). Wieland and Franke (Wieland & Franke, 1928) showed increased oxygen consumption in iron(II) tartrate solutions that had added copper(II), but the oxidation reactions in this system were more likely Fenton chemistry driven (Scheme 3, reaction 5) rather than photochemically driven.

The influence of copper(II) on the stability of glyoxylic acid appeared to be medium dependent. That is, in the ET samples, the presence of copper(II) allowed glyoxylic acid to accumulate rather than fluctuate in concentration (open circles, Figure 3b and 4) and prevented the formation of formic acid (Table 1). This increased stability of glyoxylic acid was most likely a consequence of copper(II) preventing the accumulation of hydrogen peroxide and thereby preventing the oxidation of glyoxylic acid to formic acid.

5. Conclusion

The stability of the glyoxylic acid generated on the storage of tartaric acid solutions in outdoor conditions has been linked to the levels of hydrogen peroxide in these samples. This hydrogen peroxide was also generated as a consequence of the oxidative degradation of tartaric acid and perhaps also the related degradation of ethanol in the relevant samples. Sunlight was found to be essential for the production of glyoxylic acid, as was the presence of molecular oxygen. Ethanol limited the production of glyoxylic acid, while copper(II) removed the instability caused by hydrogen peroxide but slowed the overall production of glyoxylic acid. Evidence is presented for iron contamination in the tartaric acid being responsible for the photo-initiation of the oxidative reactions and for both photodegradation and Fenton chemistry being responsible for the subsequent production of glyoxylic acid in the absence of ethanol. In the presence of ethanol, photodegradation is the main mode of tartaric acid degradation.

This work shows that solutions of tartaric acid, whether aqueous or 12% aqueous ethanol, are not stable when stored in outdoor conditions. This is also likely to be the case when

stored indoors and under light for extended periods. Although iron contamination was most likely required for the initiation of the oxidation reactions, the level of iron present (< 0.2 μM) would be much lower than expected in all commercial water supplies. Furthermore, higher levels of iron contamination would require lower levels of light exposure to initiate the degradation of tartaric acid. This work is of particular significance to solutions of tartaric acid that may be stored prior to their addition to food or beverages, such as wine.

Acknowledgements

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Legends to Figures and Schemes

Scheme 1: Photo degradation of iron(III) α-hydroxy organic acids after Balzani and Carassiti (1970), and Abrahamson et al. (1994).

Scheme 2: The proposed degradation of the tartaric acid via photochemical and Fenton chemistry mechanisms.

Scheme 3: Udenfriend (1954) reactions, where tartaric acid/light act as a reducing agent for iron(III).

Scheme 4: Production of dihydroxymaleic acid from the hydroxyl radical as generated by Fenton chemistry (Koppenol, 1993; Wardman & Candeias, 1996).

Scheme 5: Oxidation of ethanol by the hydroxyl radical (Asmus, Mockel & Henglein, 1973).

Figure 1: The chromatograms generated at 210 nm from the IEC analysis of ET (a) and T (b) after ten days of outdoor exposure. The peaks were assigned as: **1** tartaric acid; **2** hydrogen peroxide; **3** mono-ethyl tartrate; **4** formic acid; **5** oxalic acid; **6** unidentified and **7** glyoxylic acid.

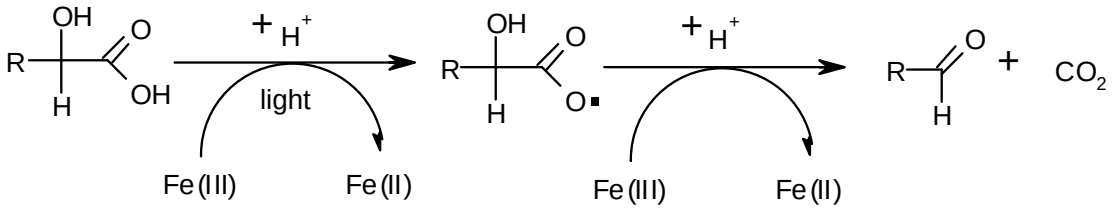
Figure 2: The percentage loss of tartaric acid during the 10 day outdoor storage of samples.

Figure 3: The concentration profiles for hydrogen peroxide (a), glyoxylic acid (b) and formic acid (c) during the ten day outdoor exposure of ET (○) and T (●).

