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**STUDIES ON THE USE OF ASCORBIC ACID  
IN CONTROLLING OXIDIZED FLAVORS IN MILK**

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STRAHMORE

STUDIES ON THE USE OF ASCORBIC ACID  
IN CONTROLLING OXIDIZED FLAVORS IN MILK

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## INTRODUCTION

It is generally considered that oxidized flavor is the most serious flavor defect in commercially pasteurized milk; because it occurs frequently, it is a very objectionable defect and no easy and practical means for preventing its development in commercial pasteurized milk is being used at present.

Many investigators have shown that metal contamination, particularly by copper, is capable of inducing an oxidized flavor in milk, but since there is no general agreement at present among the many investigators as to the exact cause or causes of the oxidized flavor, further studies are needed to establish these facts.

The addition of ascorbic acid to milk has proven to be effective in preventing the development of oxidized flavors, and since ascorbic acid is a vitamin normally present in milk, it seemed that further studies should be conducted on the use of ascorbic acid as a practical means for preventing the defect in commercial pasteurized milk.

The purposes of the study herein reported are (1) to determine the amount of ascorbic acid which must be added to milk processed by the Oklahoma A. and M. College milk plant to prevent the oxidized flavor development, (2) to determine the amount of ascorbic acid required to prevent the oxidized flavor development induced by varying amounts of added copper, (3) to determine the effect of the temperature of storage on the rate and extent of the oxidized flavor development in milk and (4) to study the possible role of the enzyme ascorbic acid oxidase in the development of oxidized flavor in milk.

## REVIEW OF LITERATURE

The question as to what constitutes an oxidized flavor has been a subject of doubt and controversy for many decades. Nelson and Dahle (30) concluded that oxidized flavor is not a single flavor, but rather can be applied to a group of flavors, namely, astringent, papery, metallic and oily and tallowy. Leeder and Herreid (26) are in agreement and designate oxidized flavor by several names, such as tallowy, metallic, cappy, cardboard and papery. They state that it resembles old stale fat and describe it as a tendency to cause a puckery sensation that is lasting and difficult to rinse from the mouth. Thurston, et al (43) are not in agreement and believe that oxidized flavor is not identical with the tallowy flavor of oxidized butterfat, but a separate and distinct off-flavor. The modern trend is to consider oxidized flavors as one of degree and not one of quality.

The constituent affected when an oxidized flavor occurs is not at present fully understood. Tracy (45) theorized that the unsaturated fatty acid (olein) in the butterfat undergoes oxidation, resulting in an oxidized flavor. This theory was accepted and followed until the middle thirties, when Thurston et al (43) advanced the relation of lecithin, chief phospholipid of milk, as the cause of oxidized flavor.

They prepared a washed butterfat in order to remove any substance absorbed on the fat globule. Water at 100°F. was added to dilute the cream. This was separated with a De Laval #17 separator, rediluted and re-separated until the cream that had been washed fourteen times or more showed no trace of phosphorous, which would indicate the sample was relatively free of phospholipids. The washed butterfat was then mixed with fresh, good-flavored skim milk and contaminated with copper, and air was bubbled through it in an

attempt to induce an oxidized flavor. The oxidized flavor could not be induced from the lecithin-free milk, but was readily induced in the control sample. As a result of this finding, they concluded that lecithin rather than butterfat causes oxidized flavors.

Henderson and Roadhouse (19) showed that milk fat of milk that had developed an oxidized flavor had passed only a fraction of its induction period (that period when the fat is not subject to oxidation), and deemed it unlikely that the oxidation of the milk fat was great enough to produce the oxidized flavor. The facts that at least one double bond exists in the milk-lecithin molecule, that lecithin in purified state oxidizes readily, and that lecithin makes up the outer portion of the butterfat globule and is therefore exposed to the action of oxidizing agents before the butterfat, are evidence that lecithin, and not butterfat, is the constituent affected when oxidized flavors develop.

It has been shown by numerous investigators that different lots of milk treated in an identical manner produce varying degrees of oxidized flavor. Dahle (8) classified milk in relation to the development of oxidized flavor in the following manner:

1. Spontaneous Milk--milk that develops oxidized flavors without the aid of catalysts.
2. Susceptible Milk--milk that does not produce spontaneous oxidation but does produce oxidation in the presence of certain metal catalysts.
3. Non-Susceptible Milk--milk that does not develop oxidized flavors even when contaminated with certain metals.

The first two classifications are the most important. The third is rarely seen. The spontaneous oxidized flavor is the main concern, since it



affects the producer, distributor and consumer. The susceptible concerns only the dealer and consumer. Fortunately, the amount of milk that develops oxidized flavors spontaneously is relatively small, and when this milk is mixed with normal milk, it remains normal in flavor.

### Factors Associated With Oxidized Flavor Development

Metal Contamination - In a review of the literature on oxidized flavors in dairy products Brown, et al (3) stated that of all the factors concerned with bringing about oxidative changes in milk, metallic contamination, particularly by copper, is at present most important. The control of copper contamination in processing becomes increasingly more important as the sanitary quality of the products improve. The role of metals, especially copper, in producing oxidized flavors is believed due to their catalytic effect.

In a summation of metal contamination, Hunziker (20) concluded the following in regard to usage of metals and their effects on oxidized flavors:

1. Copper, iron and zinc - unfit for dairy use.
2. Nickel, aluminum and aluminum alloys - unfit for use with high acid milk.
3. Tin and tinned copper - can be used without ill effects.

More recent work by Sommer (38) surveyed the various metals with regard to oxidized flavor and reports that iron, copper, copper alloys, nickel, zinc and lead are unfit for dairy use. The use of stainless steel and glass are highly recommended.

Bacterial Content - Various investigators have reported that the development of oxidized flavors in milk has been more prevalent since the standards for

milk were so adjusted as to emphasize low bacterial counts; a high quality milk with a low bacterial count is more subject to oxidation than milk with a high bacterial count.

Brown, et al (3) found that milk with a high bacterial count rendered milk less susceptible to oxidized flavor, either by growth of bacterial cells using up oxygen or by lowering the oxidation-reduction potential.

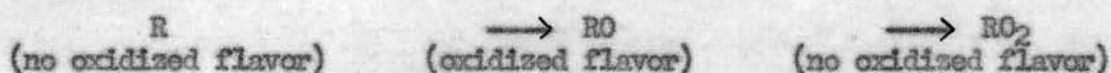
Dahle (8) found that counts of 150,000 to 200,000 per ml. were needed to protect raw milk from oxidation. Sommer (38) agreed that bacterial contamination and growth utilize the available oxygen in the milk and make conditions less favorable for the development of a tallowy flavor. He further stated that a dairy with high producing cows in the early stages of lactation, using properly washed and sterilized utensils, clean milking methods and proper cooling of the milk, is therefore more likely to experience trouble with a tallowy flavor than a dairy with scrub cows and indifferent care of the milk.

Cone and Babcock (5) studied the role of oxidase-reducing bacteria, using Pseudomonas putrefaciens and Pseudomonas fragi. They concluded that the absence and not the presence of bacteria is a cause of oxidized flavor development.

Oxidation-Reduction Potential - Sommer (38) concluded that fresh milk has a positive potential and that any cause tending to raise this potential will result in the formation of an oxidized flavor. The addition of a small amount of copper, or partly oxidized fat, raises the potential and results in oxidized flavors.

Greenbank (15) stated that oxidized flavor is the result of a mild oxidation of some minor milk constituent or constituents. He supported this

conclusion by showing that in a reducing environment such as that caused by mechanical removal of air, or the removal of oxygen by bacterial growth, or the presence of antioxidants, the flavor does not readily develop. However, under conditions favoring milk oxidation, such as low temperature of storage and the presence of certain metals that catalyze oxidation reactions, the oxidized flavor developed readily. He assumed that two stages of oxidation take place, first, a milk oxidation producing oxidized flavors, and second, complete oxidation producing a tasteless milk free from off-flavors. He symbolizes it as follows:



Thus when mild oxidizing conditions exist the oxidized flavor develops. Under more strongly oxidizing conditions the reaction proceeds to a more complete stage and the flavor does not develop.

#### Individual Cow (Breed, Age, Period of Lactation)

Guthrie and Brueckner (17) used milk produced by cows in the Cornell Experiment Station herd in an attempt to determine what effect individual cow's milk had on oxidized flavors. The milk was drawn directly into sterile, dark colored bottles free from exposure to air or metal contamination and then pasteurized at 143° F. for 30 minutes within two hours after milking. The milk was then cooled, held at 40° F. for three days, and scored for development of oxidized flavor. They found that 21% of a herd of 155 cows gave milk developing a distinct oxidized flavor and an additional 10% gave a slight oxidized flavor. They concluded that individual cows are irregular in their production of milk developing oxidized flavors. They also found no correlation between breed, age, and period of lactation with the formation of oxidized flavors.

Feed - England and Wiedimer (10) found that milk is more susceptible to oxidized flavors in the winter than at other seasons and attributed it to lack of green feeds in the diet, and that milk from cows consuming green feeds high in carotene and ascorbic acid is more resistant to oxidized flavor development than milk from cows consuming dry feeds.

Dahle (8) found that oxidized flavors could be eliminated by feeding alfalfa molasses silage. Babcock and Heller (2) did not agree with Dahle (8) but found that feeding molasses alfalfa silage, soybean silage, or corn silage had no significant effect on the copper tolerance of the milk produced.

Henderson and Roadhouse (19) found that milk from cows on a sub-maintenance ration was especially susceptible to oxidation because such milk had a high-proportion of unsaturated fats. Sommer (38) found sub-maintenance feeding more common than might be supposed when possible factors in tallowy flavor development are considered. Under-feeding may exist in times of feed shortage, drought, or when feed prices are high in relation to milk prices. It is also common during the first month of the lactation period in the case of high producing cows. In this case, the impulse to secrete milk is so high no matter how copious the feeding may be the cows are drawing upon body reserves to maintain the milk flow. As a result of this condition and because of more sanitary methods used in milk production by progressive dairymen, the trouble with tallowy flavor is more likely to be encountered by the better dairies.

Plant Operations - Pasteurization has been found by many investigators to be associated with oxidized flavors. Roland (35) found oxidized flavor the predominating off-flavor in 139 samples of commercial pasteurized milk from dairies in nineteen different cities during the winter of 1935-1936.

Studies by Nelson (31) demonstrated that highgrade cow's milk develops an oxidized flavor after pasteurization and that the intensity of the oxidized flavor increased during storage. This also indicated that it would be beneficial for milk dealers to deliver milk as soon after pasteurization as possible to avoid development of the oxidized flavor before the milk is consumed.

Gjessing and Trout (11) found that in milk pasteurized in the presence of copper at  $145.4^{\circ}$  F. for 30 minutes there occurred a destruction of ascorbic acid and a concurrent development of oxidized flavor. With milk pasteurized at  $167^{\circ}$  F. for 30 minutes under similar conditions the ascorbic acid was not destroyed and no oxidized flavor developed.

Sharp, et al (37) found that a pasteurizing temperature of  $145^{\circ}$  F. for 30 minutes caused the greatest copper contamination. Milk from the same herd was pasteurized at  $145^{\circ}$  F. for 30 minutes and  $170^{\circ}$  F. for 30 minutes. The results showed that milk pasteurized at lower temperatures developed off-flavors on storage, while the off-flavor was retarded in the milk pasteurized at the higher temperature. They concluded that pasteurized milk was more subject to development of oxidized flavor than was raw milk since the ascorbic acid is oxidized and this oxidation is hastened if copper equipment is used.

