SOME FACTORS AFFECTING THE ASCORBIC ACID CONTENT OF MILK AND ITS POSSIBLE ASSOCIATION WITH THE OXIDIZED FLAVOR

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Thesis

Respectfully submitted to the Graduate School of Michigan State College of Agriculture and Applied Science in partial fulfillment of the requirement for the Degree of Master of Science.

By

Erland C. Gjessing

1938

THESIS

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INTRODUCTION

Recent developments in the chemical aspects of vitamin C, in its isolation and in the exact chemical methods for its determination have made it possible to estimate rapidly and with a high degree of accuracy the amount of vitamin C, ascorbic acid, in a great variety of food products.

The importance of vitamin C in human nutrition is a well established fact. That of average milk is considerably below the human requirements and especially those of the child. However, the amount of vitamin C as such is not necessarily the chief object sought in its estimation. Recent years' studies on the structure and chemistry of vitamin C have shown characteristics regarding its oxidation-reduction potentials, its activation and inactivation by specific enzymes, as well as its response to metals. The role of vitamin C in the regulation of colloidal conditions and, recently, the suggestion that the vitamin itself might act as a catalyst are also of considerable interest to ascertain phases of market milk investigation.

These observations indicate that the determination of vitamin C under various conditions might throw some light on some of the physical and chemical problems in milk to which vitamin C undoubtedly is closely related and which are of major importance in the dairy industry.

Lately, several investigations have dealt with the vitamin C in milk as affected by breed, lactation, season and flavors. However, very little work has been done extensively or in detail in regard to the relationship between flavor and vitamin C in milk from individual cows, or to the effect of various heat treatments of the milk upon the stability of vitamin C. The object of this experiment has been to study the vitamin C of various milks and to correlate, if possible, the amount of this vitamin with several factors incident to production.

REVIEW OF LITERATURE

GENERAL DISCUSSION

Szent-Gyorgi (1932) undoubtedly was the first investigator who isolated vitamin C from the adrenal cortex. He named it hexuronic acid and did not try to identify it with vitamin C. The first identification appears to have been done by Waugh and Ray (1931). Shortly thereafter, Sverbely and Szent-Gyorgi (1932) proved that hexuronic acid and vitamin C were identical. Later work by these and other investigators has substantiated the identity of vitamin C and hexuronic acid, or ascorbic acid, the name which will be used frequently in this presentation. There seems to be no reasonable basis for questioning the identity of the vitamin now.

Two separate methods have been generally used in determining the amounts of vitamin C in food products. These are, by biological assay and by chemical titration.

Tillman and Hirsch (1932) were the first to show the close relationships which existed between the data secured when the amount of vitamin C was determined by titration with 2-6 dichlorophenolindophenol method and by biological assays. These close correlations were later confirmed by Bessey and King (1934) who made improvements over Tillman's method. Harris and Ray (1933) also confirmed these findings.

Several other methods, Tauber and Kleiner (1935), Tauber (1935), Tauber and Kleiner (1935), Gothlin (1933), Martini and Bonsignore (1934) and Joseph (1936) have been developed in the determinations of vitamin C. However, the titration method is by far the most extensively used

within recent years. Some factors obtain in the titration method which may give rise to errors. King (1936) classified them as follows: "(a) Other substances may be present which reduce the reagent; (b) a portion of the vitamin C may be present in the reversibly oxidized form (which is still biologically active); (c) substances may be present which interfere with the reaction of either the oxidizing or reducing agent. In only a few cases has there been evidence of serious error when the titrations were carried out rapidly in acid solution." He believed, therefore, that due caution should be used in the interpretation of results obtained by the titration method.

Methods of determining ascorbic acid in milk by titration.

Sharp (1938) proposed a method for determining ascorbic acid in milk in which the milk was titrated directly after being acidified. This method was rapid and checked fairly well when ascorbic acid was added to milk and then redetermined.

Whitnah and associates (1936) and Russell and associates (1936) precipitated the proteins with trichloroacetic acid and then titrated the filtrate with 2-6 dichlorophenolindophenol. Both of these procedures were proposed originally by Bessey and King (1934).

Amount of vitamin C in raw milk.

1. Effect of ration. The laboratory syntheses of vitamin C is accomplished by the use of carbohydrates. Micheel and associates (1934), in one of the technical syntheses of vitamin C, used d-glucose as the initial product. According to these investigators, it was unreasonable to suspect that the living organism might be able to

synthesize vitamin C under certain conditions pertaining to the diet. Guha and Ghash (1936) showed that subcutaneous and intravenous injections of mannose in rats was followed by an increase in the vitamin C content of the various organs. However, the ability or non-ability of the living organism to synthesize vitamin C was not fully clarified.

No very extensive work has been done on the effect of the ration of the dairy cow on the amount of vitamin C in milk. Riddell and associates (1936) have made studies of the influence of the ration on the ascorbic acid content of the milk. They fed a dry ration plus silage and pasture to one group of cows and a dry ration plus no silage or pasture to a second group, using the double reversal system. They concluded from the results obtained that the rations studied had no significant influence on the ascorbic acid content of the milk.

Russell and associates (1936), in a study of the effects of breed characteristics and stages of lactation on the ascorbic acid content of the milk, stated that after the first two months of lactation the ascorbic acid content of the milk was apparently dependent solely upon the ascorbic acid content of the ration fed the cow.

Whitnah and associates (1936) regarded an increased intake of ascorbic acid in the feed to be accompanied by an increased excretion of ascorbic acid in the urine, but with no increased secretion in the milk. According to Riddell and associates (1936) no significant increase in the ascorbic acid content of the milk occurred when the cow's rations were changed from a good winter ration to pasture. They explained this on the basis that the cow was able to synthesize ascorbic acid from the feeds of the winter ration.

In studies on the relationship between off flavors and the ascorbic acid content of milk, Brown and associates (1937) showed that feeding tomato juice or lemon juice removed the milk's susceptibility to become oxidized. They explained this as due to the increased ascorbic acid content of the milk. Anderson (1937), in the feeding of carrots to cows, secured similar data, explaining them as did the above investigators.

Stanislaw and associates (1937) stated that their work indicated there was no evidence of any effect of pasture feeding on the amount of vitamin C in the milk.

2. Effect of breed. Russell and associates (1936) found on the average the following amounts of vitamin C in milk from cows of different breeds:

Holstein,	Av.	10.0	mg.	ascorbic	acid	per	quart
Ayrshire,	"	12.9	"	"	"	"	"
Guernsey,	"	13.0	"	"	"	"	"
Jersey,	"	13.3	"	"	"	"	"
Brown Swiss,	"	14.8	"	"	"	"	"

They claim that this difference in the ascorbic acid content could not be explained on the basis of the different amounts of milk produced by the cows as two of the different breeds (Brown Swiss and Holstein) during the experimental period produced the same amount of milk.

Whitnah and Riddell (1936) found that the average values of ascorbic acid for the different breeds were as follows:

Holstein,	Av.	23.5	mg.	ascorbic	acid	per	liter
Ayrshire,	"	24.1	"	"	"	"	"
Guernsey,	"	26.0	"	"	"	"	"
Jersey,	"	29.2	"	"	"	"	"

These investigators stated that the high values for this particular

Jersey herd which was investigated in this work was significant. The average ascorbic acid content of milk for all cows and for all months tested was 25.5 mg. ascorbic acid per liter.

Stanislaw and associates (1937) concluded that the vitamin C content of milk was not affected by breed.

3. Effect of stage of lactation and season. Russell etal. (1936) concluded from their work that the stage of lactation appeared to have a more definite effect upon the ascorbic acid content of milk than the breed differences. The ascorbic acid content was found to be relatively higher during the early stages of lactation, but decreased to a minimum after about two months of lactation, and then increased to a maximum with later stages of lactation. In the same work a few samples of colostrum were also titrated and in each case the vitamin C content was found to be unusually high. This high ascorbic acid content was believed to indicate a certain degree of storage of this substance by the cow during the preparturition period.

Whitnah and Riddell (1937) stated that the early stages of lactation coincided with the low ascorbic acid content of the milk.

Stanislaw and associates (1937) stated that in three out of four cases studied the ascorbic acid content of colostrum was higher than that of average milk.

Ferdinand (1936) has shown that the vitamin C content of cows and humans milk, estimated by the methylen blue titration, was lower in the early spring than in winter.

4. Effect of mastitis. Stanislaw and associates (1937) found that the quarter infected with mastitis yielded milk definitely poorer in ascorbic

acid than that secreted by the normal udder.

5. Effect of bacteria. Little work has been done to show the effect of bacteria upon ascorbic acid. Thatschenko (1936) showed that lactic acid bacteria of the type Bulgaricus acidophilus and B. acidolphilus leichmani reduced the reversible oxidizable form of ascorbic acid to dehydroascorbic acid. Lominski (1936) showed that ascorbic acid in high dilution inactivates bacteriophage. This was probably due to the reducing acition of ascorbic acid since other reducing substances produce similar effects. Gagyi and Ujsagly (1936) found that certain bacteria possessed a capacity for destroying vitamin C. In experiments carried out by incubating suspensions of virulent organisms with vitamin C solutions, they found that the destruction was a partly reversible process and that a certain quantity of vitamin C would be restored on reduction with hydrogen or hydrogen sulphide.

6. Presence of dehydrascorbic acid in milk. Stanislaw and associates (1937) showed, in determining the two forms of vitamin C, ascorbic acid and dehydroascorbic acid, by treating milk with hydrogen sulphide, that as milk was drawn from the normal udder it contained only the reduced form of ascorbic acid. Their results, in part, are as follows:

Reduced ascorbic acid,	2.17 mg. ascorbic acid per 100 ml.
Total ascorbic acid,	2.21 " " " " " "

The effect of heat treatment upon the stability of ascorbic acid.

Considerable work has been done on the general effect of heat on ascorbic acid. However, little of this work has been done in respect to specific time-temperature relationships.

