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SUBZERO-TEMPERATURE PRESERVATION OF REACTIVE FLUIDS IN THE UNDERCOOLED STATE

II. THE EFFECT ON THE OXIDATION OF ASCORBIC ACID OF FREEZE CONCENTRATION AND UNDERCOOLING

Ross H.M. HATLEY [a], Felix FRANKS [a,b] and Hazel DAY [b]

[a] *Pafra Ltd, Biopreservation Division, 150 Cambridge Science Park, Cambridge CB4 4GG and* [b] *Department of Botany, University of Cambridge, Cambridge CB2 3DA, U.K.*

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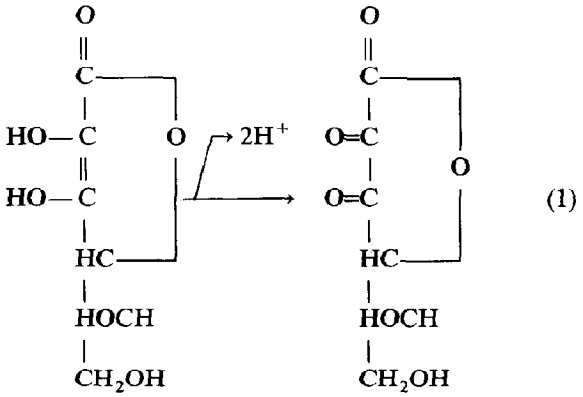
The rate of oxidation of ascorbic acid has been measured in both frozen and undercooled solutions. A new interpretation is advanced for changes in the rate of ascorbic acid oxidation in freeze-concentrated solutions. The results obtained with undercooled solutions indicate a rate reduction in line with that predicted by the Arrhenius equation. It is also demonstrated that undercooling provides a method for greatly extending the shelf life of reactive fluids.

1. Introduction

L-Ascorbic acid (vitamin C) is oxidised in the presence of oxygen to dehydroascorbic acid [1].



(1)

Many foods stored in the frozen state contain L-ascorbic acid (AA) in concentrations of up to 40 mg/100 ml. In these foods the stability of AA increases as the temperature is lowered [2,3]. In contrast, however, at low concentrations (below 1.7 mg/100 ml), freezing of a dilute solution results in a decrease in the stability of AA [4] and hence an increase in the rate of oxidation. The increase in rate of oxidation in dilute solutions is likely to be caused by freeze concentration [5]. The decrease in rate in concentrated solutions has been explained by Thompson and Fennema [6] as being a result of a lower oxygen level in the freeze-concentrated solution. When frozen to any given temperature, dilute and concentrated solutions will both reach the same concentration with respect to AA, but to achieve this the volume of residual unfrozen water will be larger in the originally more concentrated solution [7]. Both solutions originally had almost the same oxygen concentrations, but now the concentration of oxygen in the unfrozen portion of the previously dilute solution will be higher and the reaction consequently more rapid than in the more concentrated solution.

Although an increase in O_2 concentration also occurs in the concentrated solution, the resulting rate enhancement is less than the decrease caused by lowering the temperature, so that an overall decrease in rate is observed.

Previous studies of the oxidation of AA at subzero temperatures have been unable to separate the effects of temperature and freeze concentration [4,6]. The development of a method which allows solutions to be undercooled to as low as $-40°C$ [8,9] now permits the effect of temperature alone to be studied [10,11]. The experiments reported here describe the use of this technique to study the oxidation of AA at subzero temperatures.

The effect of the addition of hydrogen peroxide to concentrated solutions of AA, at subzero temperatures and in the frozen state, was also investigated, and it provides more information about the role of freeze concentration in this reaction.

From the data obtained for the undercooled samples it has been possible to measure rates of oxidation of AA at subzero temperatures in a solution free of cryoprotectants, which can themselves affect the reaction rate [12]. A comparison can then be made between theoretical values, obtained by extrapolation of Arrhenius plots, and measured values.

2. Materials and Methods

The two solution concentrations used in this study were similar to those employed by Thompson and Fennema [6], but with the addition of hydrogen peroxide.

Reaction mixture 1 ($1\times$) consisted of 40 mg/100 ml AA, 0.02 M acetate buffer, and excess H_2O_2 at pH 5.5.

Reaction mixture 2 ($1/23\times$) was the same as reaction mixture 1 but 23-times more dilute.

Fresh solutions of buffer and hydrogen peroxide were prepared daily and the reaction initiated by adding AA (time zero in all experiments). After the reaction had been initiated, the reaction mixtures were subjected to one of three protocols as follows:

(A) Bulk solutions, subzero temperatures: 3 ml aliquots were poured into Universal tubes to facilitate rapid cooling. 1 min after the initiation of the reaction they were placed together into an ethylene glycol bath set at $-20°C$ for 6 min and ice crystallization initiated. They were then immediately placed into a freezer set at the required temperature.