Figure 4: The concentration profile for glyoxylic acid during the ten-day outdoor exposure of ETC (○) and TC (●).

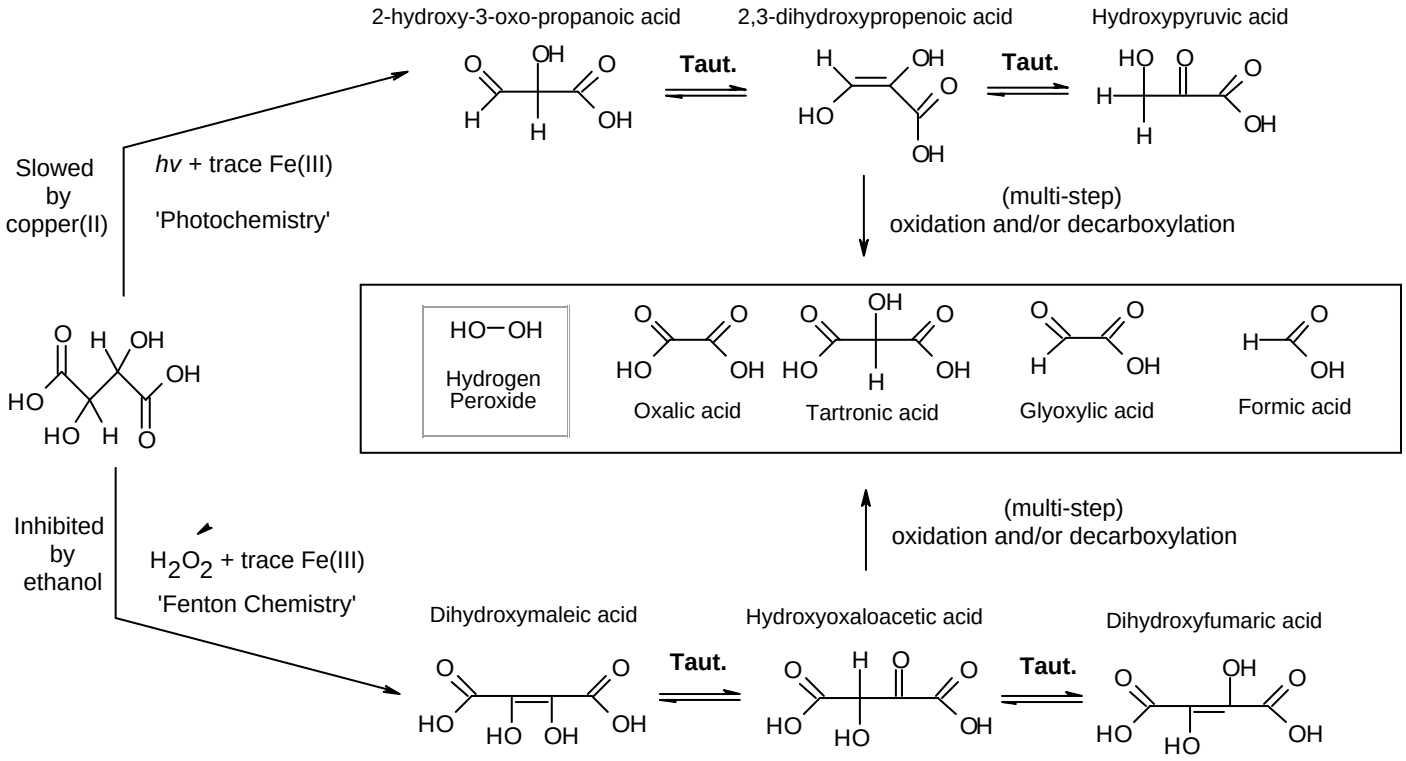
Figure 5: The concentration profile for glyoxylic acid during the 35 day outdoor storage period of ET (○) and ETC (●). The level of copper(II) in the ETC sample is 0.60 mg/L.

Figure 6: The chromatograms generated at 210 nm from the IEC-PDA analysis of T after either addition of iron(II)/hydrogen peroxide (a) or 7 hours of gamma irradiation (b). The peaks were assigned as: **1** tartaric acid; **2** hydrogen peroxide; **4** formic acid; **5** oxalic acid; **6** unidentified and **7** glyoxylic acid.