La Mer and associates (1922) were probably the first ones to study the effect of different temperatures on the vitamin C content of vegetable juices. They studied the effect of heating upon the destruction of the vitamin C in tomato juices heated for periods of one to four hours at 60°, 80° and 100° C. The results showed that the velocity of destruction, under the conditions of the experiement, decreased with the time and to a greater degree than would be expected if the reaction followed the unimolecular law of the square-root rule of Schultz. The percentage destroyed varied emperically as the square root of the time.

Schwartze and associates (1930) found by biological assays that, by boiling lightly three quarts of milk for five minutes in a glass beaker or in an aluminum pan, the vitamin C content was reduced approximately 20 per cent. In another experiment Schwartze (1931) and associates studied pasteurization of milk aerobically in aluminum, copper and tinned copper tubular pasteurizers. The destruction of vitamin C in aluminum pasteurizers was from 20 per cent to 40 per cent. This reduction was larger than that found in their previous experiments on boiling milk for five minutes. These results were rather to be expected as the duration of the exposure of pasteurization was much longer and was strictly aerobic. Tinned copper gave slightly greater losses. Pasteurizing in exposed copper cans resulted in losses of the vitamin C up to 80 to 90 per cent.

King and Waugh (1934) proved that there was no significant destruction of vitamin C in milk by the Electropure and Han Vik (flash contact) methods of pasteurization when all aluminum equipment was used.

This, according to their theory, was due to: first, a very short heating time; second, to the methods of heating; third, to a protection from the atmospheric oxygen during heating; and fourth, to a minimum exposure to metals having catalytic effects. The findings are based upon two series of animal assays of the raw and pasteurized milk and also upon the titration of the vitamin by means of 2-6 dichlorophenolindophenol method. In this experiment they also pastuerized some milk in the same types of tank for 30 minutes at 143 to 145° F., which resulted in a significant destruction of vitamin C.

Except where specified, as in the work of Waugh and King, the vitamin C determinations in the three researches reported above were done by biological assays. In the research reported below the determinations were made by the titration methods.

Whitnah and associates (1936) concluded that the stability of ascorbic acid in fresh raw milk was greater than that either in the raw or in the pasteurized samples secured at commercial pasteurizers. No immediate loss was found to result from pasteurization by the short-time high-temperature method. The 30 minutes holding process of pasteurization was not well adapted for the conservation of ascorbic acid in milk. Among all the commercial types using 30 minute holding periods only the glass lined vat produced pasteurized milk where loss of ascorbic acid on storage was as small as in the raw milk.

Sharp (1936) demonstrated that an insignificant amount of ascorbic acid was destroyed by heating for 30 minutes at 63° F. providing the milk was not metal contained. After three days the pasteurized milk contained more ascorbic acid than did similar milk raw.

Dam and Satterfield (1937) regarded the 2-6 dichlorophenolindrophenol titration method used by Sharp for the estimation of ascorbic acid in milk reliable, but only when applied to fresh milk. Difficulties with ascertaining the correct endpoint would seem to render Sharp's method less reliable for milk samples stored for three or more days.

Kon (1937) stated that pasteurization by the holder method destroyed the reversibly oxidized ascorbic acid but did not affect the reduced form of ascorbic acid in milk. The amount of destruction of ascorbic acid caused by pasteurization in the absence of catalytic metals was thought to depend upon the previous exposure of the milk to light.

Reedman (1937) stated that milk pasteurized for 30 minutes by the holding method retained over 70 per cent of its original ascorbic acid contant. Schlimmer and associates (1932) stated that short-time cooking did not reduce the ascorbic acid very much. In this experiment the holding method gave lowered ascorbic acid content.

Effect of metal contamination on the stability of ascorbic acid.

Nearly all investigators are agreed that small amounts of copper catalyze oxygen uptake of the vitamin. Several other metals will naturally have the same effect although probably not so pronounced as the copper.

Kellie and Zilva (1935) showed that ordinary laboratory distilled water contains enough quantities of copper and iron, especially copper, to catalyse the irreversible oxidation of dissolved ascorbic acid. Sharp (1936) showed the accelerating effect of soluble copper on the oxidation

of ascorbic acid. The following figures show their average results expressed as mg. ascorbic acid per liter.

Fresh pasteurized milk	20.1
After holding for 3 days at 35° F.	
Raw	11.3
Past. (145° F. 30 min.)	11.0
" " " " - 0.13 mg. Cu per liter	1.7

Whitnah and associates (1936) demonstrated that the addition of copper produced increased losses of ascorbic acid both in raw and in pasteurized milk. However, the presence of copper did not result in a serious loss in the raw milk. At least 20 times as much iron or 20 times as much chromium or nickel as copper were required to produce comparable destruction of ascorbic acid. Copper (or brass) was probably 100 fold more soluble in milk than stainless steel. Of the four metals studied, copper, iron, nickel, and chromium, copper was the only one which brought about a decreased ascorbic acid content in both pasteurized and in stored milk. This fact substantiated the earlier inference that excessive losses of ascorbic acid after pasteurization and storage indicated contamination with copper. Copper supplements did not at any time produce a detectable difference in the losses of ascorbic acid content of fresh raw milk or freshly pasteurized milk.

Schlimmer and associates (1932) have shown that the metals, copper and silver, are harmful to the ascorbic acid content of milk. Nickel, chromium, and aluminum did not show any effect upon the ascorbic acid content of milk.

Influence of aeration upon the ascorbic acid content of milk.

A report of research on the effect of aeration upon the ascorbic

acid content of milk or other food products could not be found in the literature. It is obvious, though that our present knowledge of vitamin C and its chemical characteristics permits us to conclude definitely that aeration will destroy ascorbic acid.

Already Svent-Gyorgi (1931) showed that hexuronic acid (ascorbic acid) showed a fairly high reduction potential. It lost two hydrogen atoms upon mild oxidation and, if once slightly oxidized, could be reduced again by treatment with hydrogen sulphide.

That ascorbic acid can be oxidized several ways, both reversibly and irreversibly, is a well known fact.

Roe and Barnum (1936) demonstrated that reversibly oxidized ascorbic acid has approximately one-fourth the antiscorbutic potency of ascorbic acid in its reduced form when administered to guinea pigs. Tauber and Kleiner (1935) have advised a method for determining reversibly oxidized ascorbic acid. This is accomplished by treating the substrate which contains the reversibly oxidised ascorbic acid with hydrogen sulphide which reduces the oxidized form. The reduced form then can be titrated as usual.

Sharp (1938) recently recommended a method for determining reversibly oxidised ascorbic acid in milk in which method the same principle is used.

Smith and King (1931) found that the purified preparations of ascorbic acid can be held for a period of two or three weeks without serious loss of activity, if properly protected from oxidation during laboratory manipulations and subsequent storage by the use of an atmosphere of nitrogen or carvon dioxide. Dry ice proved to be particularly suitable.

Tillman and associates (1932) demonstrated that ascorbic acid could be oxidized reversibly and irreversibly. The first kind of oxidation could be brought about by iodine, by hydrogen peroxide, or by 2-6 dichlorophenolindophenol and then reversed with hydrogen sulphide, whereas oxidation by atmospheric oxygen produced irreversible oxidation.

Influence of light on the ascorbic acid content of milk.

Kon and Watson (1936) have shown that milk giving a positive chemical test for vitamin C failed to reduce endophenol reagent after exposure to daylight through glass. The reducing power of the ascorbic acid could be restored to a varying degree by treatment with hydrogen sulphide, but irreversible losses always took place. Visible light, composed of rays of short wave length, chiefly blue and violet, was mainly responsible for the reaction. Ultraviolet rays were probably active. Yellow and red rays were almost always without effect. The action of light did not take place in the absence of oxygen. The change which took place when milk was exposed to sunlight was oxidation which was an actinic activation of oxygen. The ascorbic acid was oxidized to dehydroascorbic acid in the reversible form. Synthetic ascorbic acid added to milk behaved towards light in the same way. Pint bottles of milk exposed for one-half hour in the sun and then stored for one hour in the dark lost fully one-half of the original antiscorbutic properties. It is interesting to note the statement in this work that the amount of destruction of ascorbic acid caused by pasteurization in the absence of catalytic metals depended upon the previous exposure of the milk to light.

Kon (1937) confirmed the above findings. Riddell and Whitnah (1936) observed that exposure to sunlight might be destructive to ascorbic acid.

Effect of freezing on ascorbic acid.

Lillegren (1936), by histological comparison of the degree of healing induced in scorbutic guinea pigs by daily administration of milk and orange juice both fresh and stored at -30° C. for 24 to 36 days, demonstrated that the animals receiving the fresh material reached a given stage of healing in about two-thirds of the time needed by those receiving the frozen material.

Granat reported (1936) that lemons, frozen in their natural state and stored from October to May at temperatures below -40° C., retained their antiscorbutic activity as shown by the prevention and cure of scurvy in guinea pigs by daily dosages of three grams.

Different relationships found in respect to ascorbic acid.

Relationships between ascorbic acid and oxidized flavor. According to Sharp and associates (1936) there was a positive correlation between the rate of oxidation of ascorbic acid and the rate of development of the oxidized flavor.

In this work the addition of 100 mg. of ascorbic acid per liter greatly retarded the development of the oxidized flavor in milk.

Whitnah and associates (1937) in a study of the naturally occurring vitamin C in milk of different breeds found that the frequency with which oxidized flavor occurred was in the following order: Holstein, Ayrshire, Guernsey, Jersey. Within the breed there was no relation

between the amount of ascorbic acid in the milk and the development of the oxidized flavor.

Anderson (1937) found that carrots fed at the rate of five pounds daily greatly reduced susceptibility of the milk to become oxidized. Carrots were fed to one cow in this experiment and apparently did not give oxidized flavor in milk. This is explained according to Anderson as being due to the fact that carrots were a fairly good source of vitamin C.

Brown and associates (1937) have shown that feeding tomato juice or lemon juice reduced or eliminated the susceptibility of milk to become oxidized and have explained this effect on the vitamin C contained therein since they found that the feeding of 0.6 grains of ascorbic acid daily greatly reduced the milk's susceptibility to become oxidized.

