THE COPPER-CATALYSED OXIDATION OF ASCORBIC ACID IN FRUIT AND VEGETABLE SUSPENSIONS

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[*Accepted for Publication August 20, 1947*]

Summary

The copper-catalysed oxidation of ascorbic acid was studied in phthalate and phosphate buffers. The rate increased with concentration of copper up to ten parts per million and with increasing pH up to 6.0.

Oxalic, malic, citric, and tannic acids, sulphur dioxide, albumen, cystein, and thiourea reduced copper catalysis. Outstanding "protection" was given by thiourea.

Most fruit and vegetable tissues contain substances which reduce copper catalysis. Onion tissue gives outstanding "protection" against low concentrations of copper.

I. INTRODUCTION

Considerable attention has been given to the retention of ascorbic acid by fruit and vegetables, both fresh and processed. The stability of ascorbic acid to oxidation can vary greatly in different tissues and under different conditions of processing and storage. The factors concerned in this stability include (*a*) access of atmospheric oxygen, as determined by the structure of the tissue and by processing procedures; (*b*) oxidation catalysts, including enzymes, copper, and other substances in the tissues; and (*c*) "protective" factors.

Krishnamurthy and Giri (1941*a*) reported the existence of both oxidizing and "protective" factors in various vegetables. They separated the oxidizing enzymes from the "protective" factor by precipitation with acetone. They found that the "protective" factor was thermostable and inhibited the copper-catalysed but not the enzyme-catalysed reaction.

This paper is concerned with the copper-catalysed oxidation of ascorbic acid and its modification by various "protective" substances. Barron *et al.* (1936*a*) found that ascorbic acid is not autoxidizable in acid or neutral solutions up to pH 7. They tested the catalytic effect of salts of manganese, nickel, iron, cobalt, calcium, and copper at pH 4-6 and found that copper alone has a marked catalytic effect. Barron *et al.* (1936*b*) found that some animal and vegetable fluids contain protective agents, including gluthathione, proteins, and amino-acids, which inhibit copper catalysis. Krishnamurthy and Giri (1941*b*) found oxalic acid, xanthine, uric acid, theophylline, creatinine, antipyrine, and albumen to have

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powerful "protective" action. Kawereau and Fearon (1944) investigated various "protective" agents and found the most effective to be thiourea. They separated volatile thiol compounds from cabbage, and found them to be effective protectors of ascorbic acid.

The kinetics of the copper-catalysed oxidation have been studied by Silver-Blatt, Robinson, and King (1943). They showed that if k (the velocity constant) does not exceed 0.12 min.$^{-1}$, the reaction rate is directly proportional to the concentrations of cupric ion and ascorbic acid, but the k values depend in a rather complex manner upon the initial concentrations of ascorbic acid, hydrogen ion, and oxygen. The k values tend to decrease with increasing initial concentrations of ascorbic acid. These authors found that appreciable concentrations of hydrogen peroxide accumulate during the course of the reaction, but this does not affect the rate if k is less than 0.12 min.$^{-1}$.

Weissberger and Lu Valle (1944) found that the relation between pH and rate is rather complex. The results indicated that only the monovalent ion of ascorbic acid is the substrate of copper catalysis.

Our studies are concerned first with the copper-catalysed oxidation of ascorbic acid in buffer solutions of varying pH, and then with the "protective" effect of added substances and of fruit and vegetable suspensions. For assessing "protective" effects, it is necessary to have reference solutions in which copper catalysis is at a maximum. Phthalate buffers from pH 2.2 to 6.0 and phosphate buffers of pH 6.0 and 7.0 were found fairly satisfactory for this purpose. These solutions gave a rate of oxidation which generally increased regularly with increasing pH and was considerably higher than that given by other solutions containing "protective" substances.

II. Experimental Procedure

All media used for studying the oxidation of ascorbic acid were prepared with glass distilled water. Clark and Lubs' 0.05M phthalate buffers were prepared with a slight modification. Sulphuric acid was used in place of hydrochloric acid in buffers of pH 2.2 and 3.0. Mapson (1941) showed that chloride had a variable effect on the oxidation, increasing it in lower and decreasing it in higher concentration. By substituting sulphuric acid, oxidation in the phthalate buffers was found to decrease regularly with decreasing pH. Sorenson's M/15 phosphate buffers were also prepared.

The "protective" effect of a number of substances which occur naturally or are recommended for preservation was investigated in phosphate buffer of pH 6.0. The substances tested were starch, sucrose, glucose, oxalic acid, malic acid, citric acid, tannic acid, albumen, cystein, thiourea, and sulphur dioxide. The concentrations of the naturally occurring substances approximated to those

which might be present in fruit and vegetable suspensions. After addition of oxalic, malic, or citric acid to the buffer solution, the pH was readjusted to 6.0 with sodium hydroxide. No adjustment was required with the other substances.