(B) Bulk solutions at temperatures above 0°C: As for A, except that samples were immediately exposed to the desired temperature, without precooling.

(C) Undercooled solutions: As for A, except that 2 ml samples were emulsified into 10 ml of mineral oil, using a coaxial cylinder homogenizer. The emulsions were stabilized by placing the tubes in ice for 3 min. Subsequently they were transferred to a freezer at the required temperature.

Initial samples were taken immediately after the addition of AA to the buffer/peroxide mixture (time zero). The absorbance was measured at 265 nm by the method of Racker [13] on a Perkin-Elmer 557 double-beam, double-wavelength spectrophotometer. The absorbance obtained with this solution was then used as a standard from which the AA concentrations in partly reacted samples were calculated.

After storage at the experimental temperatures, $1/23\times$ samples which had been subjected to treatments A and B were placed in a water bath at 30°C for 5 min, poured into cuvettes, and the absorbance was measured immediately. Samples that had been emulsified were warmed in a water bath for 5 min at 30°C, then centrifuged for 5 min to separate out the aqueous phase which was then carefully removed and the absorbance recorded. $1\times$ samples were similarly treated but subjected to a 10-fold dilution with buffer before being placed in the spectrophotometer.

The concentration of AA per ml buffer was obtained for all samples by comparing the absorbance value of the sample with that of the corresponding standard sample. Rate constants (k) were calculated at each temperature for each treatment (fig. 1) using the line of best fit (estimated variance 1.7×10^{-3}) on the assumption that the reaction follows pseudo-first-order kinetics [6].

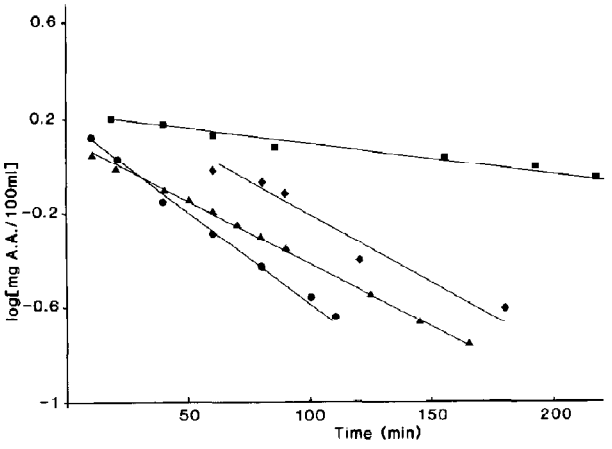


Fig. 1. Change in AA concentration with time for representative samples: (●) −20°C; (▲) 25°C, (◆) −10°C and (■) −5°C (emulsified sample).

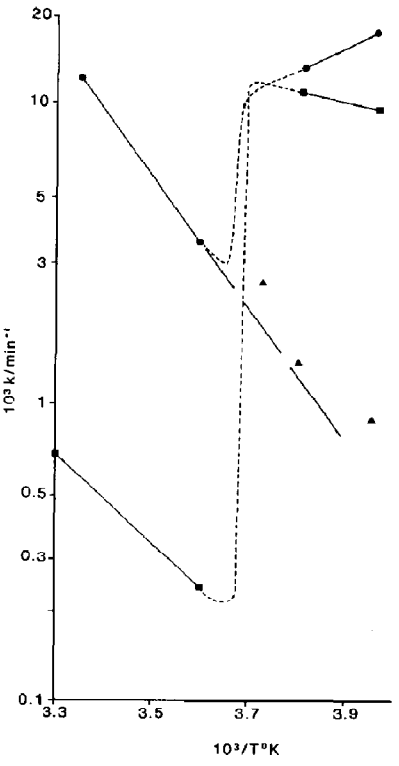


Fig. 2. The effect of temperature upon reaction rate: (▲) undercooled samples, (■) 1×samples, (●) 1/23×samples.

3. Results

The results obtained are summarised in fig. 2 and table 1. Measurements were made within the temperature range 30 to −20°C. At higher temperatures the reaction became too rapid to be measured by the techniques employed in this investigation. Below −20°C the reaction was either too slow to be measured conveniently (emulsified samples) or the threat of AA crystallisation was too great, as the solution was then below its eutectic point [6] (bulk samples).