Relationship between vitamin C and flavor in milk. It might be expected that a high vitamin C content in foods such as milk would give good flavor. Garret (1937) and associates found a significant correlation coefficient of over 0.6 based on data from all cows, 28, indicating that a definite relationship existed between vitamin C and flavor of milk from individual cows. When the vitamin C content was high the flavor of the milk was usually good.

Relationship between ascorbic acid and enzymes. Szent-Gyorgi (1932) gave evidence that there was in the cabbage leaf a highly active enzyme which in the presence of oxygen rapidly oxidized hexuronic acid (ascorbic acid) to its reversible oxidation product. The name hexoxidase was used for this enzyme.

Tauber and Kleiner (1935) succeeded in preparing from hubbard squash an enzyme which caused the ascorbic acid to react directly with free oxygen.

Bessey and King (1934) in the chemical determination of vitamin C used trichloracetic acid in macertaing and extracting the tissues, as the acid protected the vitamin C from rapid oxidative reaction which occurred upon liberation of the active enzymes from the macerated tissue cells.

Shrinivasan (1935) proved the presence of an enzyme in drumsticks juice which was inactive when treated with trichloracetic acid. Roe and Barnum (1936) attributed the antiscorbutic effect of reversibly oxidized ascorbic acid to an enzyme in the blood which had the power to reduce the reversibly oxidized form of the ascorbic acid.

Stotz and associates (1937) attributed the catalytic activity of squash and cauliflower juice on the oxidation of ascorbic acid to a specific oxidase, which was probably a compound of the copper present and some protein materials. The catalytic properties of copper were changed greatly by the presence of proteins. A mixture of copper and albumin assumed the characteristic properties of the enzyme. It displayed an optimum pH similar to that of the enzyme; was inactivated by heat and acid; and showed similar velocity relations to the quantity of substrate.

Sharp (1936) believed the oxidation of ascorbic acid in milk could be explained by assuming the presence of an ascorbic acid oxidase the action of which was markedly accelerated by traces of dissolved copper. He believed that slight destruction of the enzyme ascorbic acid oxidase occurred by 30 minutes' treatment at 62° to 63° C. Heating for 30 seconds or longer at 77° C. destroyed the enzyme completely.

Hopkins and Morgan (1936) have reported the isolation of an enzyme which oxidized ascorbic acid from the cauliflower, which they believed was identical with the hexoxidase of cabbage described by Szent-Gyorgi (1932) and probably also with the similar enzyme isolated from squash by Tauber and Kleiner (1935).

Sharp and associates (1936) explained the rate of oxidation of ascorbic acid in milk by assuming that the milk contained an enzyme which catalyzed the oxidation of ascorbic acid. This was probably not the same which catalyzed the oxidation of the fat to produce oxidized flavor. However, the two enzymes were quite similar in their properties.

Dam and Satterfield (1937) stated that the postulation by Sharp (1936) of the presence of an ascorbic acid oxidase in milk was difficult to reconcile with the observation that raw milk lost only 50 per cent of its reduced ascorbic acid on standing for three days at 3° C.

However, in view of the fact that several investigators have found enzymes that did oxidase the ascorbic acid, it could be justly expected that milk also contained an enzyme that acted similarly. That this enzyme was the same as that which Kende (1932) ascribed as causing the oxidation of the fat in milk remains to be demonstrated.

Relationship between ascorbic acid and glutathione. Gara and Giani (1934) found in the outer layers of the renal gland a substance which inhibited oxidation of ascorbic acid. Glycokoll alanin showed some inhibiting action; aldenyl acid showed a little stronger inhibiting action; while cystine, cysteine, and especially glutathione, exhibited a very strong inhibiting action. Glutathione, even in a concentration of 1/100,000 molar, showed a very strong inhibiting action. Bessey and King (1934) showed that there appeared to be some relationship between the ascorbic acid and glutathione contents of the suprarenals. Probably the auto-oxidation of ascorbic acid under the catalytic influence of heavy metals is impeded by glutathione. Similarly the auto-oxidation of adrenalin is inhibited by ascorbic acid and also by glutathione and cysteine. Bessey and King also demonstrated the protective action of the glutathione in retarding the auto-oxidation of ascorbic acid. Either substance alone readily took up oxygen. They stated that the stability of the heavy metal complex linkage decreased in the following order; thio, ascorbic acid, adrenalin.

Borsook and Jeffrey (1936) in experiments on blood, urine and tissue slices of animals showed that the protective action of ascorbic acid consisted of a reduction of the reversible oxidized ascorbic acid. This reduction can be affected rapidly by glutathione. Guzman and associates (1936) showed that fluids of animal and of some vegetable origin, which contain appreciable quantities of ascorbic acid, belong to a group of biological fluids which possess inhibiting mechanism, thus protecting the ascorbic acid from oxidation. This inhibiting action, which is caused by a variety of catalysts, mostly copper and hemochromogen, was probably due to glutathione.

Iodometric titrations for determination of glutathione in tissue extracts has been described by Gladys and associates (1932) Gara and Giani (1934), and Quensel and Wacholder (1935).

Buruinana (1937) reported that he could not find glutathione in humans', cows', or buffaloes' milk, but was able to find it in mares' milk.

<u>Other aspects of ascorbic acid.</u> Recent studies on ascorbic acid have shown that under certain conditions ascorbic acid could function as a catalyst or co-enzyme. Hopkins and Morgan (1936) demonstrated that the enzyme which oxidized ascorbic acid rapidly to dehydroascorbic acid brought about the oxidation of reduced glutathione only in the presence of ascorbic acid. Under these conditions the oxidation of the ascorbic acid itself began only when that of the glutathione was almost complete. This was due to the continuous reduction of the dehydroascorbic acid.

Stotz and Harper (1937) made similar conclusions which might indicate that the ascorbic acid was an important catalyst in biological oxidations.

PROCEDURE

The procedure followed in the direct determination of ascorbic acid in milk was the same as recommended by Sharp (1938). This method was checked occasionally by adding commercial ascorbic acid to milk and then redetermining the amount of ascorbic acid present by titration. The redetermination always checked within five per cent. On a few occasions the proteins were precipitated with trichloracetic acid. However, this method did not give results any better than those obtained by direct titration.

The method employed by Gladys (1936) in determining blood glutathione was used in studying the glutathione of milk. The method was checked by adding glutathione to water. In the redetermination, the glutathione checked within five per cent.

Leucocytes and bacteria were counted according to the Breed method as outlined in the Standard Methods of Milk Analysis. The methylene blue tests were made also as outlined by the Standard Methods of Milk Analysis.

The per cent lactic acid was determined according to the Mann's acid test.

Aeration of milk was accomplished by passing the milk over an experimental surface cooler.

Holder pasteurization of small lots of milk was accomplished in glass bottles which were partially submerged in a hot water bath. The commercial milk was pasteurized in glass or allegheny metal lined

vats. Flash pasteurization was brought about in a specially constructed pasteurizer in which the milk was heated to any desired temperature below 100° C. and cooled to below 5° C. in the course of 5 to 15 seconds. The apparatus was so constructed that all milk was heated and could be controlled, when desired, to a temperature within one-tenth of a degree. Controlled suction was used to draw the milk through the apparatus at any speed and, hence at any temperature desired.

The experimental irradiation of milk was accomplished by passing the milk over one side of a surface cooler toward which ultra violet rays from the Alpine Sun Lamp of the Hanovia Chemical Manufacturing Company was directed. The irradiation was performed at the highest intensity.

The copper solution was made up as follows: one gm. of $CuSo_4 5H_2O$ was made up to 100 ml. using distilled water, thus forming the stock solution. One ml. of the stock solution was then diluted to another 100 ml., using distilled water as before. One ml. of this second solution was finally added to 200 ml. of the milk, which resulted in the addition of 13 mg. per cent of copper to the milk.

The milk which was used in studying the seasonal effect upon the ascorbic acid was obtained both from the dairy barn and from the college creamery. The milk from the dairy barn was always titrated within two to four hours after it was drawn from the cow. The milk was stored in a refrigerator at 4° C. and titrated daily for four consecutive days. The milk from the college creamery was titrated within six to eight hours after being processed.

The oxidized flavor of milk was classified as follows: + + + strong to very strong flavor; + + distinct to pronounced oxidized flavor; + slightly oxidized flavor; ? doubtful oxidized flavor; - no oxidized flavor.

EXPERIMENTAL RESULTS

Part I

PROCESSING, ASCORBIC ACID, AND THE OXIDIZED FLAVOR OF MILK

The effect of copper upon the stability of ascorbic acid and upon the development of the oxidized flavor when the milk was pasteurized at high and low temperatures.

Individual samples of milk were obtained from 20 cows of the college herd and were cooled and treated at once. Each sample was divided into five lots; one lot served as a control; two lots were pasteurized at 63° C. for thirty minutes, after which 0.13 mg. per liter of copper was added to one lot; while the two remaining lots were pasteurized at 75° C. for thirty minutes, after which copper was added to one lot as above. Titrations for the ascorbic acid were made daily. The milk was studied organoleptically for oxidized flavor on the fourth day. The data obtained are presented in Tables 1, 2, 3, 4, 5 and 6, and summarized in Table 7, and are shown graphically in Figures 1 and 2.

An examination of the data of Table 3 shows the catalyzing effect of copper upon the oxidation of ascorbic acid when the milk was pasteurized at 63° C. for thirty minutes. This effect was very marked after the second and third days. On the fourth day no appreciable amount of ascorbic acid remained. Coincident with the marked decrease in ascorbic acid, the strong development of oxidized flavor was usually noted. However, this observation was not consistent in all samples as later it will be shown that with some milk one may observe very pronounced decreases in the ascorbic acid content, also in the presence of copper, without any notice-

able development whatsoever of the oxidized flavor.