Cooling of Milk - Leeder and Herreid (27) found that pasteurized milk cooled over a surface cooler lost more ascorbic acid and oxidized more rapidly than milk cooled in a pasteurizing vat. This was attributed to the absorption of oxygen by the milk during surface cooling.

Plant Sterilization - Dahlberg and Carpenter (6) found that chlorine solutions used to sterilize equipment had a corroding effect on the metal, causing it to dissolve more readily in the milk. They recommended a hot water rinse

following chlorine usage to remove the chlorine, thereby avoiding corrosion. They concluded that the first hot milk through the equipment is likely to be contaminated with metals and recommend that it be discarded.

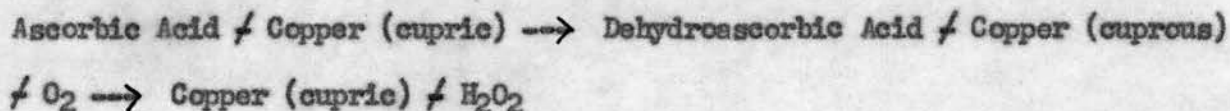
Effect of Containers and Delivery - Josephson (21) found that a 2.3% of retail milk delivered by route men is exposed to sunlight for more than two hours. The ascorbic acid loss was rapid and extensive in glass bottles exposed to sunlight, and after 30 minutes only insignificant quantities remained. The milk depleted of ascorbic acid is more susceptible to oxidized flavor development than unexposed milk.

England and Wiedner (10) found that mid-day sunlight induced oxidized flavors more readily in glass bottles than morning or afternoon sunlight, while milk in paper bottles is affected more by morning sunlight. They concluded that paper bottles offered more protection from sunlight oxidation than do glass bottles.

THE ROLE OF ASCORBIC ACID IN  
OXIDIZED FLAVOR DEVELOPMENT

Leeder and Herreid (27) concluded that ascorbic acid was an important factor in the development of oxidized flavor, mainly because its disappearance seemed to be associated with the off-flavors. Oxygen was also included because the chemical reactions accompanying ascorbic acid disappearance seemed oxidative in character. When oxygen was replaced by nitrogen, as in gas packing under a vacuum, the milk was less prone to oxidation and the vitamin C content was not lowered. Whitnah et al (47) reported an inverse ratio between the ascorbic acid content and the development of the oxidized flavor in milk from different breeds of cows. Rasmussen et al (34) observed that when milk developed an oxidized flavor the decrease in vitamin C content was coincident with the off-flavor development.

Olsen and Brown (33) postulated that in the presence of copper the following reaction takes place:



Hydrogen peroxide thus formed is a strong oxidizing substance and it further reacts with the phospholipids to give the oxidized flavor. By adding 0.1% of potassium iodide, they prevented the above reaction. The chemistry of the action of potassium iodide is explained by Mapson (29) who stated that the halides have a protective action on the oxidation of ascorbic acid by forming a copper halide complex ion.

Krukovsky and Guthrie (25), after experiments with hydrogen peroxide, did not agree with Olsen and Brown (33) and believed that any peroxide so formed would be used to oxidize the residual ascorbic acid and they further

postulated that the oxidized flavor developed when a certain equilibrium was reached between ascorbic and dehydroascorbic acids.

Krukovsky and Guthrie (24), in an extensive study of ascorbic acid in relation to oxidized flavor development, summarized the following:

- (1) The reaction producing the off-flavor was inhibited by quick and complete photochemical or chemical oxidation of ascorbic acid to dehydroascorbic acid prior to pasteurization and storage.
- (2) Partial oxidation of ascorbic acid promotes the development of the tallowy flavor.
- (3) The off-flavor is not induced by added copper (0.1 ppm.) in milk completely depleted of its total vitamin C content by quick oxidation of ascorbic acid and dehydroascorbic acid and subsequent pasteurization to destroy the dehydroascorbic acid.
- (4) "The reaction which produces the tallowy flavor could be induced again by the addition of ascorbic acid to milk which was just depleted of its ascorbic acid or of the total vitamin C content."

#### THE ROLE OF ENZYMES IN THE DEVELOPMENT OF OXIDIZED FLAVORS

Kende (22) reported oxidized flavors more prevalent during the winter when there was a scarcity of green feeds than during other seasons. He also reported that heating milk to 85° C. (185° F.) for five minutes inhibited the development of the oxidized flavor, while milk pasteurized at 63° C. (145.4° F.) for 30 minutes developed an oxidized flavor after short storage. Kende (22) further reported that 0.05 - 0.10 mg. of  $\text{CuSO}_4$  per liter was sufficient to cause the oxidized flavor in raw milk or in milk pasteurized at 63° C. (145.4° F.), while 24-40 times that amount of copper had no effect on



milk pasteurized at 35° C. Kende (22) postulated that the flavor was due to enzymatic action, naming the enzyme "oleinase." He believed this organic ferment was inactivated by the high temperature exposure.

Chilson (4) agreed with Kende (22) and believed that an oxidizing enzyme is present in skim milk which acts upon the lecithin to produce oxidized flavors. He theorized that the enzyme is catalyzed by copper and is most active at pasteurizing temperatures. He further postulated that the enzyme was destroyed at 170° F. for 10 minutes and the oxidized flavor development was thus prevented.

With slight changes as to time and temperatures of the heat treatment, the various investigators such as Guthrie and Brueckner (17), Greenbank (14), Thurston (44), and Sharp, Trout, and Guthrie (36) reported that the high heat treatments had an inhibitory effect on the development of the oxidizing flavor.

Whitnah, Ridell, and Caulfield (46), Sharp, Trout and Guthrie (36), King and Naugh (23), and Dahle and Palmer (7) observed less destruction of vitamin C when milk was heated in the absence of copper at temperatures of 160° F. (71.1° C), or above, than when pasteurized at 143° to 145° F. (61.6° - 62.8° C.) for 30 minutes.

Gjessing and Trout (11), concluded that 30° C. was the optimum temperature for vitamin C stability. They also reported that after pasteurization at 75° C. for 30 minutes the ascorbic acid exhibited marked stability in the presence of .13 mg. of copper per liter of milk with no development of oxidized flavors after three days storage at 0° C. to 5° C.

Gould and Sommer (12) made a study of the effect of heat in retarding oxidized flavors, with special attention to the cooked flavor. They found that the cooked flavor (not the heated flavor of holder pasteurized milk) was

the result of the formation of sulphides which were liberated when milk was subjected to sufficiently high temperatures. They also observed that temperatures high enough to produce the cooked flavor also inhibited the off-flavor development. They concluded that perhaps the liberated sulphides and not inactivated "olainase" as postulated by Kende (22) prevented the oxidized flavors at the higher temperatures.

Goold (13), studied oxidized flavors induced by ferrous iron and found that they were more easily retarded by high temperature treatments than were those induced by copper. He concluded that a higher temperature was needed to prevent a copper induced flavor due to the formation of a complex ion with the liberated sulphides.

Dahle (8) and Brown et al (3) agreed with Goold and Scamer (12) and believed that the enzyme theory was disproved by the various heat treatment taste which produce active reducing substances such as sulphides and sulphhydryl compounds which are capable of retarding or preventing the development of oxidized flavors.

The earliest observation that certain plant tissues catalyzed the oxidation of vitamin C was made by Szent-Gyorgyi in 1923 (40). The presence in cabbage leaves of a specific enzyme "hexoxidase," called "ascorbic acid oxidase" by later workers, was postulated by Szent-Gyorgyi (41) in 1930. Since that time, Lovett-Johnson and Nelson (28), Stots (39), and Tauber (42) have described the preparation and properties of ascorbic acid oxidase from a variety of plant sources. One of the simple preparations of the enzyme from cucumbers was described by Hand, Guthrie, and Sharp (18).

In describing the characteristics of ascorbic acid oxidase, Diemer and Zorban (9) stated that it is a protein-copper complex. They further stated that an increased copper content would increase the enzyme activity more than

40%. They reported that magnesium and zinc inactivated the enzyme in 40 to 50 minutes and that maximum enzyme activity occurred at 38° C. Diemar and Zerban (9) stated that the enzyme was relatively heat stable, being destroyed at 80° C. for a brief period.

According to a Japanese publication (1) the following was stated. "Raw milk oxidized ascorbic acid and a relatively weak ascorbic acid oxidase was obtained from it." Neuweiler (32) reported that human milk contains no ascorbic acid oxidase. Finally, Guigoz (16) and Winikoff (43) observed that the vitamin C content of human milk is nearly double that of cow's milk, human milk containing an average of 3.85 mg. per 100 ml. and cow's milk containing an average of 2.05 mg. per 100 ml.

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## METHODS

### A. The Determination of Ascorbic Acid in Market Milk

The Woessner (49) method for ascorbic acid determination in milk using the Klett-Summerson photoelectric colorimeter was the method selected for the determination of ascorbic acid in milk

#### 1. Reagents

##### a. Modified Wilberg's Reagent

Oxalic acid	0.6 gm.
Sodium chloride (C.P.)	4.8 gm.
Metaphosphoric acid (reagent grade sticks)	6.5 gm.
Distilled Water	100.0 ml.

##### b. 2, 6 Dichlorophenolindophenol crystals

##### c. Standard ascorbic acid (U.S.P.)

##### d. 0.05 N. acetic acid

##### e. Buffered sodium acetate solution

Sodium acetate	4.3 gm.
0.05 N. acetic acid	0.26 ml.
Distilled water	100.00 ml.

#### 2. Calibration

##### a. Standard dye solution

A few crystals of 2, 6 dichlorophenolindophenol was placed in five ml. of distilled water. This was then heated, filtered and diluted with enough distilled water to give a colorimeter reading of 400 on the Klett-Summerson photoelectric colorimeter, using filter 54 (green).

##### b. Standard ascorbic acid solutions

Solutions representing 5 to 50 mg. per liter of ascorbic acid in increments of five mg., were prepared in a mixture of three

parts of modified Wilberg's reagent and one part of distilled water to maintain a constant pH. Colorimeter readings were made and plotted on a graph which was used as the standard graph for ascorbic acid determinations.

### 3. Determination

- a. A twenty-five ml. quantity of milk was pipetted into a 125 ml. Erlenmeyer flask containing 75 ml. of modified Wilberg's reagent. The protein precipitate was removed by filtering through paper similar to Whatman #42. This procedure was carried out in a shaded portion of the laboratory, out of exposure to direct sunlight.
- b. Five ml. of the filtrate were then measured into a colorimeter tube and ten ml. of the indophenol-acetate solution added. The contents were mixed and read immediately.
- c. The reading obtained were then compared with the standard ascorbic acid graph and the mg. per liter of ascorbic acid were determined.

### B. Preparation of a Standard Copper Sulfate Solution

Since it was decided to use copper sulfate to catalyze the development of an oxidized flavor in the college processed milk, a standard copper sulfate solution was prepared so that each ml. when added to 1/2 pint of milk would represent a copper contamination of 1 ppm.