Acceleration values (observed rate constant/extrapolated value) were calculated and are also

Table 1

Ascorbic acid rate constants under different conditions

Concentration of ascorbic acid (mg/100 ml)	Emulsified or bulk sample	Temperature (°C)	Observed rate, k (min^{-1})($\times 10^3$)	Predicted rate, k (min^{-1})($\times 10^3$)	Acceleration value
40.0	B	30	0.69		
pH 5.5		4	0.24		
		−10	11.00	0.22	50.00
		−20	9.75	0.16	60.90
1.7	B	25	12.20		
pH 5.5		4	3.45		
		−10	13.36	1.45	10.59
		−20	17.88	0.92	19.43
1.7	E	−5	2.53	1.89	1.27
pH 5.5		−10	1.38	1.30	1.06
		−20	0.89	0.62	1.43

shown in table 1. For undercooled samples these values are approximately equal to unity. For the bulk samples the acceleration values are higher for the initially more concentrated solutions than for the dilute ones, indicating that freeze concentration is not the only factor influencing the rate in frozen AA solutions.

Data obtained for undercooled 1 × samples have not been used, as the changes in absorption over the experimental time period were too small to be measured accurately.

4. Discussion

The results for bulk samples presented here generally agree with those reported by previous investigators [6]. 1 × solutions had a lower k value in the temperature range 0–30 °C than any of the more dilute samples. Thompson and Fennema [6] suggested that this effect is due to oxygen supply becoming a limiting factor at high AA concentrations. This is an equally valid explanation of the results of our investigation.

A significant difference is apparent between the 1 × data from our experiment (table 1) and those of Thompson and Fennema (table 2) at temperatures above 0 °C. The much lower rate observed in our experiments is probably a consequence of the use of hydrogen peroxide. Thompson and Fennema [6] incorporated oxygen into the non-frozen solutions by continued shaking of the flasks, while we used hydrogen peroxide as an oxygen source. Less oxygen was thus available than would have been supplied by shaking, hence oxygen supply was more limiting in the 1 × AA solution. For 1/23 × samples above 0 °C, where the O_2 supply is not limiting, the agreement of our data with those of Thompson and Fennema indicates that the oxidation of AA follows the same pathway in both the presence and absence of H_2O_2.

At subzero temperatures in a frozen solution a totally different situation emerged, with the rates observed in the present study being higher than any data previously reported. Previous studies relied on oxygen already dissolved in the aqueous phase, with no fresh supply to replace the gas used up and therefore oxygen probably becoming limiting in the frozen solution, unless very dilute solutions were used (< 1.7 mg/100 ml). In our experi-

Table 2

Data from Thompson and Fennema [6]

Concentration of ascorbic acid (mg/100 ml)	Emulsified or bulk sample	Temperature (°C)	Observed rate, k (min^{-1})($\times 10^3$)	Predicted rate, k (min^{-1})($\times 10^3$)	Acceleration value
1.7	B	10	15.00		
pH 5.5		5	9.00		
		−1	5.80	3.3	1.8
		−10	4.30	1.1	4.2
		−17	4.40	0.4	11.0
1.7	B	10	10.00		
pH 4.6		1	3.50		
		−1	2.80	2.90	1.00
		−10	2.70	1.00	2.70
		−17	2.00	0.40	5.00
40.0	B	10	11.00		
pH 4.6		1	4.00		
		−1	1.10	2.80	0.40
		−8	0.60	1.20	0.50
		−12	0.60	0.70	0.90
		−17	0.50	0.40	1.20
		−23	0.50	0.20	2.50

ments the increase in rate of AA oxidation was due to the use of hydrogen peroxide as an oxygen source. In an unfrozen solution the oxidation of AA is pseudo-first order with respect to AA, the rate of peroxide decomposition and therefore oxygen availability being constant over the time periods and temperatures involved. Upon freeze concentration, the rate of H_2O_2 decomposition and therefore oxygen availability increase, this affecting AA oxidation.

The results from this study and a more careful examination of the data of Thompson and Fennema suggest that their explanation of the decrease in rate of AA oxidation in freeze-concentrated AA solutions does not fully explain the situation. They assume that oxygen is freeze-concentrated in the partially frozen solution and is dissolved in, suspended in or sparged through the residual liquid but remains available to take part in the reaction with AA. The rate decrease in the 1 × solution is then explained in the following way: Both dilute (1/23 ×) and concentrated (1 ×) solutions start off with the same initial oxygen concentration, both solutions being O_2 saturated. When frozen, both solutions freeze to the same AA concentration [7]. To achieve this, more water freezes in the dilute than in the concentrated solution, and therefore the oxygen concentration is higher in the dilute solution. The higher oxygen level causes the dilute solution to show an increase in rate and the concentrated solution to show a decrease in rate, compared to unfrozen solutions at 0 °C.