Some destruction of ascorbic acid, resulting from 63° C. holder or pasteurization, without copper contamination, was apparent especially on the first and second days. However, on the third day the pasteurized milk, although relatively low in ascorbic acid, had a larger amount of ascorbic acid than did the control samples. In other words, the stability of ascorbic acid was slightly less in raw milk over a three day storage period than similar milk pasteurized by the holder method.

Pasteurization of milk at 75° C. for thirty minutes resulted in a slightly greater initial destruction of the ascorbic acid than pasteurization at 63° C., but, on the other hand, the stability of the ascorbic acid present, after the high heat treatment, was greatly increased over that when the low temperature was employed. However, there was a slight decrease in the amount of ascorbic acid present during storage as shown in Table 4 and Figure 1. The increased stability of ascorbic acid at the 75° C. exposure was shown more strikingly in the cases where copper was added to the milk. There was a slight decrease in the ascorbic acid present compared with the milk that had been heated to the same temperature, but without any addition of copper. Oxidized flavor did not develop at all in the samples thus high heat treated even in the presence of copper.

An examination of Tables 1, 2, 3, 4 and 5 shows that milk from certain cows exhibited a continuous and remarkable stability in respect to ascorbic acid, regardless of the different treatments. This stability was especially noticeable in the case of cows number 30, 111 and 116, a Guernsey and two Jerseys, respectively. This outstanding stability of

the ascorbic acid of some cows' milk is demonstrated in Table 6. In view of these observations, and mindful of the relationship between the destruction of ascorbic acid and the development of oxidized flavor, it might be expected that these samples would not show any tendency to develop the oxidized flavor. However, an examination of the oxidized flavor data on milk of the different treatments showed that milk from these three cows had just as great a susceptibility for oxidized flavor as did that from any other cows. In fact, milk from cow number 116 had already shown, while raw, a tendency to develop the oxidized flavor.

These effects of copper and heat treatments upon the stability of ascorbic acid in milk from individual cows are summerized in Table 7 and are presented graphically in Figures 1 and 2.

Table 1. The ascorbic acid content and the development of the oxidized flavor in raw milk from individual cows after various storage periods.

Cow (No.)	Ascorbic acid (mg./L) First Day	Second Day	Third Day	Fourth Day	Oxidized flavor after four days
13	16.4	12.5	6.8	2.9	-
30	23.6	22.6	19.6	16.7	-
36	18.3	11.5	2.5	2.9	- ?
37	18.3	10.5	3.4	2.5	-
41	19.3	18.5	13.7	8.8	-
42	21.7	18.1	6.8	2.5	-
100	24.1	20.1	12.8	5.9	- ?
111	24.5	23.5	23.1	17.7	+ ?
116	25.5	24.1	24.1	14.7	-
117	21.2	19.5	12.8	7.8	+ ?
134	20.2	17.0	10.8	4.9	-
142	18.8	16.0	8.8	2.9	-
174	16.4	12.5	7.8	1.9	-
180	20.7	16.0	10.8	3.9	-
190	21.2	17.5	12.7	8.8	-
197	22.2	17.5	11.8	5.9	-
237	19.7	13.0	2.5	1.9	- ?
238	22.2	17.5	13.7	5.9	-
239	21.7		6.8	1.4	-
300	18.8	16.5	13.7	3.9	-
Ave.	20.7	17.1	11.0	6.2	

Table 2. The ascorbic acid content and the development of the oxidized flavor in holder pasteurized milk from individual cows after various storage periods.

Cow (No.)	Ascorbic acid (mg./L) Second Day	Third Day	Fourth Day	Oxidized flavor after four days
13	16.5	9.3	5.9	-
30	22.0	19.7	12.7	-
36	9.0	2.9	1.9	-
37	9.0	3.9	1.9	?
41	17.5	14.7	10.8	-
42	14.0	8.8	5.9	-
100	20.0	14.7	12.8	-
111	18.5	18.7	18.7	?
116	23.0	20.6	18.1	-
117	19.5	16.7	8.8	+ ?
134	14.0	10.8	6.8	-
142	16.0	10.3	5.9	-
174	11.0	5.4	2.4	-
180	18.0	12.3	9.8	-
190	17.5	13.3	11.3	-
197	18.5	13.7	10.8	-
237	10.5	2.9	2.9	-?
238	17.0	12.9	10.8	-
239	16.0	7.8	3.9	-
300	16.5	14.7	11.8	-
Ave.	15.9	11.7	8.7	

Table 3. The ascorbic acid content and the development of the oxidized flavor in copper contaminated holder pasteurized milk from individual cows after various storage periods.

Cow (No.)	Ascorbic acid (Mg./L.) Second Day	Third Day	Fourth Day	Oxidized flavor after four days
13	2.0	1.9	0.0	-
30	16.0	6.8	2.5	+ +
36	3.5	2.5	0.0	+
37	3.5	3.4	0.0	- ?
41	11.5	2.5	0.0	+ +
42	7.5	1.9	0.0	+ + +
100	10.0	1.9	0.0	-
111	16.0	5.9	2.9	+ + +
116	17.5	12.1	3.4	+
117	12.0	2.9	0.0	+ +
134	5.5	1.5	0.0	+ +
142	5.5	1.9	0.0	+ +
174	2.5	1.9	0.0	- ?
180	4.5	1.9	0.0	+ + +
190	8.0	0.9	0.0	+ + +
197	4.5	0.9	0.0	+ + +
237	3.0	0.9	0.0	+
238	7.0	1.9	0.0	+ +
239	5.0	0.9	0.0	+
300	9.0	0.9	0.0	+ + +
Ave.	7.7	2.8	—	

Table 4. The ascorbic acid content and the development of the oxidized flavor in high temperature (75° C. - 30 min.) pasteurized milk from individual cows after various storage periods.

Cow (No.)	Ascorbic acid (Mg/L) Second Day	Third Day	Fourth Day	Oxidized flavor after four days
13	13.5	11.3	10.3	-
30	19.0	18.2	16.2	-
36	11.0	9.3	8.8	-
37	12.5	10.8	9.3	-
41	15.5	12.8	12.8	-
42	14.5	13.7	11.8	-
100	17.0	16.2	14.7	-
111	18.5	18.7	16.7	-
116	18.0	17.2	14.7	-
117	17.0	13.7	11.8	-
134	15.0	12.8	10.3	-
142	11.0	10.3	10.3	-
174	12.5	10.8	10.3	-
180	14.5	14.3	12.2	-
190	17.0	14.3	13.7	-
197	14.5	14.3	12.8	-
237	13.5	11.8	9.3	-
238	15.5	14.3	12.8	-
239	16.0	14.3	12.2	-
300	12.5	12.8	10.8	-
Ave.	14.9	13.6	12.1	

Table 5. The ascorbic acid content and the development of the oxidized flavor in high temperature (75° C. - 30 min.) pasteurized copper contaminated milk from individual cows after various storage periods.

Cow (No.)	Ascorbic acid (Mg./L) Second Day	Third Day	Fourth Day	Oxidized flavor after three days
13	12.0	11.3	8.8	-
30	19.5	16.7	14.5	-
36	11.0	7.3	4.9	-
37	10.5	8.8	5.4	-
41	13.5	13.3	12.8	-
42				
100	17.0	14.7	14.3	-
111	18.5	17.2	15.7	-
116	19.0	17.7	17.7	-
117	17.0	14.3	14.7	-
134	13.0	11.8	6.4	-
142	14.0	10.8	7.8	-
174	11.0	8.8	6.4	-
180	14.0	12.8	9.8	-
190	14.5	11.8	7.8	-
197	16.0	14.3	14.3	-
237	13.0	9.8	8.8	-
238	15.5	14.7	13.3	-
239	15.5	14.7	12.8	-
300	15.0	13.3	10.8	-
Ave.	14.6	12.9	10.9	

Table 6. Variation in the stability of ascorbic acid of milk from individual cows as shown by high heat treatment and storage.

Cow (No.)	Ascorbic acid (Mg.L) Control Stored 5 days	Past. (75° C. - 30 min.) Stored 5 days	Difference as a result of heat treatment: Increase	Decrease
13	2.9	10.3	7.4	
30	16.7	16.2		- 0.5
36	2.9	8.8	5.9	
37	2.5	9.3	6.8	
41	8.8	12.8	4.0	
42	2.5	11.8	9.3	
100	5.9	14.7	8.8	
111	17.7	16.7		- 1.0
116	14.7	14.7		0.0
117	7.8	11.8	4.0	
134	4.9	10.3	5.4	
142	2.9	10.3	7.4	
174	1.9	10.3	8.4	
180	3.9	12.2	8.3	
190	8.8	13.7	4.9	
197	5.9	12.8	6.9	
237	1.9	9.3	7.4	
238	5.9	12.8	6.9	
239	1.4	12.2	10.8	
300	3.9	10.8	6.9	
Ave.			- 7.0	- 0.5

Table 7. The effect of various treatments of milk from individual cows on the stability of the ascorbic acid. (Average of milk from 20 cows).