#### Calculations for Preparing the Copper Sulfate Solution

Weight of 1/2 pint of milk = 243.8 gms. then  $243.8 \times 0.000001 =$   
 $.0002438$  gram of copper needed for each 1/2 pint of milk. The copper sulfate used was  $\text{CuSO}_4 \cdot 5 \text{H}_2\text{O}$ . One gram molecular weight of this salt is 249.71

gm. and this contains 63.57 grams of copper. One liter of the desired solution would require 0.2438 gm. of copper ( $0.0002438 \times 1000$ ).

Then:

$$0.2438 : 63.57 :: x : 249.71$$

$$X = 0.9576 \text{ gm. of } \text{CuSO}_4 \cdot 5 \text{ H}_2\text{O}$$

required to make 1 liter of the standard solution.

C. Method Used in Processing Milk at the Oklahoma A. and M. Milk Plant

The milk produced by the Oklahoma A. and M. College herd was pasteurized at 143° F. for 30 minutes in two new 100-gallon stainless steel Steriline vats. The milk was then cooled in the vats to 130° F. and pumped through stainless steel sanitary pipes over a new Cherry-Burrell tinned copper cooler. From the cooler the milk flowed into a Cherry-Burrell bottle filler with a stainless steel supply tank. All equipment used for processing the milk was made of stainless steel or well tinned copper to insure a minimum of metal contamination.

## EXPERIMENTAL

### A. Amount of Ascorbic Acid Required to Prevent an Oxidized Flavor in Pasteurized Milk

The amount of ascorbic acid required to prevent an oxidized flavor in the pasteurized milk processed by the milk plant at Oklahoma A. and M. College was studied during the period from December 4, 1947 to January 6, 1948.

For each trial, twelve 1/2 pint bottles were collected immediately after leaving the bottle filler. Ascorbic acid determinations were made on the milk immediately and two sets of six each of the bottles were then treated as follows:

Sample 1 - control - nothing added

Sample 2 - 10 mg. per liter ascorbic acid added

Sample 3 - 20 mg. per liter ascorbic acid added

Sample 4 - 30 mg. per liter ascorbic acid added

Sample 5 - 40 mg. per liter ascorbic acid added

Sample 6 - 50 mg. per liter ascorbic acid added

One set of the samples was then stored at 40° F. and the other at 50° F. for 72 hours. Organoleptic determinations for flavor were made after 24, 48, and 72 hours storage and the ascorbic acid determined after the 72 hour period.

The results obtained in 10 trials on the amount of ascorbic acid required to prevent an oxidized flavor in pasteurized milk are shown in Table 1. The organoleptic determinations for flavor made after 24 and 48 hours storage are not included in the table since the flavor determinations after 72 hours storage are representative of the results obtained after 24- and 48-hour periods. In general, 30 mg. per liter of ascorbic acid was



Date	Control Sample Nothing Added			100 mg. per liter of Ascorbic Acid Added						20 mg. per liter of Ascorbic Acid Added						30 mg. per liter of Ascorbic Acid Added						40 mg. per liter Ascorbic Acid Added						50 mg. per liter of Ascorbic Acid Added					
	Oxidized Flavor after 72 hours storage at		mg. of ascorbic acid per liter	Oxidized Flavor after 72 hours storage at		mg. of ascorbic acid per liter		Oxidized Flavor after 72 hours storage at		mg. of ascorbic acid per liter		Oxidized Flavor after 72 hours storage at		mg. of ascorbic acid per liter		Oxidized Flavor after 72 hours storage at		mg. of ascorbic acid per liter		Oxidized Flavor after 72 hours storage at		mg. of ascorbic acid per liter		Oxidized Flavor after 72 hours storage at		mg. of ascorbic acid per liter							
	40° F.	50° F.	Start	After 72 hours Storage at 40° F. 50° F.		40° F.	50° F.	Start	After 72 hours storage at 40° F. 50° F.		40° F.	50° F.	Start	After 72 hours storage at 40° F. 50° F.		40° F.	50° F.	Start	After 72 hours storage at 40° F. 50° F.		40° F.	50° F.	Start	After 72 hours storage at 40° F. 50° F.		40° F.	50° F.	Start	After 72 hours storage at 40° F. 50° F.				
12-4-47	+++	++	17.8	9.6	10.2	++	++	21.8	10.6	11.1	/	/	37.8	15.2	15.6	---	---	47.8	36.7	36.4	---	---	57.8	43.6	45.2	---	---	67.8	47.5	47.8			
12-8-47	/	/	18.1	10.3	10.6	/	/	28.1	13.7	13.8	?	---	38.1	21.4	21.9	---	---	48.1	39.6	39.9	---	---	58.1	47.3	47.6	---	---	68.1	53.4	53.9			
12-9-47	/	?	15.2	6.1	6.9	/	/	25.2	12.6	12.9	?	?	35.2	21.2	21.8	---	---	45.2	33.4	34.2	---	---	55.2	42.3	44.4	---	---	65.2	50.7	51.9			
12-11-47	++	/	14.9	3.1	4.2	/	/	24.9	11.6	11.7	---	---	34.9	20.3	21.2	---	---	44.9	36.5	37.3	---	---	54.9	47.6	49.1	---	---	64.9	51.4	53.6			
12-12-47	++	/	12.6	2.4	2.8	++	/	22.6	7.4	8.5	/	?	32.6	18.1	18.8	---	---	42.6	30.4	30.8	---	---	52.4	39.1	40.6	---	---	62.4	48.3	49.1			
12-20-47	/	/	14.6	3.3	4.1	/	---	24.6	13.2	14.6	---	---	34.6	20.5	21.1	---	---	44.6	33.1	33.9	---	---	54.6	41.7	42.3	---	---	64.6	50.3	50.4			
12-24-47	---	---	15.8	7.4	8.2	---	---	25.8	19.3	19.5	---	---	35.8	24.3	24.7	---	---	45.8	37.1	38.4	---	---	55.8	43.0	43.1	---	---	65.8	58.3	60.3			
12-30-47	---	---	16.6	12.4	12.9	/	---	26.6	19.8	20.3	---	---	36.6	27.4	28.3	---	---	46.6	37.3	37.6	---	---	56.6	45.1	46.7	---	---	66.6	53.1	54.4			
1-5-48	---	---	14.9	8.7	8.8	---	---	24.9	14.6	15.4	/	?	34.9	17.3	18.2	---	---	44.9	30.7	32.1	---	---	54.9	40.1	41.3	---	---	64.9	49.7	51.1			
1-6-48	---	---	13.6	9.7	10.3	---	/	23.6	16.1	16.8	---	---	33.6	24.7	26.8	---	---	43.6	32.4	33.1	---	---	53.6	41.6	42.3	---	---	63.6	50.1	50.9			

Key: (---) = No oxidized flavor  
(?) = Questionable Oxidized Flavor

(/) = Slight Oxidized Flavor  
(+/) = Distinct Oxidized Flavor

(+/+/) = Strong Oxidized Flavor  
(+/+/+) = Very strong Oxidized Flavor

sufficient to prevent an oxidized flavor during storage at 40° F. and at 50° F. for 72 hours. The control samples developed oxidized flavors in varying degrees ranging from questionable to strong.

The control samples stored at 40° F. developed a slight oxidized flavor in three trials, a distinct oxidized flavor in two trials, and a strong oxidized flavor developed in one trial, and no oxidized flavor in the four remaining trials. The control sample stored at 50° F. developed a questionable oxidized flavor in one trial, a slight oxidized flavor in four trials, and a distinct oxidized flavor developed in one trial, and no oxidized flavor in the remaining four trials. It seemed that the control samples stored at 50° F. developed oxidized flavors of lower intensity than those stored at 40° F.

The milk fortified with 10 mg. per liter of ascorbic acid showed definite protective properties against the development of an oxidized flavor. However, the samples stored at 50° F. developed an oxidized flavor in six of the ten trials, while the samples stored at 40° F. developed an oxidized flavor in seven of the ten trials.

The results show that milk fortified with 20 mg. per liter of ascorbic acid offered more protection against the oxidized flavor development than the milk fortified with 10 mg. per liter of ascorbic acid. The samples stored at 40° F. developed a slight oxidized flavor in three trials, a questionable oxidized flavor in two trials, and no oxidized flavor in the remaining five trials. The samples stored at 50° F. developed a slight oxidized flavor in one trial, a questionable oxidized flavor in three trials, and no oxidized flavor in the remaining six trials.

The milk fortified with 30, 40, and 50 mg. per liter of ascorbic acid did not develop an oxidized flavor in any of the samples in the ten trials after storage at 40° F. or at 50° F. for 72 hours.

The results also show that oxidized flavors of lower intensity were noted at 50° F. However, this may have resulted from the absorption of odors from the cheese cooler where the milk was stored, which may have masked the oxidized flavor in the samples stored at 50° F. Also, the flavors of the samples that did not develop the oxidized flavor were superior in those stored at 40° F. compared to those stored at 50° F.

Furthermore, the results show that the samples stored at 50° F. showed less destruction of ascorbic acid than did the samples stored at 40° F. The ascorbic acid content in 42 of the 60 samples stored at 50° F. ranged from 0.1 mg. per liter to 0.9 mg. per liter higher than those samples stored at 40° F., while in the other 18 samples stored at 50° F. the ascorbic acid content ranged from 1.0 mg. per liter to 2.2 mg. per liter higher than in those samples stored at 40° F.

### B. Protective Effect of Ascorbic Acid Under Commercial Conditions

The protective effect of ascorbic acid under actual processing practices in the college milk plant was studied during the period from December 4, 1947 to April 10, 1948.

Two one-hundred gallon lots of milk were pasteurized at 143° F. for 30 minutes. One vat of the milk was cooled, bottled and used as the control. The other vat of milk was cooled to 130° F. and then 12.90 gm. of ascorbic acid (representing 35 mg. per liter) was dissolved in 250 ml. of distilled water and added to the milk. The milk was then cooled and bottled.

Two 1/2-pint samples from each vat of milk were obtained immediately after leaving the bottle filler and one sample from each was stored at 40° F. and at 50° F. The samples were examined organoleptically for flavor after 72 hours storage.

The results obtained in 101 trials on the protective effect of ascorbic acid under commercial conditions are shown in Table 2. These results show that 35 mg. per liter of ascorbic acid added after pasteurization was sufficient to prevent the development of an oxidized flavor in milk processed under conditions which insured a minimum of metal contamination. Fifty-five per cent of the control samples developed no oxidized flavor, 11 per cent a questionable oxidized flavor, and the remaining 34 per cent developed an oxidized flavor in varying degrees, ranging from slight to strong. The flavor of the milk fortified with ascorbic acid was superior to that in the control samples even when no oxidized flavor developed in the control.