Although this can explain the rate enhancement found in the dilute solution and the rate decrease in the concentrated solution, it does not explain the extent of the rate decrease.

When acceleration values are calculated for the 1 × solution in freeze-concentrated solutions they are found to be less than unity which means that the rate of AA oxidation in the partially frozen concentrated solution is lower than that predicted by an extrapolation of the Arrhenius plot. Oxygen limitation cannot account for these low acceleration values, as the following example will demonstrate. If we take a 1 × solution and undercool it to T °C, the rate of reaction is found to be equal to that predicted by the Arrhenius equation (table 1). The AA and oxygen concentrations of the undercooled solution will be similar to those at room temperature, and the acceleration value will be approximately equal to unity. A 1 × solution freeze-concentrated to T °C has increased AA and oxygen concentrations and it will show a rate of reaction higher than the undercooled solution (although not necessarily higher than solutions at temperatures just above 0 °C). Its acceleration value should be greater than unity. Acceleration values obtained from observed data for such a solution, however, give an acceleration value of less than unity. Thompson and Fennema's interpretation of their data does not provide a correct explanation of the behaviour of freeze-concentrated solutions.

The initial assumption that oxygen is freeze-concentrated is probably incorrect [14]. The oxygen is more likely expelled from a freeze-concentrated solution and lost to the atmosphere. This would mean that a 1 × freeze-concentrated solution would have a lower oxygen/AA ratio than an undercooled solution. In the part-frozen solution AA would therefore be oxidised more slowly than in the undercooled solution with a higher oxygen content. This would result in the 1 × solution having an acceleration value of less than unity. This argument would suggest that the 1/23 × solution would exhibit an even lower rate than the 1 × solution because even more water would freeze and more oxygen would be lost. This is not observed; instead, the 1/23 × solution shows an enhanced oxidation rate. The above argument alone cannot account for the observed results, but it can act as the basis for a further explanation that can account for all the observations encountered.

It is generally accepted that oxygen is lost from frozen solutions. Therefore in the 1 × solution oxygen is probably lost to the atmosphere and a low rate of oxidation of AA follows.

The oxygen expelled from the 1/23 × solution during freezing is probably unable to escape from the ice matrix to the atmosphere. The trapped oxygen then provides a reservoir which, by diffusion into the aqueous phase, replaces the oxygen used in the AA oxidation. An increase in rate is then observed, caused by concentration of the AA

in the residual liquid portion of the partially frozen solution.

This argument is further supported by the observation that the acceleration values of 1 × solutions cooled below −17°C are greater than unity. At this temperature the percentage of water frozen would be similar to that of an originally more dilute solution frozen at a higher temperature. The freeze-concentrated 1 × solution would then display the same characteristics as the 1/23 × solution, at the higher temperature, i.e., oxygen would be trapped (but available and the rate would increase above that predicted by extrapolation of the Arrhenius plot.

In frozen samples the effect of the increased oxygen availability from the hydrogen peroxide resulted in higher reaction rates than those previously reported [6]. This implies that in the previous data for frozen solutions oxygen was the limiting factor, rather than AA. The agreement of the reaction rates in freeze-concentrated solutions, regardless of their initial concentration, here reported suggests that a value of k of about 0.13 min^{-1} is an accurate estimate for these conditions at −10°C, where O_2 is not limiting. At lower temperatures, below the AA eutectic temperature of −18°C, an accurate determination of k becomes more uncertain, as the possibility of AA crystallization cannot be discounted.

The acceleration values reported in table 1 are much higher than those reported earlier (table 2). This strengthens the argument that in previous investigations of frozen solutions oxygen availability was the limiting factor. It can also be observed that the acceleration values never drop below unity. This implies that the rate-limiting factor experienced by Thompson and Fennema does not occur in our experiments where oxygen is not limiting.

The rate constants for the undercooled samples (table 1) confirm that the reaction rate declines with temperature, as predicted by the Arrhenius relationship. This suggests that the effect of temperature alone on the reaction solely reduces the rate of the reaction but that a temperature change does not cause an alteration in mechanism.

Undercooling also enables solutions containing reactive substances to be kept for far longer than can be achieved by freezing or refrigeration. For the particular reaction described, undercooling can extend the shelf life of a dilute sample of vitamin C from a few hours, possible at present by refrigeration, to a period of years, although the practical value of undercooled storage for this particular mixture is low, because removal of oxygen would have the same effect, for other reactive systems which cannot be so easily inhibited, undercooling would be of great benefit, both for storage and for kinetic and mechanistic studies [15].

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