Treatment	Ascorbic acid (Mg./L) after First Day	Second Day	Third Day	Fourth Day
Control	20.7	17.1	11.0	6.2
Past. 62.8° C. - 30 min.	-	15.9	11.7	8.7
Past. 62.8° C. - 30 min. plus copper	-	7.7	2.8	0.0
Past. 75° C. - 30 min.	-	14.9	13.6	12.1
Past. 75° C. - 30 min. plus copper	-	14.6	12.9	10.9

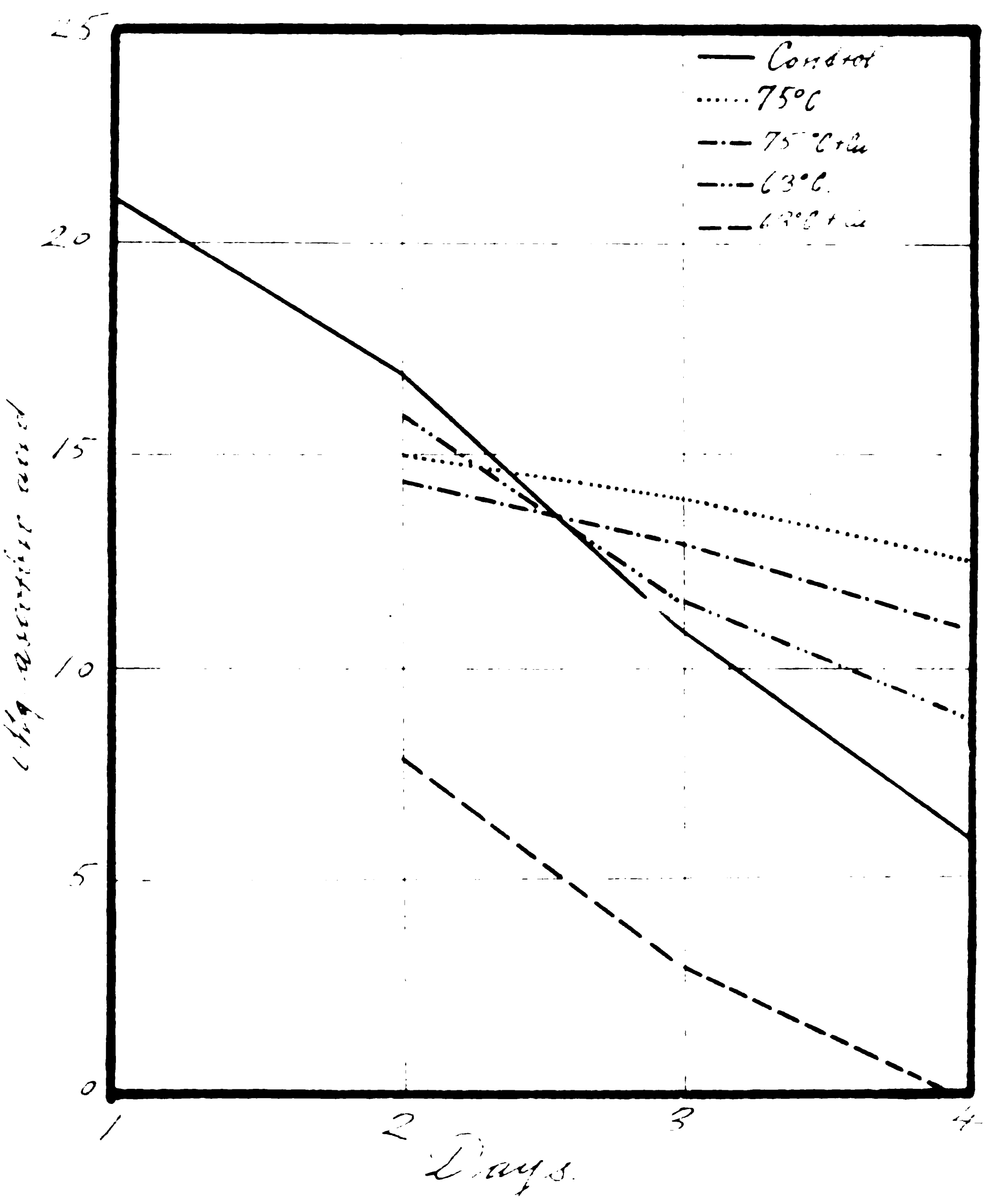

Figure 1. Showing the relative stability of ascorbic acid in milk processed at different temperatures with and without the addition of copper.

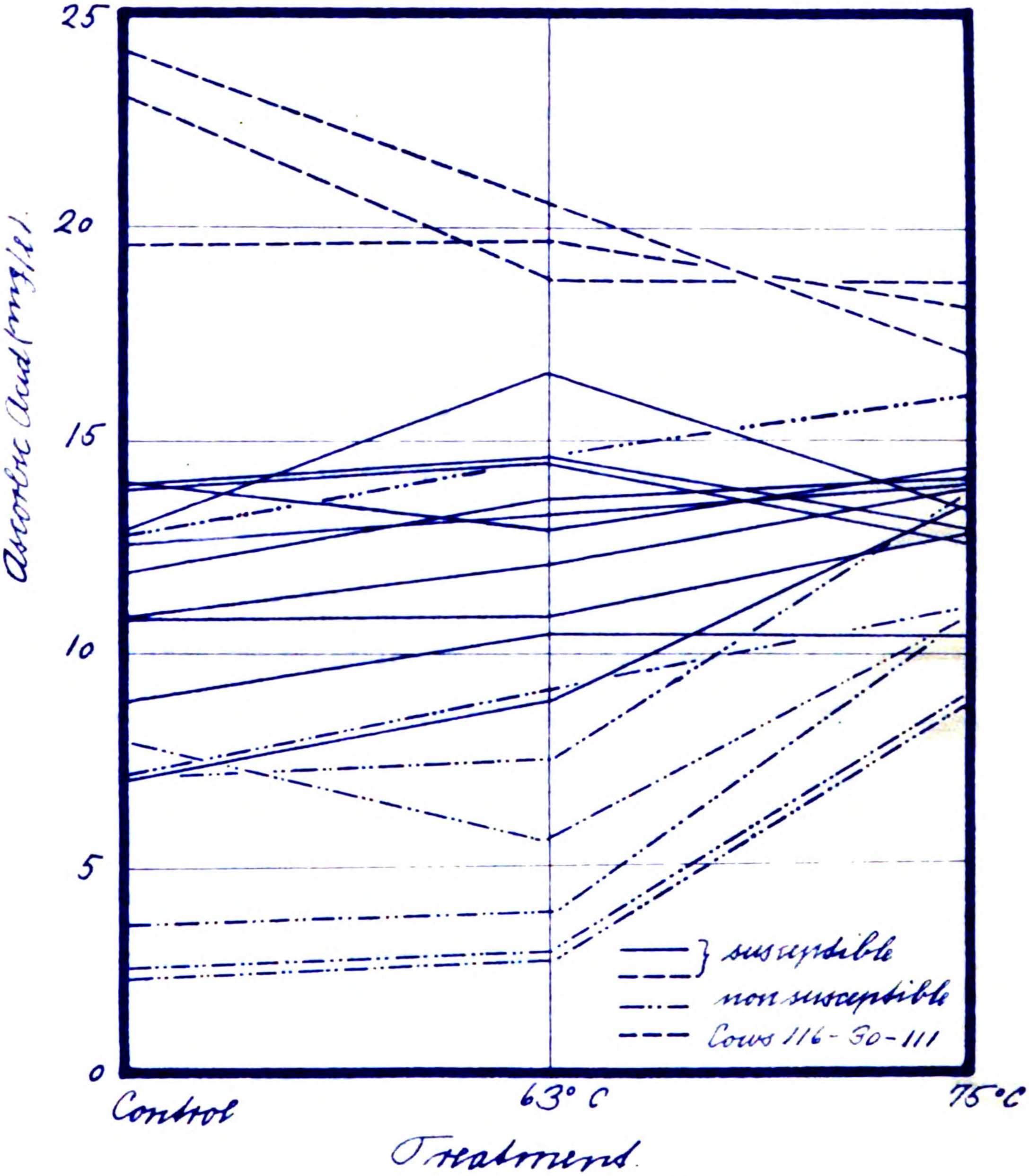

Figure 2. The stability of ascorbic acid in milk from individual cows when treated for one-half hour at 63° C. and 75° C.

The effect of flash heating of milk at various temperatures upon the stability of the ascorbic acid.

Samples of milk from individual cows were flash pasteurized at various temperatures from 60° to 97° C. for, and cooled within, a period ranging from five to fifteen seconds. Following pasteurization, the samples were stored and titrated daily for ascorbic acid. The data secured are presented in Tables 8 and 9 and are shown graphically in Figures 3 and 4.

An examination of these data shows that at least six effects were noted as a result of flash pasteurizing, at high temperatures, milk from individual cows. These are: first, the milk from all cows did not respond identically as to the stability of the ascorbic acid at similar heat treatments; second, flash pasteurization temperatures ranging from 85° to 95° C. had the most pronounced stabilizing effect upon the ascorbic acid; third, the ascorbic acid stability passed through a mixima as the flash exposure extended from 60° to 97° C.; fourth, flash exposures below 75° C. had a less stabilizing effect upon the ascorbic acid than that existing naturally in the raw milk; fifth, the critical flash exposure minima for ascorbic acid stability were between 75° and 85° C; and sixth, milk having a naturally low ascorbic acid content usually showed the largest increased stability of ascorbic acid upon flash heating than did those samples having a naturally high ascorbic acid value. These relationships can best be observed in Figures 5 and 6.

Table 8. Effect of flash heating at different temperatures upon the ascorbic acid content of milk from individual cows during November.