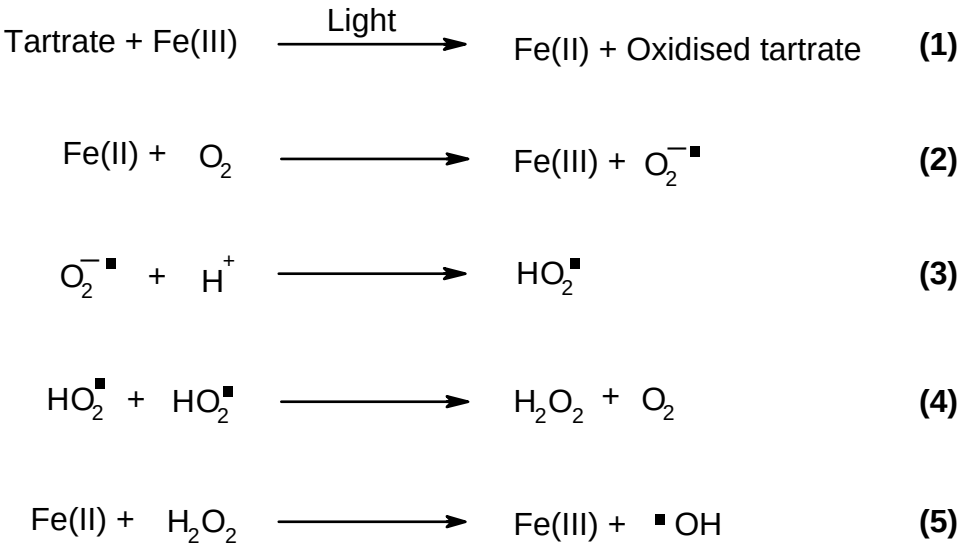


Scheme 1

Clark et al. 2005

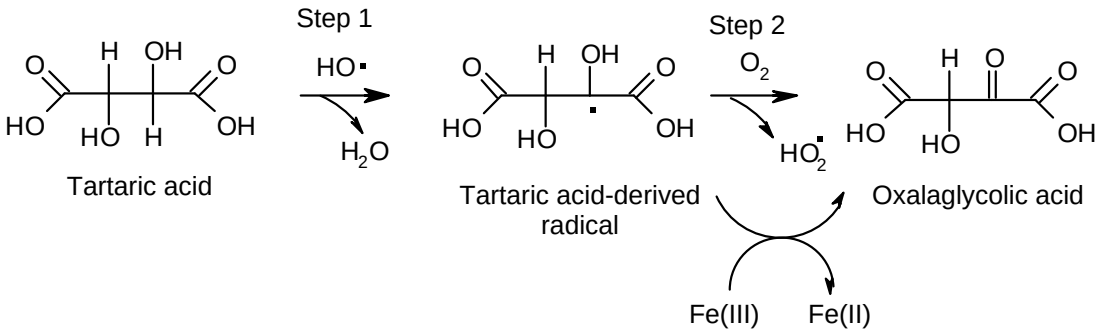

2-hydroxy-3-oxo-propanoic acid
2,3-dihydroxypropenoic acid
Hydroxypyruvic acid
Taut.
Taut.
Slowed by copper(II)
hv + trace Fe(III)
'Photochemistry'
(multi-step) oxidation and/or decarboxylation
HO—OH
Hydrogen Peroxide
Oxalic acid
Tartronic acid
Glyoxylic acid
Formic acid
Inhibited by ethanol
H2O2 + trace Fe(III)
'Fenton Chemistry'
(multi-step) oxidation and/or decarboxylation
Dihydroxymaleic acid
Hydroxyoxaloacetic acid
Dihydroxyfumaric acid
Taut.
Taut.