TABLE 2

PROTECTIVE EFFECT OF ASCORBIC ACID UNDER COMMERCIAL CONDITIONS

Date	35 mg. per liter ascorbic acid added to vat			Control vat (no ascorbic acid added)		
	Ascorbic acid per liter		Oxidized flavor	Ascorbic acid per liter		Oxidized flavor
	Start	72 hrs. storage	after 72 hrs. storage	Start	72 hrs. storage	after 72 hrs. storage
12- 4-47	52.8	39.2	---	17.8	9.6	?
12- 5	53.2	41.6	---	18.2	10.1	f
12- 8	50.2	36.4	---	15.2	6.1	f
12- 9	49.9	39.6	---	14.9	7.4	-
12-10	50.7	40.1	---	15.7	6.1	?
12-11	49.7	38.4	---	14.7	3.1	f f
12-12	52.4	33.7	---	12.6	2.4	f f
12-13	50.7	30.7	---	13.4	4.7	-
12-15	51.4	36.3	---	15.2	1.4	f
12-16	52.1	38.3	---	16.3	8.1	-
12-17	49.4	29.4	---	11.6	0.0	f f
12-18	50.3	41.3	---	15.3	4.7	?
12-19	51.6	39.7	---	16.1	4.4	f
12-20	50.1	43.4	---	14.9	3.6	f

-- = no oxidized flavor

f = slight oxidized flavor

f f = strong oxidized flavor

? = questionable oxidized flavor

f f = distinct oxidized flavor

f f f = very strong oxidized flavor

TABLE 2

PROTECTIVE EFFECT OF ASCORBIC ACID UNDER COMMERCIAL CONDITIONS (Continued)

Date	35 mg. per liter ascorbic acid added to vat			Control vat (no ascorbic acid added)		
	Ascorbic acid per liter		Oxidized flavor	Ascorbic acid per liter		Oxidized flavor
	Start	72 hrs. storage		Start	72 hrs. storage	
12-22	49.6	38.7	---	12.1	6.4	-
12-23	48.4	33.2	---	9.4	1.1	f
12-24	52.6	44.3	---	15.8	7.4	-
12-25	51.2	40.4	---	15.2	8.1	-
12-26	53.2	40.3	---	17.6	10.8	-
12-27	50.2	37.4	---	14.9	6.1	?
12-29	47.4	30.1	---	12.4	3.6	f
12-30	50.4	38.7	---	16.6	12.4	-
12-31	50.4	34.2	---	17.2	11.6	-
1 - 5-48	51.4	36.8	---	14.9	8.7	-
1 - 6	48.4	37.1	---	13.6	9.7	-
1 - 7	51.2	38.4	---	16.2	5.2	+++
1 - 8	50.4	47.7	---	15.8	7.1	f
1 - 9	50.4	40.2	---	15.1	11.7	-
1 -10	50.9	46.4	---	16.1	9.8	-
1 -12	50.3	32.3	---	15.7	3.7	++

(Continued)

TABLE 2

PROTECTIVE EFFECT OF ASCORBIC ACID UNDER COMMERCIAL CONDITIONS (Continued)

Date	35 mg. per liter ascorbic acid added to vat			Control vat (no ascorbic acid added)		
	Ascorbic acid per liter		Oxidized flavor after 72 hrs. storage	Ascorbic acid per liter		Oxidized flavor after 72 hrs. storage
	Start	72 hrs. storage		Start	72 hrs. storage	
1-13	48.4	36.1	---	13.1	4.4	+
1-14	44.4	29.7	---	9.1	1.2	+
1-15	47.3	34.1	---	12.6	8.4	-
1-16	50.6	41.4	---	14.7	10.3	-
1-17	49.3	38.6	---	14.4	10.6	-
1-18	52.7	44.4	---	16.7	12.6	-
1-19	51.2	39.4	---	16.5	11.4	-
1-20	48.3	37.9	---	14.1	9.6	-
1-21	53.7	48.4	---	18.6	6.8	+
1-22	58.4	49.6	---	21.7	1.7	+++
1-23	51.0	37.4	---	16.4	3.1	++
1-24	54.6	39.9	---	18.3	2.5	+++
1-25	50.1	46.3	---	16.7	8.4	-
1-26	49.6	35.4	---	14.8	6.4	-
1-27	50.4	42.6	---	15.3	6.8	?
1-28	51.3	39.8	---	16.2	8.7	-

(Continued)

TABLE 2

PROTECTIVE EFFECT OF ASCORBIC ACID UNDER COMMERCIAL CONDITIONS (Continued)

Date	35 mg. per liter ascorbic acid added to vat			Control vat (no ascorbic acid added)		
	Ascorbic acid per liter		Oxidized flavor	Ascorbic acid per liter		Oxidized flavor
	Start	72 hrs. storage		Start	72 hrs. storage	
2-5	54.6	48.3	---	19.3	8.8	?
2-6	50.3	33.6	---	15.3	5.1	/
2-7	50.7	32.1	---	15.8	5.2	//
2-9	51.3	38.4	---	16.1	8.2	-
2-10	50.7	36.9	---	16.3	12.4	-
2-11	51.2	39.3	---	16.6	16.6	/
2-12	51.9	39.5	---	15.9	6.8	//
2-13	53.6	43.6	---	18.4	12.6	-
2-14	54.1	41.7	---	19.3	15.7	-
2-16	52.1	39.6	---	17.4	8.9	-
2-17	52.6	41.4	---	17.1	10.7	-
2-18	48.7	36.4	---	14.1	3.4	//
2-19	48.4	38.1	---	15.3	6.6	?
2-20	51.4	42.6	---	16.4	11.6	-
2-21	52.4	45.3	---	17.6	10.4	-
2-23	50.3	38.7	---	14.9	2.7	/

(Continued)



TABLE 2

PROTECTIVE EFFECT OF ASCORBIC ACID UNDER COMMERCIAL CONDITIONS (Continued)

Date	35 mg. per liter ascorbic acid added to vat			Control vat (no ascorbic acid added)		
	Ascorbic acid per liter		Oxidized flavor	Ascorbic acid per liter		Oxidized flavor
	Start	72 hrs. storage		Start	72 hrs. storage	
2-24	50.6	40.1	---	14.1	5.2	/
2-25	45.4	28.6	---	11.1	1.3	//
2-26	51.6	39.4	---	16.2	8.7	-
2-27	54.1	43.6	---	18.7	9.4	?
2-28	51.3	41.2	---	16.6	4.8	/
3- 1-48	50.7	33.6	---	14.1	6.4	/
3- 2	47.7	32.1	---	13.1	9.4	-
3- 3	52.1	44.3	---	16.1	10.3	-
3- 4	50.6	37.4	---	15.7	6.4	//
3- 5	50.8	39.6	---	16.7	8.3	/
3- 6	48.7	29.4	---	13.3	6.3	?
3- 8	49.6	32.7	---	14.1	8.4	-
3- 9	52.6	40.3	---	16.7	7.3	/
3-10	49.8	30.7	---	13.4	3.4	?
3-11	50.1	39.7	---	15.2	8.6	-
3-12	50.7	37.3	---	14.8	10.4	-

(Continued)

TABLE 2

PROTECTIVE EFFECT OF ASCORBIC ACID UNDER COMMERCIAL CONDITIONS (Continued)

Date	35 mg. per liter ascorbic acid added to vat			Control vat (no ascorbic acid added)		
	Ascorbic acid per liter		Oxidized flavor	Ascorbic acid per liter		Oxidized flavor
	Start	72 hrs. storage		Start	72 hrs. storage	
3-13	50.4	36.3	----	15.7	7.1	-
3-15	50.2	31.4	----	15.6	8.3	-
3-16	52.4	40.1	----	17.1	9.6	?
3-17	49.7	36.4	----	14.4	9.4	-
3-18	53.6	41.4	----	18.1	10.7	-
3-19	52.1	39.6	----	17.8	6.6	+
3-20	55.8	47.6	----	20.2	14.7	-
3-22	54.5	44.3	----	20.4	16.3	-
3-23	50.6	39.6	----	16.5	3.2	+
3-24	52.4	41.6	----	15.3	9.6	-
3-25	51.7	36.3	----	16.3	8.4	-
3-26	52.8	40.0	----	17.3	11.7	-
3-29	54.2	46.3	----	19.2	14.7	-
3-30	56.0	41.3	----	21.6	17.4	-
3-31	59.3	48.1	----	24.1	7.3	+

(Continued)

TABLE 2

PROTECTIVE EFFECT OF ASCORBIC ACID UNDER COMMERCIAL CONDITIONS (Continued)

Date	35 mg. per liter ascorbic acid added to vat			Control vat (no ascorbic acid added)		
	Ascorbic acid per liter		Oxidized flavor	Ascorbic acid per liter		Oxidized flavor
	Start	72 hrs. storage		Start	72 hrs. storage	
4-1-48	58.6	49.3	---	23.6	13.7	-
4-2	53.3	42.1	---	18.4	9.7	-
4-3	52.7	46.2	---	17.7	10.3	-
4-5	55.9	39.7	---	21.1	14.2	-
4-6	49.3	27.6	---	14.6	8.6	-
4-7	51.4	40.1	---	16.7	11.4	-
4-8	56.3	43.4	---	21.6	15.2	-
4-9	56.9	46.1	---	21.4	13.4	-
4-10	57.2	50.1	---	22.6	15.2	-

C. Amount of Ascorbic Acid Required to Prevent a Copper Induced Oxidized Flavor

1. The protective influence of 35 mg. per liter of ascorbic acid on milk contaminated with varying amounts of copper

Since 35 mg. per liter of ascorbic acid was found to be sufficient for the prevention of oxidized flavors in the milk processed at the Oklahoma A. and M. College milk plant it was deemed advisable to determine if that level of ascorbic acid would prevent oxidized flavors induced by added copper.

Thirty-five mg. per liter of ascorbic acid was added to the pasteurized milk prior to bottling (as in experiment B). For each trial, ten 1/2-pint bottles were collected immediately after leaving the bottle filler. Two sets of five each of the bottles were then treated as follows, using a standard copper sulfate solution as the source of the copper:

Sample 1 - control - nothing added

Sample 2 - 0.25 ppm. copper added

Sample 3 - 0.50 ppm. copper added

Sample 4 - 0.75 ppm. copper added

Sample 5 - 1.00 ppm. copper added

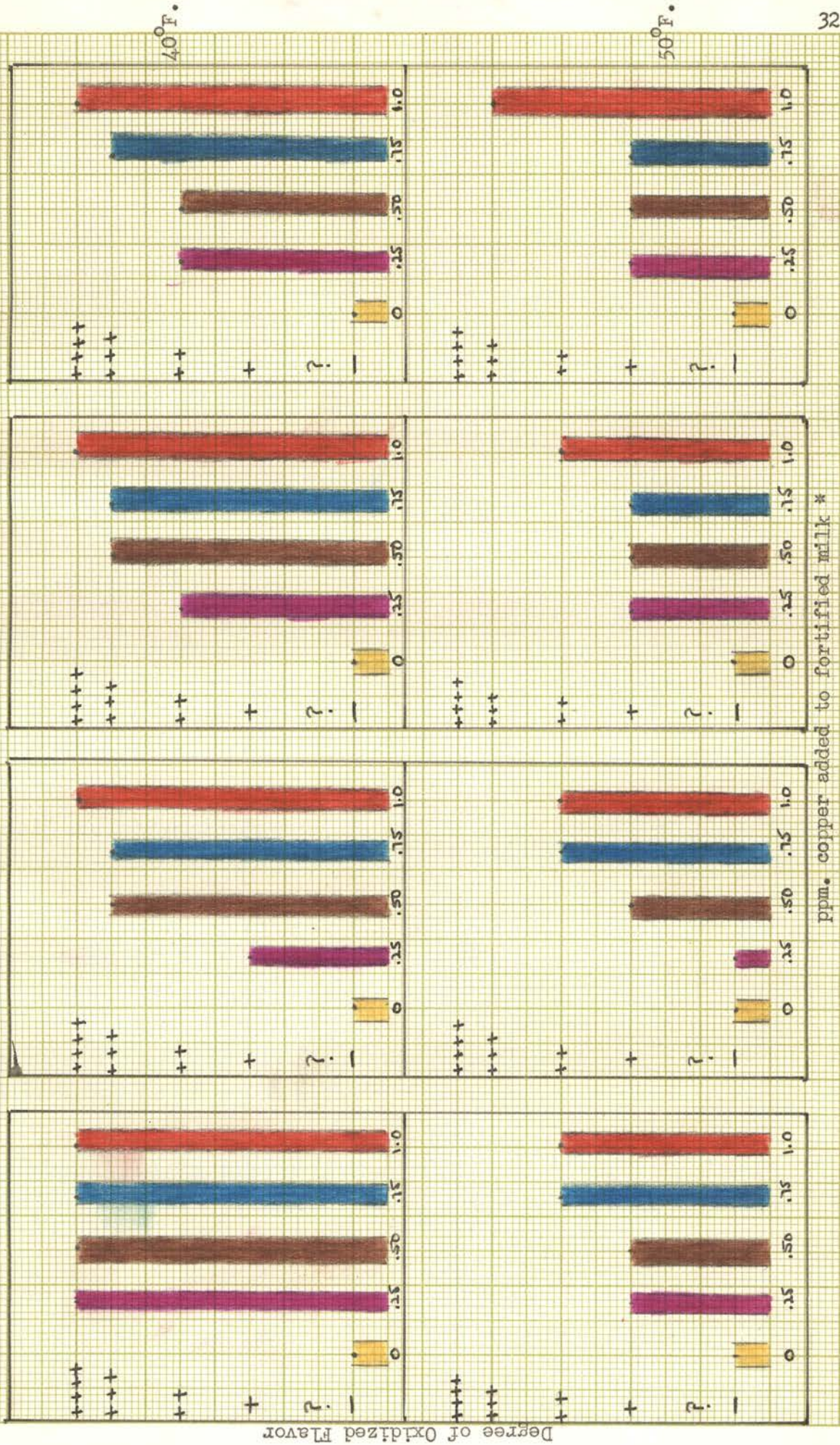
One set of the samples was then stored at 40° F. and the other set at 50° F. for 72 hours. Organoleptic determinations for flavor were made after 24, 48, and 72 hours storage.

The results obtained in four trials on the protective influence of 35 mg. per liter of ascorbic acid on milk contaminated with varying amounts of copper are shown in Bar graph 1. The graph does not include flavor observations at 24 and 48 hours because the flavor observations made after 72 hours storage were representative of the results obtained after 24 and 48 hours. The results show that the control samples which contained 35 mg. per liter

BAR GRAPH 1

THE PROTECTIVE INFLUENCE OF 35 mg. PER LITER OF ASCORBIC ACID  
ON MILK CONTAMINATED WITH VARYING AMOUNTS OF COPPER

\*Note: All milk used was fortified with 35 mg. per liter with ascorbic acid



ppm. copper added to fortified milk \*

of ascorbic acid but no added copper did not develop an oxidized flavor in any of the four trials when stored at either 40° F. or at 50° F. for 72 hours, whereas the milk samples with 0.25 to 1.0 ppm. of copper added all developed oxidized flavors in varying degrees during the storage. It appeared that the intensity of the oxidized flavor which developed during storage was closely correlated with the amount of copper contamination.

The samples contaminated with 0.25 ppm. of copper and stored at 40° F. developed a slight oxidized flavor in one trial, a distinct oxidized flavor in two trials, and a very strong oxidized flavor developed in the other trial. The samples contaminated with 0.25 ppm. of copper and stored at 50° F. developed a slight oxidized flavor in three trials and no oxidized flavor in the remaining trial.

The samples contaminated with 0.5 ppm. of copper and stored at 40° F. developed a distinct oxidized flavor in one trial, a strong oxidized flavor in two trials and a very strong oxidized flavor in the other trial. The samples stored at 50° F. and contaminated with 0.50 ppm. of copper developed a slight oxidized flavor in all four trials.

The samples contaminated with 0.75 ppm. of copper and stored at 40° F. developed a strong oxidized flavor in three trials and a very strong oxidized flavor in the other trial. The samples stored at 50° F. and contaminated with 0.75 ppm. of copper developed a slight oxidized flavor in two trials and a distinct oxidized flavor in the other two trials.

Milk contaminated with 1.0 ppm. of copper and stored at 40° F. developed a very strong oxidized flavor in all of the four trials. The samples stored at 50° F. and contaminated with 1.0 ppm. of copper developed a distinct oxidized flavor in three trials and a strong oxidized flavor developed in the remaining trial.

The results also show that apparently, oxidized flavors of lower intensity developed in the samples of milk stored at 50° F. than in those stored at 40° F. However, this was later attributed to the absorption of odors from the cheese cooler where the milk was stored, which may have masked the oxidized flavor in the samples stored at 50° F. The flavor was superior in the unoxidized milk stored at 40° F. than in the unoxidized milk stored at 50° F.

2. Amount of Ascorbic Acid Required to Prevent an Oxidized Flavor Induced by 1.0 ppm. Added Copper

Since 35 mg. per liter of ascorbic acid showed inadequate protective properties against copper induced oxidized flavor, another series of experiments were performed using varying levels of ascorbic acid and a constant copper contamination of 1.0 ppm.

The milk used was pasteurized at 143° F. for 30 minutes in the Oklahoma A. and M. College milk plant. For each trial ten 1/2-pint bottles were collected immediately after leaving the bottle filler. Two sets of five each of the bottles were then treated as follows, using a standard copper sulfate solution as the source of the copper:

Sample 1 - control - 1.0 ppm. copper added

Sample 2 - 1 ppm. copper added / 50 mg. per liter of ascorbic acid

Sample 3 - 1 ppm. copper added / 100 mg. per liter of ascorbic acid

Sample 4 - 1 ppm. copper added / 150 mg. per liter of ascorbic acid

Sample 5 - 1 ppm. copper added / 200 mg. per liter of ascorbic acid

One set of the samples was then stored at 40° F. and the other at 50° F. for 72 hours. Organoleptic determinations for flavor were made after 24, 48, and 72 hours storage.

The results obtained in 10 trials on the amount of ascorbic acid required to prevent an oxidized flavor induced by 1.0 ppm. of added copper are shown in Table 3. The results do not include flavor observations after 24 and 48 hours because the flavor observations after 72 hours storage was representative of the results obtained after 24 and 48 hours. However, the rate and extent of the oxidized flavor development in the milk varied inversely with the amount of ascorbic acid added. In general, 150 mg. of ascorbic acid per liter was sufficient to prevent an oxidized flavor when the milk was contaminated with 1.0 ppm. of copper.

The control samples which were contaminated with 1.0 ppm. of copper but not fortified with ascorbic acid all developed a strong oxidized flavor at both 40° F. and at 50° F. Milk fortified with 50 mg. per liter of ascorbic acid and stored at 40° F. developed a strong oxidized flavor in seven trials, a distinct oxidized flavor in two trials, and a slight oxidized flavor in the other trial. The milk containing 50 mg. per liter of ascorbic acid and stored at 50° F. developed a strong oxidized flavor in one trial, a distinct oxidized flavor in seven trials, and a slight oxidized flavor in the remaining trial. The samples stored at 50° F. apparently developed oxidized flavors of lower intensities than did those stored at 40° F.

Milk fortified with 100 mg. per liter of ascorbic acid and stored at 40° F. did not develop an oxidized flavor in six trials, but it developed a slight oxidized flavor in one trial and a questionable oxidized flavor in the other three trials. The milk containing 100 mg. per liter and stored at 50° F. did not develop an oxidized flavor in seven trials, but it developed a slight oxidized flavor in two trials and a questionable oxidized flavor in the remaining trial.



**TABLE 3**

AMOUNT OF ASCORBIC ACID REQUIRED TO PREVENT AN OXIDIZED FLAVOR  
INDUCED BY 1.0 PPM. OF ADDED COPPER

All milk treated subsequent to pasteurization with 1 ppm. "cu"

Date	Control Sample		50 mg. per liter ascorbic acid added		100 mg. per liter ascorbic acid added		150 mg. per liter ascorbic acid added		200 mg. per liter ascorbic acid added	
	Oxidized Flavor after 72 Hours Storage at									
	40° F.	50° F.	40° F.	50° F.	40° F.	50° F.	40° F.	50° F.	40° F.	50° F.
10-28-47	###	###	+	+	?	?	-	-	-	-
11-4	###	###	##	##	-	-	-	-	-	-
11-6	###	###	##	##	?	-	-	-	-	-
11-13	###	###	##	##	+	+	-	-	-	-
11-18	###	###	##	##	-	-	-	-	-	-
11-20	###	###	##	##	-	-	-	-	-	-
11-22	###	###	##	+	-	-	-	-	-	-
11-24	###	###	##	##	?	-	-	-	-	-
12-4	###	###	##	##	-	+	-	-	-	-
12-6	###	###	##	##	-	-	-	-	-	-

- = No oxidized flavor

? = Questionable

+ = Slight

## = Distinct

## = Strong

### = Very strong

Milk fortified with 150 mg. per liter and 200 mg. per liter of ascorbic acid did not develop an oxidized flavor at 40° F. or at 50° F. It was also observed that the milk fortified with 200 mg. per liter of ascorbic acid had a better flavor than the milk fortified with 150 mg. per liter of ascorbic acid.

The results show that while 50 mg. per liter and 100 mg. per liter of ascorbic acid did not completely protect the milk from the development of the oxidized flavors, it did, however, lower the intensities of the flavors produced and milk fortified with 100 mg. per liter of ascorbic acid offered more protection against the oxidized flavor development than did the milk fortified with 50 mg. per liter of ascorbic acid.

### 3. Protective effect of 50 mg. per liter of ascorbic acid on milk with varying amounts of copper added

The milk used was the regular pasteurized milk processed by the Oklahoma A. and M. milk plant. For each trial twelve 1/2-pint bottles were collected immediately after leaving the bottle filler. Three sets of four each of the bottles were then treated as follows:

Sample 1 -- control -- 50 mg. per liter ascorbic acid added

Sample 2 -- 0.25 ppm. copper added / 50 mg. per liter of ascorbic acid

Sample 3 -- 0.50 ppm. copper added / 50 mg. per liter of ascorbic acid

Sample 4 -- 0.75 ppm. copper added / 50 mg. per liter of ascorbic acid

One set of the bottles was then stored at 32° F., one set at 40° F., and the other set at 50° F. for 72 hours. Organoleptic determinations for flavor were made after 24, 48, and 72 hours storage.

Since it was believed that the milk stored at 50° F. in the cheese cooler was absorbing odors which were masking the intensity of the oxidized flavor developed at that temperature, it was deemed advisable to store the

milk at 50° F. in a thermostatically controlled incubator in the milk cold room. A storage temperature of 32° F. was also included to determine the influence of storage temperatures on the rate and extent of oxidized flavor development.

The results obtained in 20 trials on the protective effect of 50 mg. per liter of ascorbic acid on milk with varying amounts of copper added are shown in Table 4. The table does not include flavor observations after 24 and 48 hours because the flavor observations after 72 hours storage are representative of the results obtained after 24 and 48 hours. However, it was observed that the rate and extent of the oxidized flavor that developed in the milk was closely correlated with the amount of copper contamination. In general, 50 mg. per liter of ascorbic acid was sufficient to prevent an oxidized flavor in the milk contaminated with 0.25 ppm. of copper.