Temperature (° C.)	Ascorbic acid (Mg./L) 1st day	2nd day	3rd day	4th day	5th day	Ave. 5 days	Increase or decrease from control (%)
Cow 246							
65 - 68	11.8	3.9	1.9	0.0	0.0	3.5	-40.0
74 - 75	14.8	5.8	1.4	0.0	0.0	4.6	-22.0
83 - 85	13.8	9.8	7.6	2.3	0.0	6.7	13.5
89 - 91	14.8	11.7	8.1	7.1	0.9	8.5	44.0
93.5- 95	15.3	11.2	8.1	6.6	0.9	8.4	42.0
96 - 97	13.8	10.2	8.6	8.1	0.9	8.3	41.0
Control	15.3	2.4	1.9	0.0	0.0	5.9	
Cow 37							
65 - 67	18.3	13.2	9.5	3.3	0.0	8.8	-14.8
74 - 77	17.8	16.6	10.9	5.6	2.8	10.4	3.0
84 - 86	18.8	15.1	15.2	13.3	6.2	13.7	35.6
89 - 91	14.8	15.1	15.7	14.2	7.5	13.5	33.7
93 - 95	17.8	15.6	15.2	14.2	9.5	14.5	43.6
96 - 97	16.8	14.2	14.3	12.3	9.5	13.4	32.7
Control	18.8	12.7	11.4	6.6	0.9	10.1	
Cow 30							
65 - 67	23.7	18.5	16.1	15.6	12.3	17.3	- 9.4
74 - 75.5	22.7	20.0	19.5	17.9	14.2	18.9	- 1.0
83.5 - 85	23.7	21.5	19.9	18.9	18.9	20.6	7.9
89.5 - 89	22.7	20.0	20.4	19.4	18.9	20.3	6.2
94 - 95	22.7	20.5	19.9	18.9	17.9	20.0	4.7
97	22.7	20.5	19.0	19.4	15.2	19.4	1.6
Control	23.7	20.5	18.0	17.0	14.7	19.1	
Cow 261							
65 - 66	21.3	15.6	9.5	2.3	0.0	9.7	-12.6
75 - 76	19.3	14.1	9.5	3.3	0.0	9.2	-17.1
85 - 86	19.8	17.6	16.1	8.5	0.9	12.5	12.6
89 - 90	19.8	18.5	15.7	10.9	2.8	13.5	21.6
94 - 95	20.8	16.6	15.7	14.2	7.5	14.9	34.2
96.5 - 97	20.8	15.6	14.3	12.8	7.5	14.2	27.9
Control	21.7	14.6	11.9	6.6	0.9	11.1	
Cow 144							
62 - 63	12.3	6.8	1.9	0.0	0.0	4.2	-44.7
74 - 75	16.3	11.7	6.6	0.9	0.0	7.1	- 6.5
83.5 - 84.5	14.8	14.6	12.4	8.5	0.9	10.2	34.2
89 - 90.5	14.3	13.7	11.9	10.9	0.9	10.3	35.5
93 - 9415	14.3	13.7	11.4	10.4	4.7	11.9	56.5
96 - 97	15.3	12.7	12.3	10.9	8.5	11.9	56.5
Control	15.3	10.3	3.8	0.9	0.0	7.6	

Table 8 continued from previous page.

Temperature (° C.)	Ascorbic acid (Mg/L) 1st day	2nd day	3rd day	4th day	5th day	Ave. 5 days	Increase or decrease from control (%)
Cow 116							
64 - 67	23.7	20.5	16.6	12.8	14.2	17.6	- 5.4
74 - 75	23.7	20.9	19.0	15.2	14.7	18.7	- 0.5
83 - 85	23.7	21.9	20.4	18.9	18.9	20.8	11.8
90 - 91	23.2	21.9	20.9	19.8	19.4	21.0	12.9
94 - 95	22.7	20.9	21.8	20.8	21.0	21.4	15.0
96 - 97	21.7	19.5	19.0	18.9	18.9	19.6	5.4
Control	25.2	21.5	19.9	15.6	15.1	18.6	

Table 9. Effect of flash heating at different temperatures upon the ascorbic acid content of milk from individual cows during July.

Temperature (° C.)	Ascorbic acid (Mg/L) 1st day	3rd day	4th day	Ave. day	Increase or decrease from control (%)
Cow 246					
60 - 63	10.1		1.0	5.5	6.0
70 - 72	12.5		2.4	7.5	44.0
80 - 82	13.5		6.7	10.1	94.0
89 - 91	15.4		12.9	14.1	171.0
95 - 96	14.9		12.5	13.7	163.0
Control	9.1		1.4	5.2	
Cow 226					
60 - 62	16.8	12.5	7.7	12.3	- 8.8
69 - 71	17.3	17.8	13.9	16.3	21.0
79 - 81	19.7	18.7	15.9	18.1	34.0
88 - 90	20.2	19.2	16.3	18.5	37.0
94 - 96	19.7	19.2	16.3	18.4	36.0
Control	18.7	13.5	8.2	13.5	
Cow 46					
60 - 63	11.1	6.2	3.8	7.1	- 4.0
70 - 73	13.9	10.6	8.6	11.3	53.0
80 - 82	13.9	12.0	13.0	12.9	74.0
88 - 91	12.9	12.5	13.0	12.8	73.0
94	13.9	12.5	13.9	13.4	81.0
Control	11.1	6.7	4.3	7.4	
Cow 134					
61 - 62	5.8	3.4	1.9	3.5	-24.0
71 - 74	10.1	7.7	5.8	7.9	72.0
79 - 81	10.1	9.6	10.1	9.9	115.0
88 - 90	11.1	11.1	12.9	11.7	154.0
95 - 96		11.5	12.0	11.7	154.0
Control	7.2	4.3	2.4	4.6	
Cow 14					
60 - 62	7.7	2.9	2.4	4.3	0.0
72 - 75	9.6	7.7	6.2	7.8	81.0
80 - 83	9.1	8.6	9.1	8.9	106.0
88 - 90	9.1	10.1	10.1	9.8	128.0
96 - 97	10.1	9.6	10.6	10.1	135.0
Control	8.1	2.9	1.0	4.3	
Cow 146					
60 - 62	16.8		12.0	14.4	12.7
70 - 73	18.3		16.3	17.3	5.0
79 - 81	19.2		19.2	19.2	16.0
88 - 91	19.7		19.2	19.5	18.0
94 - 96	18.7		19.7	19.2	16.0
Control	18.7		14.4	16.5	

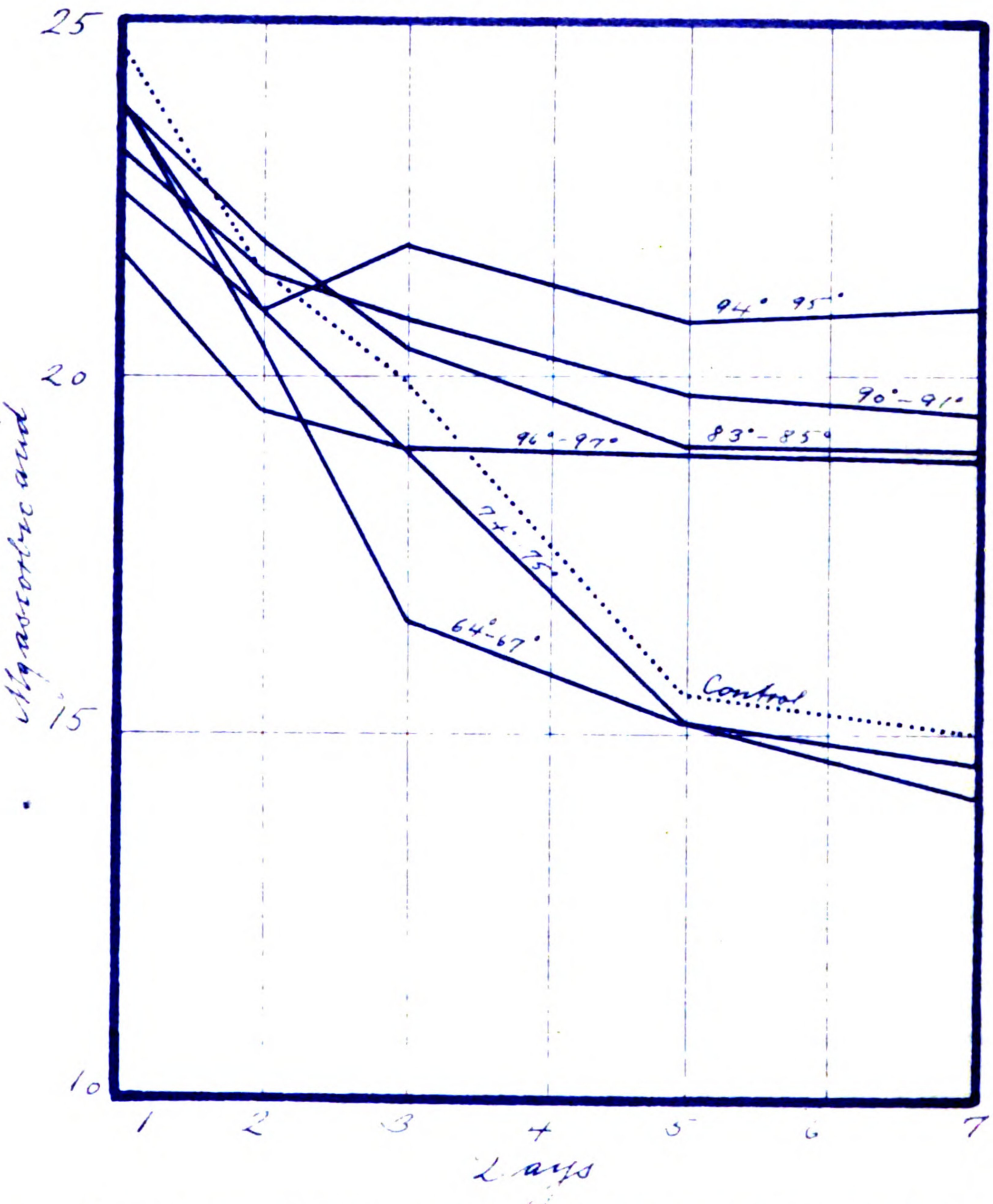

Figure 4. Showing the effects of various flash pasteurizing temperatures upon the ascorbic acid content of milk from cow No. 116 when stored for several days.