Scheme 2



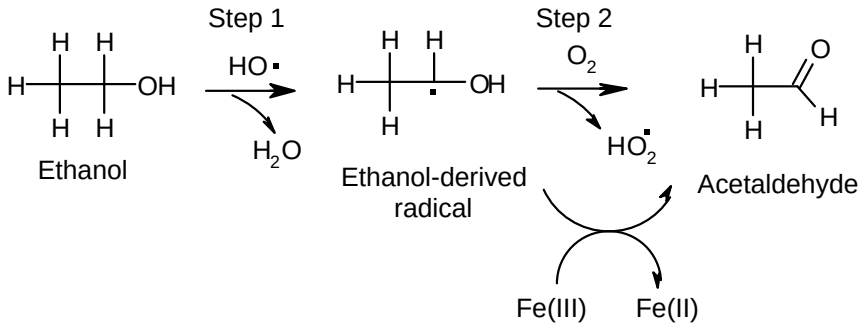
Scheme 3

Clark et al. 2005



Scheme 4

Clark et al. 2005



Scheme 5

Clark et al. 2005

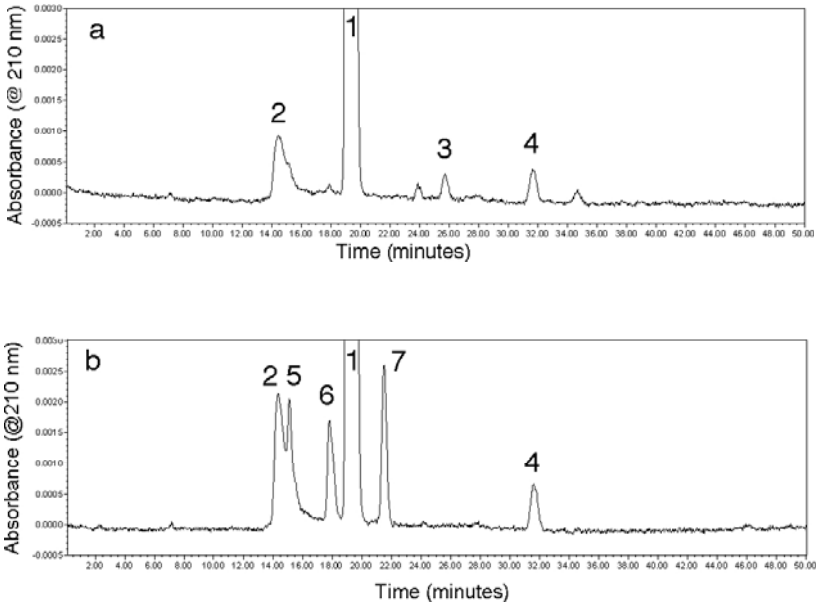


Figure 1

Clark *et al.* 2006

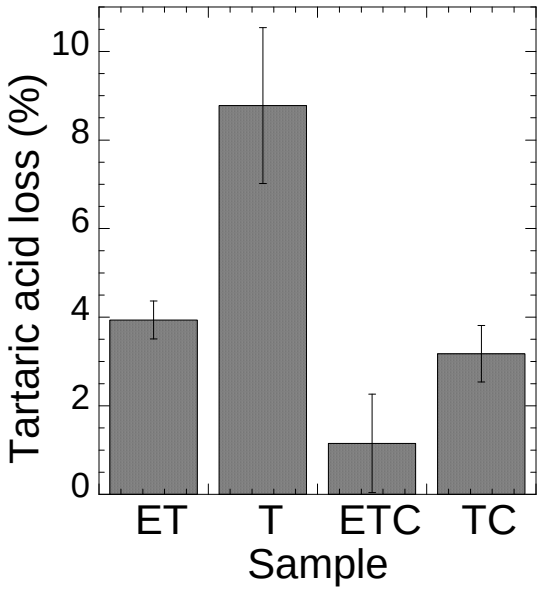
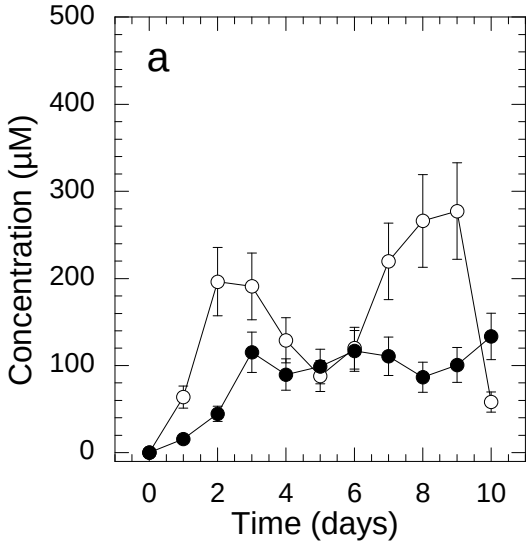