The fruit and vegetable suspensions were prepared by disintegrating one part by weight of tissue with four parts of glass distilled water in the Waring Blendor. They were boiled for two minutes to inactivate the enzymes. (Practically identical rates of oxidation were obtained after boiling for twenty minutes). The rose hip suspension was prepared from one part of rose hips and nine parts of water as more concentrated suspensions were too viscous to handle. Only one sample of each fruit and vegetable was investigated.

Studies of the oxidation of ascorbic acid were made as follows: Fifty millilitres of solution or suspension, containing 0, 0.5, 1, 2, 5, and 10 parts of copper per million were measured into a 100 ml. florence flask and brought to 40°C. in a constant temperature bath. The copper was added as copper sulphate. Air was bubbled at the rate of approximately 12 ml. per second. After adding 2 ml. of 0.5 per cent. ascorbic acid, a 5 ml. aliquot was pipetted into 10 ml. of 3 per cent. metaphosphoric acid and titrated with approximately 0.001N 2.6-dichlorophenolindophenol. Subsequent aliquots were pipetted at intervals up to 30 minutes and titrated with the dye.

When the logarithm of the ascorbic acid concentration c was plotted against time t, an approximately linear curve was obtained, indicating a first order reaction. The velocity constant k was calculated from the formula $1/t \log_e C_o/C_t$ and expressed as min.$^{-1}$.

To avoid foaming in some of the suspensions, a few drops of caprylic alcohol were added to the medium. This was found not to affect the rate appreciably.

In these studies, the initial concentration of ascorbic acid was approximately 20 mg. per 100 ml. in all preparations except the rose hip suspension. In most cases this concentration of ascorbic acid was derived almost wholly from the added ascorbic acid, as little ascorbic acid remained in the suspension after blending. The exceptions were diluted orange juice and rose hip suspension. The former retained approximately 20 mg. of ascorbic acid per 100 ml. and did not require any further addition. The latter retained approximately 80 mg. of ascorbic acid per 100 ml.

III. Oxidation in Pure Solutions

The velocity constants in phthalate and phosphate buffers are given in Table 1, and the effect of pH on the velocity constant with one and ten parts of copper per million is given in Figure 1.

In all buffers the velocity constant increased considerably with increasing copper concentration. Copper was present in the control buffers up to 0.2 part per million, and was probably responsible for the slight oxidation. For each level of copper the velocity constant increased with increasing pH up to pH 6. The velocity constant was still higher at pH 7 for the lower levels of copper (up to 2 p.p.m.), but it fell off at the higher levels, probably due to precipitation.

TABLE 1

VELOCITY CONSTANT IN PHTHALATE AND PHOSPHATE BUFFERS

Buffer	Added Copper (p.p.m.)	100 k at pH 2.2	3.0	4.0*	5.0	6.0	7.0
Phthalate	0	0.0	0.1	0.4 (0.2)	1.4	1.9	
	0.5	0.2	0.7	4.1 (2.6)	10.3	17.8	
	1.0	0.2	1.2	5.3 (3.6)	14.4	25.1	
	2.0	0.4	2.0	8.5 (5.2)	17.9	33.0	
	5.0	0.8	5.0	15.4 (8.2)	27.4	43.2	
	10.0	1.7	11.7	26.5 (11.3)	41.2	127.3	
Phosphate	0					0.5	0.9
	0.5					34.6	53.0
	1.0					49.0	72.2
	2.0					77.9	102.5
	5.0					120.3	118.5
	10.0					161.2	131.3

*The velocity constants in brackets are those obtained when 100 mg. of ascorbic acid per 100 ml. are added initially.

At pH 6, copper catalysis was definitely greater in phosphate than in phthalate buffer. This indicates a specific effect of the anion in addition to the effect of the hydrogen ion. However, catalysis in these buffers still provides an approximate standard for reference, as it is considerably greater than that occurring in the presence of certain "protective" substances which reduce the concentration of free copper ions by chemical combination.

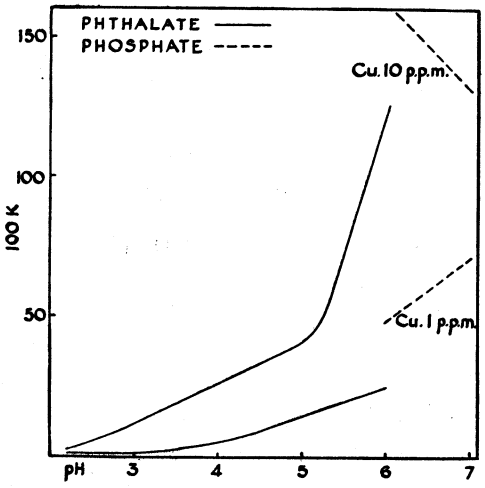

Fig. 1.—The effect of pH on the velocity constant of the oxidation of ascorbic acid by copper.