The control samples which contained 50 mg. per liter of ascorbic acid but no added copper did not develop an oxidized flavor after 72 hours storage when stored at 32° F., 40° F. or 50° F. The samples fortified with 50 mg. per liter of ascorbic acid and then contaminated with 0.25 ppm. of copper did not develop an oxidized flavor after 72 hours storage at any of the storage temperatures used.

The milk fortified with 50 mg. per liter of ascorbic acid and then contaminated with 0.50 ppm. of copper and stored at 32° F. developed no oxidized flavors in four trials, a questionable oxidized flavor in three trials, a slight oxidized flavor in nine trials, a distinct oxidized flavor in three trials and a strong oxidized flavor in the other trial. When stored at 40° F. no oxidized flavor developed in two trials, a questionable oxidized flavor developed in three trials, a slight oxidized flavor in eleven trials, a distinct oxidized flavor in one trial and a strong oxidized

**TABLE 4**

**PROTECTIVE EFFECT OF 50 MG. PER LITER OF ASCORBIC ACID  
ON MILK WITH VARYING AMOUNTS OF COPPER ADDED**

All milk treated subsequent to pasteurization with 50 mg. per liter ascorbic acid

Date	Control Sample			0.25 ppm. Copper Added			0.50 ppm. Copper Added			0.75 ppm. Copper Added		
	Oxidized Flavor after 72 Hours Storage at											
	32° F.	40° F.	50° F.	32° F.	40° F.	50° F.	32° F.	40° F.	50° F.	32° F.	40° F.	50° F.
2-5-48	-	-	-	-	-	-	/	/	?	##	##	##
2-6	-	-	-	-	-	-	/	/	?	##	##	##
2-10	-	-	-	-	-	-	?	/	?	##	##	##
2-12	-	-	-	-	-	-	##	##	##	##	##	##
2-16	-	-	-	-	-	-	/	/	-	##	##	##
2-17	-	-	-	-	-	-	-	?	?	##	##	##
2-19	-	-	-	-	-	-	##	##	##	##	##	##
2-23	-	-	-	-	-	-	##	/	/	##	##	##
2-25	-	-	-	-	-	-	?	-	-	##	##	##
2-27	-	-	-	-	-	-	-	-	-	##	##	##
3-1-48	-	-	-	-	-	-	-	?	/	##	##	##
3-3	-	-	-	-	-	-	##	##	##	##	##	##
3-5	-	-	-	-	-	-	/	/	/	##	##	##
3-8	-	-	-	-	-	-	/	/	?	##	##	##
3-9	-	-	-	-	-	-	/	/	/	##	##	##
3-10	-	-	-	-	-	-	?	/	/	##	##	##
3-15	-	-	-	-	-	-	-	?	?	##	##	##
3-17	-	-	-	-	-	-	/	##	?	##	##	##
3-18	-	-	-	-	-	-	/	/	##	##	##	##
3-29	-	-	-	-	-	-	/	/	/	##	##	##

- = No oxidized flavor      / = Slight oxidized flavor      ## = Strong oxidized flavor  
 ? = Questionable oxidized flavor      // = Distinct oxidized flavor      ### = Very strong oxidized flavor

flavor in the remaining three trials. When stored at 50° F. no oxidized flavor developed in three trials, a questionable oxidized flavor developed in seven trials, a slight oxidized flavor in six trials and a distinct oxidized flavor in the remaining four trials.

The milk fortified with 50 mg. per liter of ascorbic acid and then contaminated with 0.75 ppm. of copper developed oxidized flavors in varying degrees ranging from distinct to very strong in all the twenty trials.

The results show no uniform correlation between the temperature of storage and the intensity of the oxidized flavor development in milk with the various levels of copper contamination. The lower incidence of oxidized flavor development at 50° F. found in previous experiments was not apparent when the milk was stored at 50° F. in a thermostatically controlled incubator in the milk cold room. It would seem that the odors absorbed from storage in the cheese cooler masked the oxidized flavors in the samples stored there.

4. Protective effect of 75 mg. per liter of ascorbic acid on milk with varying amounts of copper added

The milk used was the regular pasteurized milk processed by the Oklahoma A. and M. milk plant. For each trial, twelve 1/2-pint bottles were collected immediately after leaving the bottle filler. Three sets of four each of the bottles were treated as follows:

Sample 1 - control - 75 mg. per liter ascorbic acid added

Sample 2 - 0.25 ppm. copper + 75 mg. per liter ascorbic acid added

Sample 3 - 0.50 ppm. copper + 75 mg. per liter ascorbic acid added

Sample 4 - 0.75 ppm. copper + 75 mg. per liter ascorbic acid added

One set of bottles was stored at 32° F., one set at 40° F., and the other set at 50° F. Organoleptic determinations for flavor were made after 24, 48, and 72 hours storage.

The results obtained in 20 trials on the protective effect of 75 mg. per liter of ascorbic acid on milk with varying amounts of copper added are shown in Table 5. The results do not include the flavor observations after 24 and 48 hours because the flavor observations after 72 hours storage are consistent with the results obtained after 24 and 48 hours. However, the rate and extent of the oxidized flavor that developed in the milk was closely correlated with the amount of copper contamination. In general, 75 mg. per liter of ascorbic acid was sufficient to prevent an oxidized flavor in the milk contaminated with 0.50 ppm. of copper.

The control which contained 75 mg. per liter of ascorbic acid but no added copper and the samples contaminated with 0.25 and 0.50 ppm. of copper and stored at 32° F., 40° F., and 50° F. did not develop an oxidized flavor in any of the twenty trials after 72 hours storage.

The samples contaminated with 0.75 ppm. of copper and stored at 32° F. developed a questionable oxidized flavor in three trials, a slight oxidized flavor in eight trials, a distinct oxidized flavor in eight trials, and a strong oxidized flavor in the other trial. The samples stored at 40° F. did not develop an oxidized flavor in one trial, developed a questionable oxidized flavor in one trial, a slight oxidized flavor in nine trials, a distinct oxidized flavor in eight trials and a strong oxidized flavor in the remaining trial. With the samples stored at 50° F., no oxidized flavor developed in two trials, a questionable oxidized flavor developed in three trials, a slight oxidized flavor in twelve trials, a distinct oxidized flavor in two trials, and a strong oxidized flavor in the other trial.

TABLE 5

PROTECTIVE EFFECT OF 75 MG. PER LITER OF ASCORBIC ACID  
ON MILK WITH VARYING AMOUNTS OF COPPER ADDED

All milk treated subsequent to pasteurization with 75 mg. per liter ascorbic acid

Date	Control Sample			0.25 ppm. Copper Added			0.50 Copper Added			0.75 ppm. Copper Added		
	Oxidized Flavor after 72 Hours Storage at											
	32° F.	40° F.	50° F.	32° F.	40° F.	50° F.	32° F.	40° F.	50° F.	32° F.	40° F.	50° F.
2-5-48	---	---	---	---	---	---	---	---	---	##	##	##
2-6	---	---	---	---	---	---	---	---	---	##	##	##
2-10	---	---	---	---	---	---	---	---	---	##	##	+
2-12	---	---	---	---	---	---	---	---	---	+	##	?
2-16	---	---	---	---	---	---	---	---	---	##	##	##
2-17	---	---	---	---	---	---	---	---	---	+	+	?
2-19	---	---	---	---	---	---	---	---	---	?	---	---
2-23	---	---	---	---	---	---	---	---	---	##	##	##
2-25	---	---	---	---	---	---	---	---	---	##	##	##
2-27	---	---	---	---	---	---	---	---	---	+	+	?
3-1-48	---	---	---	---	---	---	---	---	---	+	+	##
3-3	---	---	---	---	---	---	---	---	---	##	##	##
3-5	---	---	---	---	---	---	---	---	---	+	+	---
3-8	---	---	---	---	---	---	---	---	---	?	+	+
3-9	---	---	---	---	---	---	---	---	---	##	##	##
3-10	---	---	---	---	---	---	---	---	---	+	+	+
3-15	---	---	---	---	---	---	---	---	---	+	+	+
3-17	---	---	---	---	---	---	---	---	---	##	+	+
3-18	---	---	---	---	---	---	---	---	---	?	?	+
3-19	---	---	---	---	---	---	---	---	---	+	+	+

- = No oxidized flavor    + = Slight oxidized flavor    ## = Strong oxidized flavor  
 ? = Questionable oxidized flavor    ## = Distinct oxidized flavor    ### = Very strong oxidized flavor

The results show that the temperature of storage apparently had no significant influence on the rate or extent of oxidized flavor development. There appeared to be no difference in the flavor of the unoxidized milk stored at 32° F. or at 40° F., but the milk at those temperatures was superior in flavor to the unoxidized milk stored at 50° F.



D. The Relationship of Ascorbic Acid Oxidase to the Development of Oxidized Flavors in Milk

Krukovsky and Guthrie (24), as well as several other investigators, reported that rapid oxidation of ascorbic acid in milk occurs concurrently with the development of oxidized flavors, and that the oxidation of ascorbic acid is a key factor in the development of oxidized flavors in milk.

The possibility that enzymes cause oxidized flavors in milk has been postulated by Kende (22) and Chilson (4). Since the enzyme ascorbic acid oxidase is capable of rapidly oxidizing ascorbic acid, a series of experiments was carried out with the purpose of determining if the enzyme ascorbic acid oxidase in milk is related to the oxidized flavor development.

1. Protective effect of high temperatures exposure against oxidized flavor development

Since Dismar and Zerban (9) reported that the enzyme ascorbic acid oxidase is destroyed at 176° F. for brief periods of exposure, an experiment was performed to determine the influence of such heat treatment on the development of oxidized flavors in milk.

Two liters of milk, representing portions of the milk from four cows, were obtained directly from the milking machine in a three-liter Erlenmeyer flask during the afternoon milking. All the equipment used in handling the milk was made of glass to protect the milk against any metal contamination.

The milk was divided into four lots by placing 500 ml. of the milk in each of four one-liter Erlenmeyer flasks. Two lots of the milk were then laboratory pasteurized in a water bath at 143° F. for 30 minutes, and the other two lots of the milk were laboratory pasteurized in a water bath at 176° F. for five minutes. The four lots of milk were then cooled to approximately 60° F. with tap water. Each lot of milk was then further divided into three 1/3

pint samples and each lot treated as follows:

Sample 1 - control - pasteurized at 143° F. for 30 minutes

Sample 2 - pasteurized at 143° F. for 30 minutes / 0.25 ppm. copper added

Sample 3 - control - pasteurized at 176° F. for 5 minutes

Sample 4 - pasteurized at 176° F. for 5 minutes / 0.25 ppm. copper added

The samples were then stored at 32° F., 40° F., and 50° F. for 72 hours. Organoleptic determinations for flavor were made after 24, 48, and 72 hours storage.

The results obtained in 10 trials on the protective effect of high temperature exposure against oxidized flavor development in milk are shown in Table 6. The table does not include the flavor observations after 24 and 48 hours because the flavor observations after 72 hours storage were consistent with the results obtained after 24 and 48 hours. In general, pasteurization at 176° F. for five minutes protected the milk against the development of oxidized flavors even when the milk was contaminated with 0.25 ppm. of copper.

The control samples pasteurized at 143° F. for 30 minutes and stored at 32° F., 40° F., and 50° F. did not develop any more than a questionable oxidized flavor after 72 hours storage in any of the ten trials. The questionable oxidized flavor was present in only two of the samples stored at 32° F., four stored at 40° F., and in one stored at 50° F. The control samples pasteurized at 176° F. for five minutes did not develop an oxidized flavor after storage at 32° F., 40° F., and 50° F. for 72 hours in any of the ten trials.