Figure 2

Clark et al. 2006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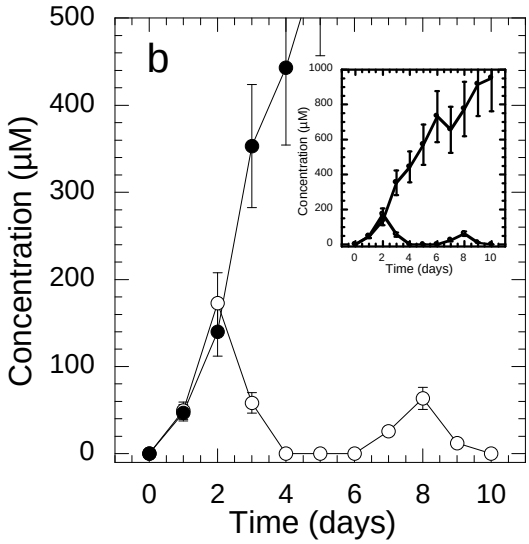
Concentration (µM)
Time (days)
0
100
200
300
400
500
0
2
4
6
8
10


b
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Time (days)
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200
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500
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Concentration (µM)
Time (days)
0
200
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600
800
1000
0
2
4
6
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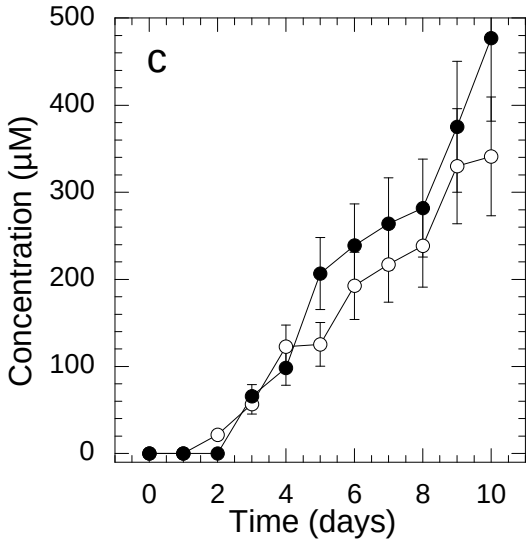
c
Concentration (µM)
Time (days)
0
100
200
300
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Figure 3, Clark *et al.* 2006