Most of the velocity constants refer to an initial concentration of 20 mg. of ascorbic acid per 100 ml. At a higher concentration (100 mg./100 ml.), the velocity constant is definitely less.

The velocity constants in phosphate buffer of pH 6.0 with various added substances are given in Table 2. The "protective" effects can be estimated by comparison with the velocity constant in the control buffer.

TABLE 2

VELOCITY CONSTANT IN PHOSPHATE BUFFERS AT pH 6.0 WITH ADDED SUBSTANCES

Added Substances	100 k with Added Copper Equivalent to					
	Nil	0.5 p.p.m.	1.0 p.p.m.	2.0 p.p.m.	5.0 p.p.m.	10.0 p.p.m.
Control	0.5	34.6	49.0	77.9	120.3	161.2
Starch (1%)	1.4	28.4	42.0	63.5	96.9	102.6
Sucrose (1%)	1.6	36.5	46.1	53.0	102.5	148.0
Glucose (1%)	0.7	35.8	51.7	65.6	115.1	123.6
Oxalic acid (0.1%)	0.1	2.1	3.6	5.8	8.4	9.3
Malic acid (0.2%)	2.8	17.1	21.6	30.6	44.0	55.3
Citric acid (0.2%)	1.4	6.2	7.3	9.2	10.4	12.5
Tannic acid (0.02%)	0.0	1.7	2.6	4.5	7.9	10.2
Albumen (0.2%)	0.0	0.3	0.6	1.5	8.9	53.5
Cystein, HCl (0.02%)	0.3	0.3	1.2	1.6	5.0	9.2
Thioureau (0.02%)	0.0	0.1	0.0	0.1	0.4	0.5
Sulphur dioxide (0.02%)	0.0	19.5	24.5	35.7	44.9	57.6

The "protective" effects of starch, sucrose, and glucose were found to be negligible, as the velocity constants differed only slightly from those of the control buffers. All the other substances showed some "protective" effect. Good "protection" was afforded, at the concentrations tested, by oxalic acid, citric acid, tannic acid, and cystein. Albumen was very effective in low concentrations of copper, but less effective at higher concentrations. Outstanding "protection" was afforded by thiourea, which at a concentration of 0.02 per cent. resulted in negligible oxidation with up to two parts of added copper per million and only slight oxidation with ten parts per million.

The "protective" effect of cystein is not confined to the reduced form, as the disulphide cystin was found to be almost as effective. With ten parts of copper per million, an equivalent concentration of cystin gave a value of 12.4 for 100 k.

IV. OXIDATION IN FRUIT AND VEGETABLE SUSPENSIONS

The velocity constants in fruit and vegetable suspensions are given in Table 3. In some cases the centrifuged liquor was used, as the whole suspension was difficult to pipette and titrate. The suspensions were centrifuged at 2,000 r.p.m. Centrifuging tends to reduce the protective effect.

TABLE 3

VELOCITY CONSTANT IN SUSPENSIONS BOILED FOR TWO MINUTES

Preparation	pH	Original Copper (p.p.m.)	100k with Added Copper Equivalent to				
			Nil	0.5 p.p.m.	1.0 p.p.m.	2.0 p.p.m.	5.0 p.p.m.	10.0 p.p.m.
Apple suspension	3.2	0.2	1.3	1.5	1.9	2.5	4.3	8.5
Orange juice suspension	3.6	0.1	0.0	0.6	1.5	2.8	4.7	6.6
Rose hip liquor	3.6	0.2	0.3	0.8	1.6	1.8	3.2	3.9
Tomato suspension	4.3	0.2	0.7	2.2	4.6	6.3	8.8	10.5
Orange rind liquor	5.1	0.2	0.2	1.3	2.9	6.4	12.9	18.7
Onion suspension	5.3	0.1	0.0	0.0	0.0	0.5	4.4	7.1
Parsley liquor	5.5	—	0.3	0.8	1.1	2.6	12.5	22.7
French bean liquor	5.5	0.2	0.3	0.5	0.8	2.9	7.9	11.0
Potato suspension	5.7	0.2	0.9	0.9	0.9	2.1	4.0	8.0
Cabbage suspension	6.0	0.2	0.4	0.8	0.9	3.1	6.7	13.0
Asparagus liquor	6.0	0.3	0.5	1.0	2.0	4.9	11.9	19.9
Swede turnip suspension	6.0	0.1	0.2	1.7	3.1	5.8	11.8	17.0
Silver beet liquor	6.7	0.4	1.2	1.4	1.7	2.2	5.7	13.2

Most of the tissues contain substances which reduce the copper-catalysed oxidation of ascorbic acid (as compared with oxidation in phthalate and phosphate buffers). The rate of oxidation in onion tissue was negligible with one part of copper per million and very low with higher concentrations. Rose hips gave a particularly low rate in ten parts of added copper per million. Orange juice also gave a low rate of oxidation. Tomato gave comparatively poor protection against low concentrations of added copper.