The samples pasteurized at 143° F. for 30 minutes and then contaminated with 0.25 ppm. of copper developed oxidized flavors in all trials in varying degrees, ranging from slight to strong. There was no appreciable difference in the intensities of the oxidized flavors developed at 32° F., 40° F., or 50° F.

TABLE 6

PROTECTIVE EFFECT OF HIGH TEMPERATURE EXPOSURE  
AGAINST OXIDIZED FLAVOR DEVELOPMENT

Date	Pasteurized at 143° F. for 30'			Pasteurized at 143° F. for 30'			Pasteurized at 176° F. for 5'			Pasteurized at 176° F. for 5'		
	Untreated Control			+ 0.25 ppm. copper added			Untreated Control			+ 0.25 ppm. copper added		
	Oxidized flavor after 72 hrs. storage at			Oxidized flavor after 72 hrs. storage at			Oxidized flavor after 72 hrs. storage at			Oxidized flavor after 72 hrs. storage at		
	32°F.	40°F.	50°F.	32°F.	40°F.	50°F.	32°F.	40°F.	50°F.	32°F.	40°F.	50°F.
3-2-48	---	---	---	##	##	##	---	---	---	---	---	---
3-3	---	?	---	##	##	##	---	---	---	---	---	---
3-5	---	?	---	##	##	##	---	---	---	---	---	---
3-6	?	---	---	##	##	##	---	---	---	---	---	---
3-9	?	?	?	##	##	##	---	---	---	---	---	---
3-11	---	---	---	##	##	##	---	---	---	---	---	---
3-12	---	---	---	##	##	##	---	---	---	---	---	---
3-13	---	?	---	##	##	##	---	---	---	---	---	---
3-16	---	---	---	##	##	##	---	---	---	---	---	---
3-17	---	---	---	+	+	+	---	---	---	---	---	---

- = No oxidized flavor      / = Slight oxidized flavor      ## = Strong oxidized flavor  
 ? = Questionable oxidized flavor      # = Distinct oxidized flavor      ### = Very strong oxidized flavor

The samples pasteurized at 176° F. for five minutes and then contaminated with 0.25 ppm. of copper did not develop an oxidized flavor after 72 hours when stored at 32° F., 40° F., or 50° F. In all milk pasteurized at 176° F. for five minutes and then contaminated with 0.25 ppm. of copper, the characteristic "cooked flavor," reported by many investigators to be caused by the liberated sulphides when milk is heated to high temperatures, generally disappeared after 24-hour storage.

## 2. A Study of oxidized flavor in human milk

Since Neuweiler (32) reported that human milk contains no ascorbic acid oxidase, a series of experiments was performed, using human milk with the purpose of determining if milk containing no ascorbic acid oxidase would develop an oxidized flavor. The human milk used in this experiment was donated by the lactating mothers at the Oklahoma A. and M. College maternity ward, through the cooperation of the College Infirmary staff. The milk was collected after the afternoon feeding and placed in two ounce green "Duraglass" bottles. Two ounces of human milk was used for each trial. Each of the two-ounce samples of milk was divided into two lots of one ounce each. One lot was then left untreated and was used as the control, and the other lot was contaminated with 0.25 ppm. of copper. The samples were then stored at 40° F. for 72 hours. Organoleptic flavor determinations were made after 24, 48, and 72 hours storage.

The results obtained in 17 trials showed that oxidized flavors failed to develop either in the control or in the copper contaminated samples in any of the trials during 72 hours storage at 40° F. There appeared to be no difference in flavor between the samples containing copper and those with no added copper.

3. The relationship of temperature of exposure of milk to the oxidation of ascorbic acid

Since the rapid oxidation of ascorbic acid in milk seems to be associated with the development of an oxidized flavor, a series of experiments was performed to determine if high temperature exposures would prevent the oxidation of the ascorbic acid in milk.

One liter of milk representing portions of the milk from three cows was obtained directly from the milking machines in a two-liter Erlenmeyer flask during the afternoon milking. All the equipment used in handling the milk was made of glass to protect the milk against any metal contamination. One hundred and fifty ml. portions of the milk were then dispensed in 1/2 pint milk bottles, the bottles then placed in a water bath and heated to the following temperatures:

Sample 1 - 77° F.

Sample 2 - 86° F.

Sample 3 -104° F.

Sample 4 -122° F.

Sample 5 -140° F.

Sample 6 -158° F.

Sample 7 -167° F.

Sample 8 -176° F.

Sample 9 -185° F.

Sample 10-194° F.

Sample 11-203° F.

Ascorbic acid determinations were made on each sample immediately after the desired temperature was reached.

The results obtained in four trials on the relationship of temperature of exposures of milk to the oxidation of ascorbic acid are shown on Table 7, and the average destruction of ascorbic acid is shown on Graph A. In general, the ascorbic acid content of the milk remained constant at temperatures above 176° F. The oxidation of ascorbic acid to dehydroascorbic acid varied directly with the temperature of exposure, until a temperature of 140° F. was reached, with an average total destruction from the 77° F. to the 140° F. exposure of 6.85 mg. per liter of ascorbic acid. From 140° F. to 176° F. the destruction of ascorbic acid was very slight, with an average of only 0.10 mg. per liter of ascorbic acid. The destruction of ascorbic acid was most rapid between 104° F. and 140° F. with an average destruction of 4.8 mg. per liter of ascorbic acid. The ascorbic acid remained constant at temperatures above 176° F.

4. The influence of an inhibitor of ascorbic acid oxidase on the oxidation of ascorbic acid in milk

Since the rapid destruction of ascorbic acid in milk may possibly be enzymatic in action, an experiment was performed to demonstrate the influence of an inhibitor of the enzyme on the extent of destruction of ascorbic acid in milk, using sodium diethyl-dithio carbamate as the inhibitor of ascorbic acid oxidase.

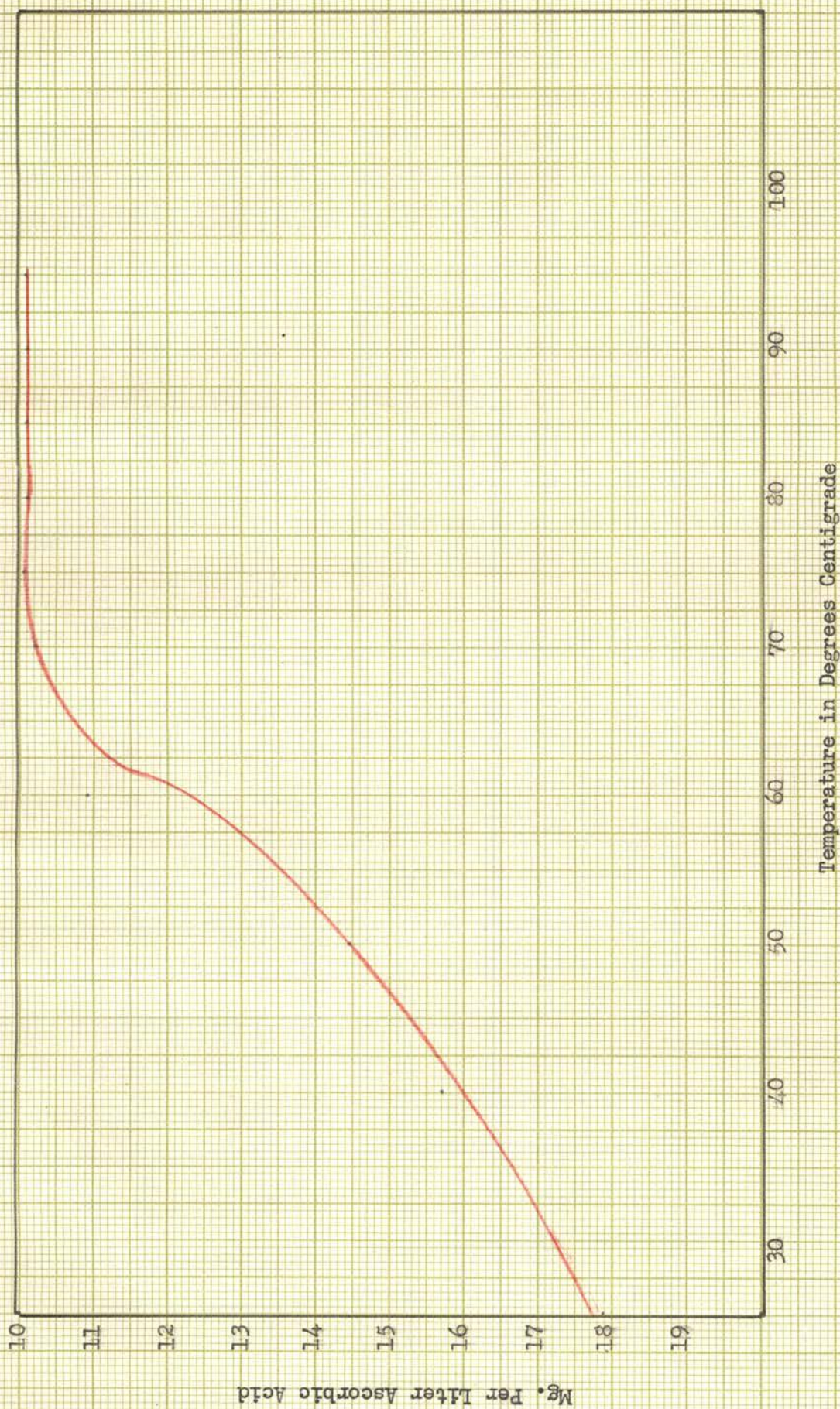
Two liters of milk, representing portions of the milk from six cows, were obtained directly from the milking machines in a three-liter Erlenmeyer flask during the afternoon milking. All the equipment used in handling the milk was made of glass to protect the milk against any metal contamination.

The milk was then fortified with 25 mg. per liter of ascorbic acid and divided into two lots of one liter each. One lot of the milk was laboratory pasteurized at 143° F. for 30 minutes and the other lot of milk was

TABLE 7

THE RELATIONSHIP OF TEMPERATURE OF EXPOSURES OF MILK  
TO THE OXIDATION OF ASCORBIC ACID

Date	<u>Temperatures of Exposure</u>										
	<u>77°F.</u>	<u>86°F.</u>	<u>104°F.</u>	<u>122°F.</u>	<u>140°F.</u>	<u>158°F.</u>	<u>167°F.</u>	<u>176°F.</u>	<u>185°F.</u>	<u>194°F.</u>	<u>203°F.</u>
Mg. per Liter of Ascorbic Acid											
3-8-48	19.2	18.6	17.4	12.6	10.4	10.0	9.8	10.0	10.0	10.0	10.0
3-13	17.2	17.0	15.8	14.3	9.8	9.0	8.8	9.0	9.0	9.0	9.0
3-15	17.2	16.4	14.8	13.8	12.2	10.8	10.4	10.4	10.4	10.4	10.4
3-19	17.8	17.0	15.8	13.0	11.2	11.0	10.8	11.0	11.0	11.0	11.0
Average	17.85	17.25	15.70	14.42	10.90	10.2	9.95	10.1	10.1	10.1	10.1

GRAPH A  
AVERAGE ASCORBIC ACID DESTRUCTION



Laboratory pasteurized at 176° F. for five minutes and both lots then cooled to approximately 60° F. Ascorbic acid determinations were made on both lots of milk immediately after pasteurization. For each trial, each lot of milk was divided into four samples by dispensing 50 ml. quantities into 2 oz. green Duraglass bottles. Three bottles of each sample were then prepared and treated as follows:

- Sample 1 - control - pasteurized at 143° F. for 30 minutes
- Sample 2 - 0.1% sodium diethyl-dithio carbamate added
- Sample 3 - 0.1% sodium diethyl-dithio carbamate / 0.25 ppm. copper added
- Sample 4 - 0.25 ppm. copper added
- Sample 5 - control - pasteurized at 176° F. for five minutes
- Sample 6 - 0.1% sodium diethyl-dithio carbamate added
- Sample 7 - 0.1% sodium diethyl-dithio carbamate / 0.25 ppm. copper added
- Sample 8 - 0.25 ppm. copper added

The samples were then stored at 40° F. and ascorbic acid determinations were made after 24, 48, and 72 hours, using one bottle of each sample for each determination to avoid any mechanical destruction of ascorbic acid.