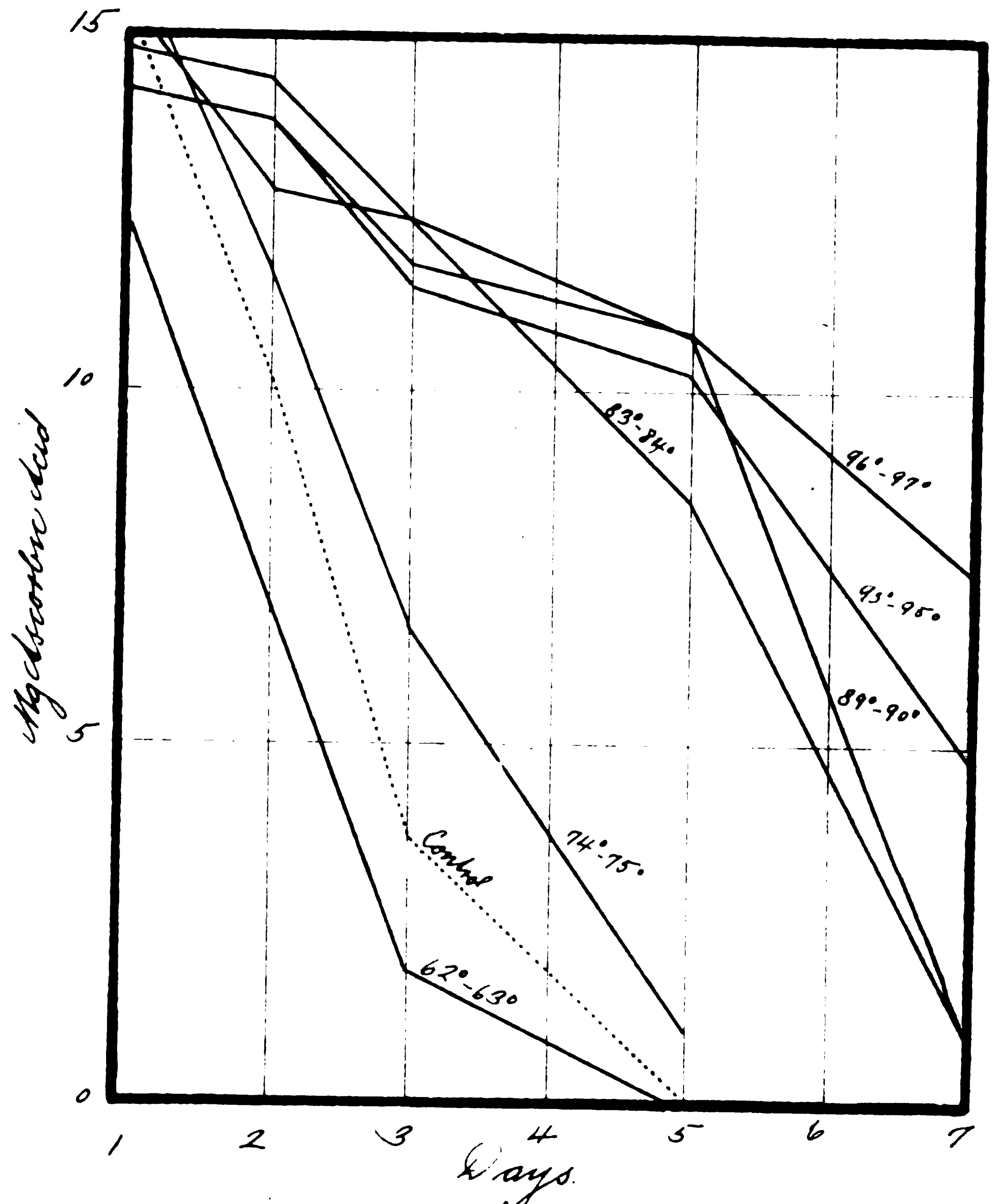

Figure 3. Showing the effects of various flash pasteurizing temperatures upon the ascorbic acid content of milk from cow No. 144 when stored for several days.

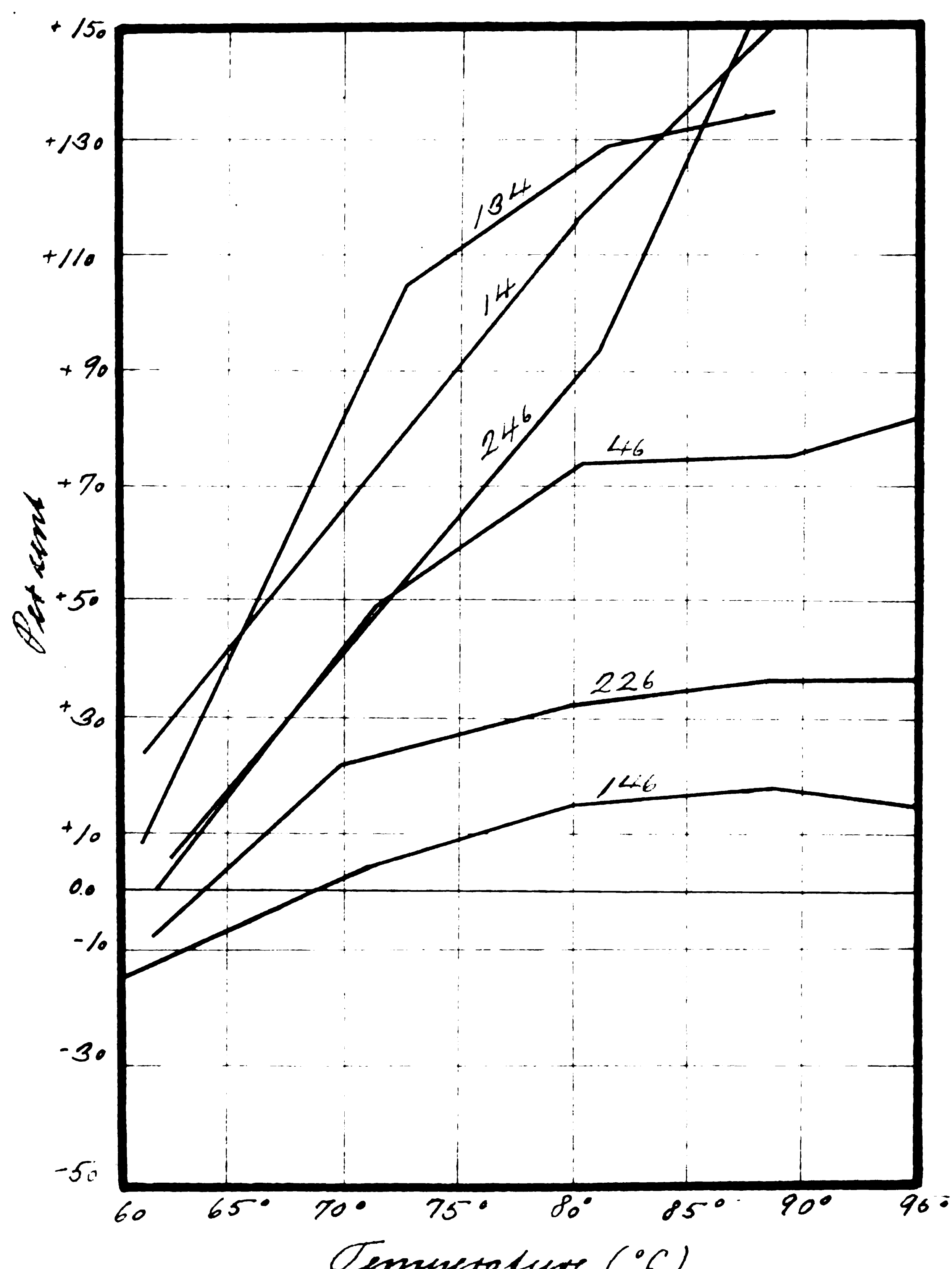

Figure 5. Showing percentage variations from the control samples of the ascorbic acid content of individual cows milk when treated at different temperatures (November).

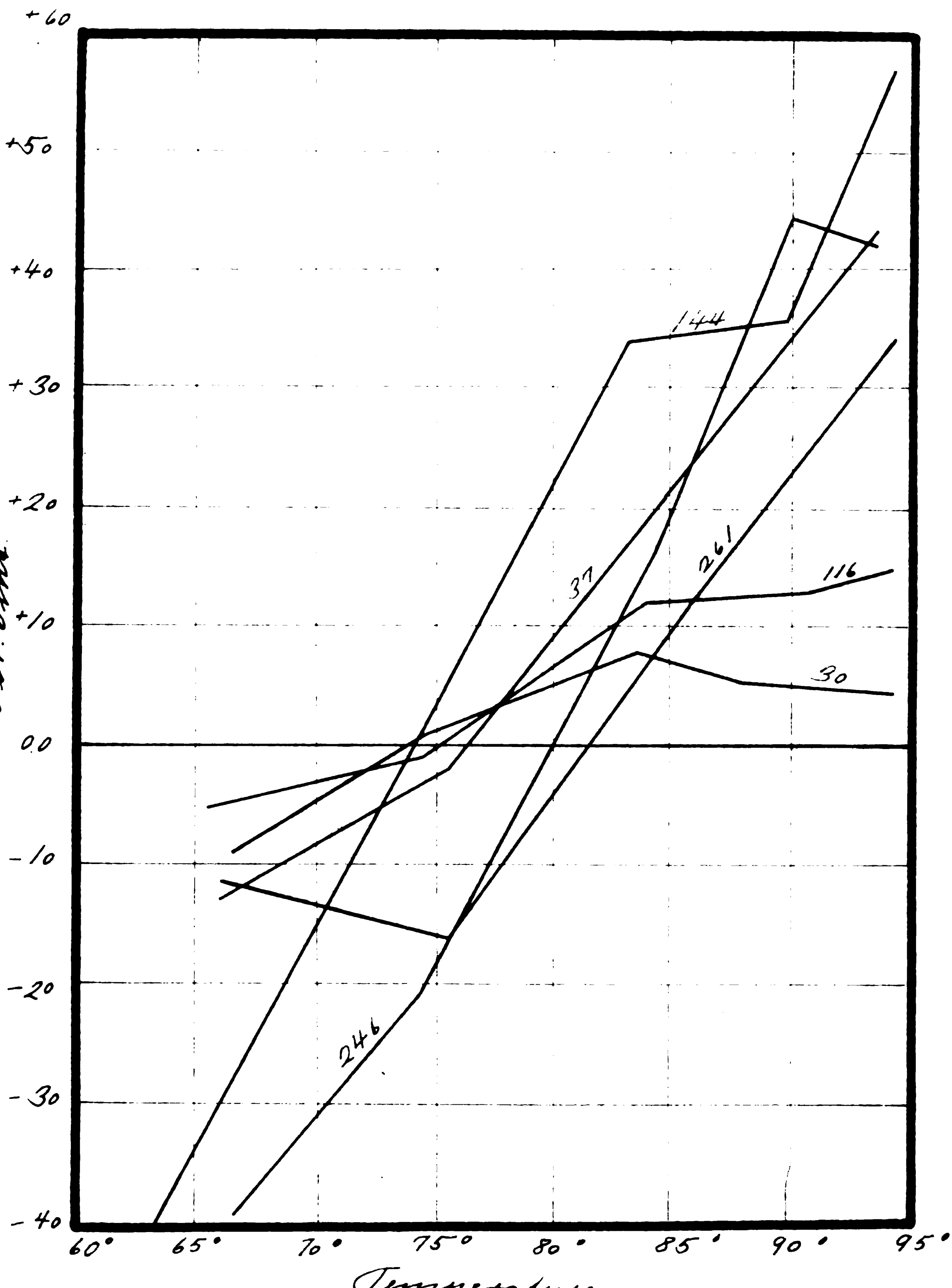

Figure 6. Showing percentage variations from the control sample of the ascorbic acid content of individual cows' milk when treated at different temperatures (July).