As the rate of the copper-catalysed oxidation varies considerably with pH, the "protective" effect of a particular suspension can only be estimated by comparing its velocity constant with that of a phthalate or phosphate buffer of the same pH. The latter can be obtained from the curves in Figure 1. Using these data, the ratio k_s/k_b, where k_s is the velocity constant of the suspension and k_b is the velocity constant of a phthalate or phosphate buffer of the same pH, has been calculated for one and ten parts of copper per million. The results are given in Table 4.

From Table 4 it appears that the comparatively low rate of oxidation in orange juice is due as much to the low pH as to the effect of "protective" substances. In the rose hip suspension, the comparatively high initial concentration of ascorbic acid (80 mg./100 ml.) also reduced the velocity constant. The "protective" effect is particularly noticeable in the products of high pH.

Organic acids, tannins, and sulphur compounds probably all contribute to the "protective" effect. One would generally expect their specific effect to be less at lower pH, due to the reduced ionization and copper binding power.

TABLE 4

THE PROTECTIVE EFFECT IN FRUIT AND VEGETABLE SUSPENSIONS

Preparation	Reference Buffer	pH	k_s/k_b with Added Copper Equivalent to 1 p.p.m.	k_s/k_b with Added Copper Equivalent to 10 p.p.m.
Apple suspension	Phthalate	3.2	1.07	0.57
Orange juice suspension	„	3.6	0.48	0.32
Rose hip liquor	„	3.6	0.52	0.19
Tomato suspension	„	4.3	0.62	0.34
Orange rind liquor	„	5.1	0.19	0.43
Onion suspension	„	5.3	0.00	0.13
Parsley liquor	„	5.5	0.06	0.30
French bean liquor	„	5.5	0.04	0.14
Potato suspension	„	5.7	0.04	0.08
Cabbage suspension	„	6.0	0.04	0.10
Asparagus liquor	„	6.0	0.08	0.16
Swede turnip suspension	„	6.0	0.12	0.13
Silver beet liquor	Phosphate	6.7	0.03	0.09

There appeared to be little specific "protection" in the apple suspension. As shown previously, the "protective" effect of 0.2 per cent. malic acid is comparatively low. Moreover, there is some evidence of a thermostable catalyst besides copper in the apple suspension. The velocity constant (1.3) for the apple suspension without added copper is higher than would be expected from its copper content (0.2 p.p.m.) particularly as an additional one part of copper per million only increased it to 1.9.

The high "protection" of onion tissue is probably due largely to the volatile sulphur compounds associated with pungency. A buffered distillate of the same volume and pH as the original suspension was found to give complete "protection" against one part per million of added copper ($100\ k = 0.0$). Suspensions of non-volatile residue obtained by prolonged boiling or by blending oven dried tissue still showed some "protective" effect. With one part of added copper per million, $100k_s = 2.1$ and $k_s/k_b = 0.12$.

V. DISCUSSION

It is probable that the copper-catalysed oxidation of ascorbic acid takes place in two stages. Cupric ion oxidizes the ascorbic acid to dehydroascorbic acid and is reduced to cuprous. The cuprous is then oxidized to cupric by atmospheric oxygen. Silverblatt, Robinson, and King (1943) showed that if k is not greater than 0.12 min.$^{-1}$, the rate is proportional to the concentration of cupric ion. Most of the "protective" substances are known to form complexes which reduce the concentration of free cupric ions.

The results for fruit and vegetable suspensions indicate generally a reduced copper catalysis compared with the reference buffers. In most cases the effect of low concentrations of copper is still quite appreciable. This applies par-

ticularly to tomato products. The relation of these studies to copper contamination in fruit and vegetable products can only be approximately assessed. Oxidation can be minimized by rigorous exclusion of air, but where this is impossible, copper contamination should be reduced to a minimum.

This paper makes a further contribution to the already considerable literature on copper catalysis and the effect of "protective" substances. Various additional substances and natural products have been tested for their "protective" effect. In addition, an attempt has been made to estimate the "protective" effect more precisely by comparison with copper catalysis in certain buffer solutions of the same pH. These solutions were found to give approximately maximum catalysis.

VI. Acknowledgment

The work described in this paper was carried out as part of the research programme of the Division of Food Preservation and Transport, C.S.I.R.

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