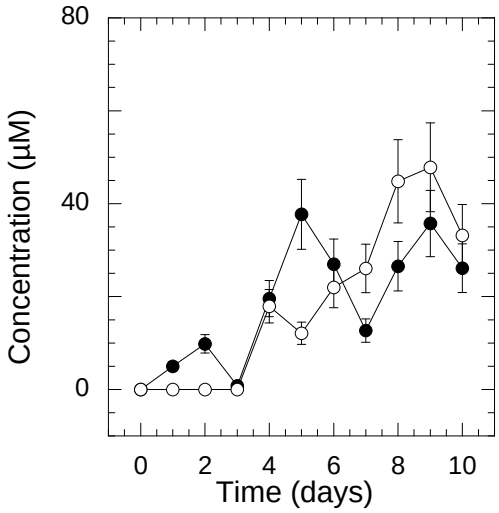

Figure 4

Clark *et al.* 2006

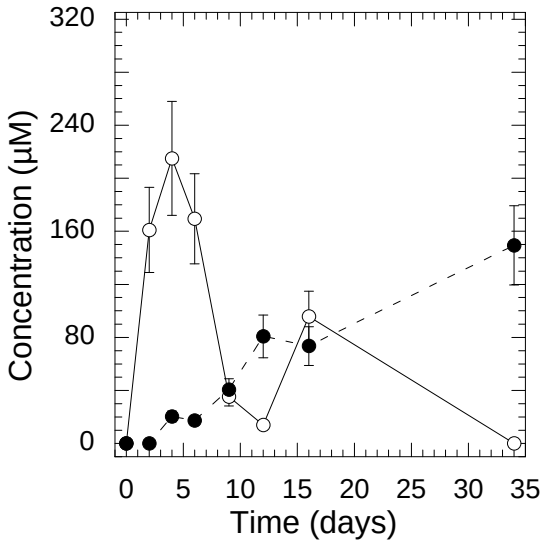

Figure 5

Clark *et al.* 2006

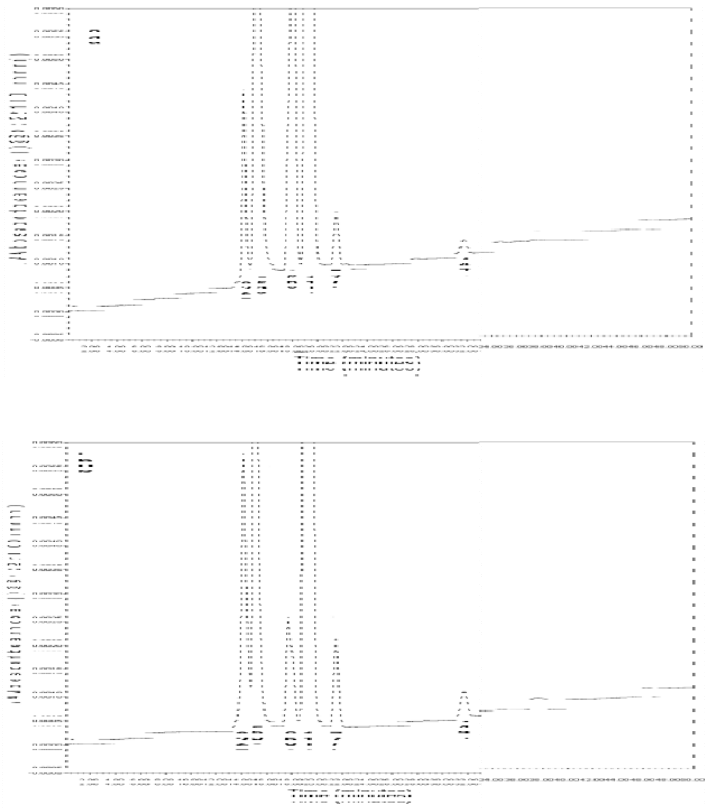

Figure 6

Clark et al. 2006

Table1: Maximum concentration (μM) for hydrogen peroxide, glyoxylic acid and formic acid in the various tartaric acid samples.

ET – 12 % aqueous ethanol, 0.011 M potassium hydrogen tartrate and 0.008 M tartaric acid, **T** – 0.011M potassium hydrogen tartrate and 0.008 M tartaric acid, **C** – 0.6 mg/L copper(II), **Tr** – detection of formic acid below 30 μM, **aq.** - aqueous and **nd** – not detected

Sample	**Storage**	**Hydrogen peroxide**	**Glyoxylic acid**	***Formic acid***
ET	outdoors	280 ± 60	180 ± 30	340 ± 70
ETC	outdoors	nd	40 ± 8	Tr
T	outdoors	130 ± 30	1000 ± 200	500 ± 100
TC	outdoors	nd	40 ± 8	nd
ET	darkness (25°C)	nd	nd	nd
ETC	darkness (25°C)	nd	nd	nd
ET	darkness (45°C)	nd	nd	nd
ETC	darkness (45°C)	nd	nd	nd
T	darkness (25°C)	nd	nd	nd
TC	darkness (25°C)	nd	nd	nd
Water	outdoors	nd	nd	nd
Water+C	outdoors	nd	nd	nd
12% aq. ethanol	outdoors	nd	nd	nd
12% aq. ethanol +C	outdoors	nd	nd	nd

Table 2: Influence of increased glass surface area and the preparation of samples under sterile conditions on the production of glyoxylic acid, hydrogen peroxide and formic acid.

Measurements were taken after the samples were left outdoors for four days. **T** - 0.011M potassium hydrogen tartrate and 0.008 M tartaric acid.

Sample	**Hydrogen peroxide (μM)**	**Glyoxylic acid (μM)**	**Formic acid (μM)**
T	100 ± 20	470 ± 60	160 ± 30
T(sterile conditions)	90 ± 20	480 ± 40	140 ± 30
T+broken glass	98 ± 9	540 ± 30	150 ± 40