The results obtained in four trials on the influence of sodium diethyl-dithio carbamate, an inhibitor of ascorbic acid oxidase, on the oxidation of ascorbic acid in milk are shown on Table 8. The results indicate that the addition of 0.1% of sodium diethyl-dithio carbamate to milk pasteurized at 143° F. for 30 minutes was sufficient to prevent the oxidation of the oxidation of the ascorbic acid even when the milk was contaminated with 0.25 ppm. of copper.

In the samples of milk pasteurized at 143° F. for 30 minutes and then stored at 40° F. for 72 hours, the control samples showed an average ascorbic

acid destruction of 14.4 mg. per liter, while the samples treated with 0.1% of sodium diethyl-dithio carbamate showed an average ascorbic acid destruction of 0.55 mg. per liter. The samples treated with 0.1% sodium diethyl-dithio carbamate and then contaminated with 0.25 ppm. of copper showed an average ascorbic acid destruction of 0.27 mg. per liter, while the samples contaminated with 0.25 ppm. of copper showed an average ascorbic acid destruction of 25.7 mg. per liter.

Results clearly show that 0.1% of sodium diethyl-dithio carbamate when added to milk pasteurized at 143° F. for 30 minutes was sufficient to prevent the oxidation of the ascorbic acid even when the milk was contaminated with 0.25 ppm. of copper.

In the samples pasteurized at 176° F. for five minutes and then stored at 40° F. for 72 hours, the control samples showed an average ascorbic acid destruction of 0.70 mg. per liter and those treated with 0.1% of sodium diethyl-dithio carbamate showed an average ascorbic acid destruction of 0.57 mg. per liter. The samples treated with 0.1% sodium diethyl-dithio carbamate and then contaminated with 0.25 ppm. of copper showed an average ascorbic acid destruction of 0.80 mg. per liter and those contaminated with 0.25 ppm. of copper showed an average ascorbic acid destruction of 0.90 mg. per liter. The results clearly show that pasteurization at 176° F. for five minutes was sufficient to prevent the oxidation of the ascorbic acid in the samples with and without copper added.

The above results would seem to indicate that the destruction of ascorbic acid is possibly due to enzymatic action because in all the milk heated at a temperature sufficient to destroy the enzyme (176° F. for five minutes) the ascorbic acid content showed no significant decrease during the 72 hour holding period while in the control samples pasteurized at 143° F. for 30 minutes

and with copper added there was a striking decrease in the ascorbic acid content during the same holding period. Further evidence that the destruction of ascorbic acid in milk is due to enzymatic action is apparent from the fact that when an inhibitor for the enzyme (sodium diethyl-dithio carbamate) was added there was no significant destruction of ascorbic acid in the milk pasteurized at 143° F. for 30 minutes, while in comparable samples without the inhibitor added there was a striking decrease in the ascorbic acid destruction. The flavor of the milk treated with 0.1% sodium diethyl-dithio carbamate was very undesirable and therefore it could not be used in commercial pasteurized milk to prevent the oxidation of ascorbic acid.

## DISCUSSION

The results indicate that the development of the oxidized flavors in milk is closely associated with the ionization of copper and the destruction of ascorbic acid. This is clearly shown by the fact that 35 mg. per liter of ascorbic acid, when added to milk without copper added, was sufficient to prevent the development of oxidized flavors. However, when 0.25 ppm. of copper was added to the milk, it required 50 mg. per liter of ascorbic acid to prevent the development of the oxidized flavors. The milk to which 0.5 and 1.0 ppm. of copper was added required 75 mg. per liter and 150 mg. per liter, respectively, of ascorbic acid to prevent the off-flavor development. Since ascorbic acid is a reducing agent, it seems logical to assume that by adding ascorbic acid to milk in amounts sufficient to create a reducing system (lowering the O-R potential) an oxidized flavor can be prevented. Since copper tends to raise the O-R potential in milk, an increase in copper content requires an increase in the amount of ascorbic acid that must be added to the milk to prevent the development of the oxidized flavor.

The various temperatures of storage (32° F., 40° F., and 50° F.) used in this study seemed to have had very little effect upon the intensity of the oxidized flavors developed. The lower incidence of oxidized flavor development at 50° F. encountered in some of the experiments when the milk was stored in the cheese cooler was not apparent when the milk was stored in a thermostatically controlled incubator in the milk cold room. This would seem to indicate that the odors absorbed from storage in the cheese cooler masked the oxidized flavors in the samples stored there.

Although the ascorbic acid content of the milk stored for 72 hours at 50° F. was slightly higher than the ascorbic acid content of the milk stored

at 40° F., it is hardly practical to assume that the slightly greater content of ascorbic acid (from 0.1 mg. to 2.2 mg. per liter) in the samples stored at 50° F. would have any protective effect against the development of an oxidized flavor. Since the flavor of the unoxidized milk stored at 32° F. and 40° F. was superior to the unoxidized milk stored at 50° F., it appears that perhaps an increase in bacterial growth in the samples stored at 50° F. was responsible for the flavor difference noted; however, this assumption was not substantiated by bacterial count.

The possibility that enzymes cause the oxidized flavors in milk was first postulated by Kende (22). He believed that when milk was heated to 176° F. the enzyme was destroyed and the oxidized flavor thus prevented. However, an investigation by Gould and Sommer (12) on the cooked flavor in milk showed that sulphides are liberated at a temperature of 176° F. They postulated that the liberated sulphides were strong reducing substances, and therefore prevented the oxidized flavor development. Several investigators have reported that the destruction of ascorbic acid in milk occurs concurrently with development of the oxidized flavor and some of them suggested that it may have been due to enzymatic action. It seems logical to assume that perhaps the enzyme involved was ascorbic acid oxidase. The results herein presented clearly show that pasteurization at 176° F. for five minutes prevented the development of an oxidized flavor in milk contaminated with 0.25 ppm. of copper. In all milk contaminated with 0.25 ppm. of copper, the characteristic "cooked flavor," reported by Gould and Sommer (12) to be caused by the "liberated sulphides," disappeared after 24 hours storage. The protective action attributed to the "liberated sulphides" would seem to be ineffective after 24 hours storage since the results reported herein showed that the "cooked flavor" had disappeared after that period.

Experiments using human milk, which contains almost twice as much ascorbic acid than does cow's milk (16) (48), showed that an oxidized flavor failed to develop in the control samples or in the samples contaminated with 0.25 ppm. of copper after 72 hours storage. Since human milk does not contain the enzyme ascorbic acid oxidase (32), and further since human milk did not develop an oxidized flavor even when contaminated with 0.25 ppm. of copper, these results would seem to indicate the possible importance of ascorbic acid oxidase in the development of oxidized flavor in milk.

In studying the relation of the oxidation of ascorbic acid to various temperature exposures, the results showed that the ascorbic acid content of the milk remained constant at temperatures above 176° F. Since Diemar and Zerban (9) have reported that the enzyme ascorbic acid oxidase is destroyed at 176° F. and since the results herein reported showed that when milk was heated to 176° F. there was no further oxidation of ascorbic acid, it would seem that possibly the oxidation of ascorbic acid in milk was due to the destruction of the enzyme ascorbic acid oxidase at the higher temperature and not to the formation of liberated sulphides. The results also show that inasmuch as the destruction of ascorbic acid in the milk was not proportional to the temperature increase above 140° F., it therefore appears that the destruction of ascorbic acid was due to enzymatic action.

In the experiments with sodium diethyl-dithio carbamate, an inhibitor of ascorbic acid oxidase, the results showed that 0.1 per cent of sodium diethyl-dithio carbamate when added to milk pasteurized at 143° F. for 30 minutes was sufficient to prevent the oxidation of the ascorbic acid even when the milk was contaminated with 0.25 ppm. of copper while in the milk without the added inhibitor, there was a pronounced decrease in ascorbic acid

content during storage for 72 hours at 40° F., and when contaminated with 0.25 ppm. of copper the milk showed an even greater destruction of ascorbic acid. This would seem to illustrate that the destruction of ascorbic acid in milk was due at least in part to the presence of the enzyme ascorbic acid oxidase.

## CONCLUSIONS

1. Thirty-five mg. per liter of ascorbic acid added to milk after pasteurization in a milk plant is sufficient to prevent the development of the oxidized flavor in the milk during 72 hours storage at 40° F. or at 50° F.
2. The rate and extent of the oxidized flavor development is closely correlated with the amount of copper added; as the amount of added copper is increased the amount of ascorbic acid required to prevent the oxidized flavor also increases.
3. The flavor of those samples of milk which do not develop an oxidized flavor is superior in the samples stored at 32° F. or at 40° F. to that of the samples of the same milk stored at 50° F.
4. There appears to be no close correlation between the temperature of storage of milk within the range of 32° F. to 50° F. and the rate and extent of development of oxidized flavors.
5. Pasteurization of milk at 176° F. for five minutes prevents the development of oxidized flavors even when 0.25 ppm. of copper is added.
6. The protective effect of high temperature pasteurization may be due to the inactivation of ascorbic acid oxidase.
7. Human milk, which contains almost twice the amount of ascorbic acid than does cow's milk and no ascorbic acid oxidase, does not develop an oxidized flavor even when contaminated with 0.25 ppm. of copper. This indicates that possibly ascorbic acid oxidase is a factor in the oxidized flavor development.



8. The destruction of ascorbic acid in milk during heating occurs rapidly until a temperature of  $140^{\circ}$  F. is reached, above which temperature the rate of destruction decreases until a temperature of  $176^{\circ}$  F. is reached; when the heating is continued above  $176^{\circ}$  F., no further destruction of ascorbic acid occurs. These data indicate that the destruction of ascorbic acid is probably due to the enzyme ascorbic acid oxidase which is inactivated at a temperature of  $176^{\circ}$  F.

9. The addition of 0.1 per cent of sodium diethyl-dithio carbonate (an inhibitor of the enzyme ascorbic acid oxidase) to milk pasteurized at  $143^{\circ}$  F. for 30 minutes with or without added copper prevents the destruction of ascorbic acid during 72 hours storage at  $40^{\circ}$  F. Therefore, the oxidation of ascorbic acid in milk seems to be due at least in part to the enzyme ascorbic acid oxidase.

10. The development of an oxidized flavor in milk may be due to the destruction of ascorbic acid by the enzyme ascorbic acid oxidase.

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