<u>The effect of various heat treatments upon the ascorbic acid content of milk and upon the development of oxidized flavor when copper was added before and after heating.</u>

<u>Stability of ascorbic acid.</u> This experiment was run in June, 1937. The milk was obtained from the College Dairy Barn and was mixed from three different breeds, namely, Jersey, Guernsey and Brown Swiss. The milk was treated and the ascorbic acid value determined the same day as milking.

The milk was divided into two groups, I and II, of six lots each, A, B, C, D, E and F. The lots were again divided into six portions, 1, 2, 3, 4, 5 and 6 of 200 ml. each. To the six 200 ml. portions 0, 13, 26, 39, 52 and 65 mg/% of copper were added respectively. The copper was added to the milk comprising Group I before heating and to that of Group II after heating. Lots A, B, C, D, E and F of both the Groups I and II, were heated for exactly ten minutes at 65°, 70°, 75°, 80°, 85° and 90° C., respectively.

The milk was heated in glass bottles in steam-heated water baths, and following heat treatment, was stored at 4° C. When the copper was added after heating, it was added to the milk while still warm immediately after heating, and then the milk was put into the refrigerator.

The determinations for ascorbic acid were run on four consecutive days. The samples were scored for oxidized flavor after three and after four days.

The experimental data are tabulated in Table 10 and plotted on Figures 7, 8, 9 and 10.

Figure 7 represents the picture obtained by plotting the ascorbic

acid content of milk against temperatures for the different amounts of copper added before heating. From this figure may be observed that 80° C. was found to be the optimum temperature for the stability of ascorbic acid regardless of the different amounts of copper added. When increasing amounts of copper were added, the ascorbic acid content decreased, but, in all cases, 80° C. was the optimum, temperature of ascorbic acid stability.

From Figure 8, representing similar heat treatments except that the copper was added after heating, may be seen also that at 80° C. the maximum stability of ascorbic acid was secured. However, as it may be seen, the ascorbic acid did not decrease much after the temperature of 80° C. had been exceeded.

In studying these figures some characteristic differences worthy of note should be mentioned. The figure representing the samples to which the copper was added after heating shows very little difference in the ascorbic acid content for the different additions of copper at temperatures at 80° C. and over, while the figure representing the samples to which the copper was added before heating shows a very wide variation in the ascorbic acid content for the different additions of copper at temperatures of 80° C. or over. These differences between the two series show up under all conditions studied with no exception.

In the figures representing copper additions before and after heating, similar wide variations in the ascorbic acid content of milk heated to temperatures below 80° C., for instance, 65° C., were noted. However, when the temperature of 80° C. had been exceeded, those samples

which had had copper added after heating showed very little variation in the ascorbic acid content for the different additions of copper, while those that had had copper added before heating showed the same wide variation as those heated to temperatures below 80° C.

From the above discussion it follows that a temperature of 80° C. for 10 minutes seems to be an exposure during which marked changes take place in milk as far as factors affecting the stability of ascorbic acid are concerned.

The ascorbic acid content of milk to which different amounts of copper had been added after heating tended to remain at a relatively high level at the 80° C. exposure. This tendency may be noted from the data presented in Tables 11 and 12.

All the data indicated that copper, when added below temperatures of 80° C., whether before or after heating, had the same destructive effect upon the ascorbic acid. When the milk was heated at temperatures beyond 80° C., copper, regardless of the time added, had little effect upon the destruction of ascorbic acid.

Copper added before heating had quite a different effect at the 80° C. exposure on the ascorbic acid stability than when added before heating. The decrease in ascorbic acid was smaller at temperatures above 80° C. than at temperatures below 80° C., but was considerably larger than those that had had the copper added after heating.

The differences in the ascorbic acid values between the controls and the samples to which copper was added increased after the second day in the same relative amount both in those samples which had had the copper

added before or after heating at temperatures above 80° C. This peculiarity can justify the conclusion that the difference between the samples which had had the copper added before or after heating at temperatures above 80° C. was due to causes during heating while the copper was present, and was due to a very small extent, if at all, to any other cause.

Data showing the stability of ascorbic acid, when copper was added after heating, are illustrated in Figure 9. It appears from these graphs of this figure that a temperature of 80° C. for ten minutes is consistently the most favorable to the stability of ascorbic acid. Temperatures of 85° C. and 90° C. are also good for stabilizing the ascorbic acid ranking in respective order as to their importance. All three of these temperatures, with 80° C. ranking first, apparently have a stabilizing effect upon the ascorbic acid, regardless of the amount of copper present.

A temperature of 75° C., however, shows some peculiarities. This temperature is apparently able to stabilize the ascorbic acid just as well in general as the three above mentioned temperatures when copper is present in amounts of 26 mg/% or less, but if the copper content is higher, the temperature exhibits a sudden drop in its stabilizing effect. In other words, 75° C. with heating time of 10 minutes, is one of the temperatures at which important changes take place regarding ascorbic acid. Probably these changes occur between 75° C. and 80° C.

The lines indicating the ascorbic acid values for 65° C. and 70° C. are equally interesting, as noted in Figure 9. These temperatures showed extremely small ascorbic acid stabilizing power. Their lines

lying considerably lower show entirely different characteristics than do the lines for the other temperatures. Also they follow each other very closely.

The temperatures show the same characteristics in general in regard to the stability of ascorbic acid when copper was added before heating (Figure 10). Those of 65° C. and 70° C. follow each other closely and show different characteristics that do the other temperatures. That of 80° C. also was the temperature at which the most stabilizing effect regarding ascorbic acid occurred. The other temperatures, 85° C., 90° C. and 75° C. rank in the same order, as above mentioned, in importance.

Oxidized flavor. The theory has been generally accepted that heating to sufficiently high temperatures prevents development of oxidized flavor even when copper was present. Data secured on the development of oxidized flavor are presented in Table 10.

The data show that the development of oxidized flavor tended to follow the destruction of ascorbic acid, but did not reach a minimum point of greatest development at any of the temperatures studied. As the temperatures increased, the milk became less and less susceptible to oxidized flavor, even in the presence of large amounts of copper. At temperatures over 85° C. the milk did not develop any oxidized flavor, when copper was added before heating. When copper was added after heating, some of the milk became oxidized even at 90° C. as the data of Table 10 indicates, but to a smaller degree than any milk heated to lower temperatures.

An important difference between the stability of ascorbic acid and the development of oxidized flavor was noted. The ascorbic acid reached its maximum stability at a temperature below 85° C., at 80° C. in this experiment, while the oxidized flavor reached a minimum or failed completely to develop at a temperature above 85° C., at 90° C. in this experiment. This observation is of especial interest in view of the theory that has been proposed that the enzyme which causes oxidized flavor is similar in many respects to the one which oxidizes ascorbic acid. This theory is not here being retired or a new one proposed, but it does seem that, upon heating, the stability of ascorbic acid and the milk's susceptibility to acquire oxidised flavor have two entirely different critical temperatures.

Table 10. The effect of adding copper before and after heat treatment upon the stability of the ascorbic acid and upon the development of the oxidized flavor of milk.

Treatment		Copper added before heating				Copper added before heating			
Temp. (° C. 10 min.)	Copper added*	Ascorbic acid (Mg/L) 1st day	Ascorbic acid (Mg/L) 2nd day	Oxidized flavor 3rd day	Oxidized flavor 4th day	Ascorbic acid (Mg/L) 1st day	Ascorbic acid (Mg/L) 2nd day	Oxidized flavor 3rd day	Oxidized flavor 4th day
65	1	19.0	15.9	-	-	18.0	14.9	-	-
	2	15.0	8.9	-	-	14.5	8.9	?	+ +
	3	11.5	3.4	-	+	11.5	3.9	?	+ +
	4	6.0	1.9	-	+	9.0	2.4	-	+ +
	5	5.5	1.5	-	+	7.5	1.9	-	+ +
	6	lost				6.0	1.9	-	+ +
70	1	19.0	15.9	-	-	19.1	16.4	-	-
	2	14.5	8.9	-	+	14.0	9.4	?	+
	3	11.0	4.9	-	+	12.5	3.9	?	+
	4	7.5	1.9	-	+	10.5	2.4	-	+ +
	5	4.0	1.9	-	+	9.0	1.9	-	+ +
	6	2.5	2.4	-	+	6.5	1.9	-	+ + +
75	1	19.0	17.9	-	-	19.0	17.4	-	-
	2	19.0	15.4	-	-	18.5	14.4	-	-
	3	16.0	12.4	-	+	18.5	14.9	-	+
	4	13.0	7.9	-	+ +	16.0	11.4	++	+ +
	5	11.5	4.9	-	+ +	14.0	6.9	++	+ + +
	6	7.0	1.9	-	+++	9.0	1.9	-	+ + +
80	1	19.5	17.9	-	-	lost			
	2	18.0	15.9	-	-	18.0	15.9	-	-
	3	17.0	14.9	-	-	17.5	14.4	-	-
	4	15.0	13.9	-	+ +	19.0	14.9	-	+ +
	5	15.0	11.9	-	+ +	17.5	14.9	-	+ +
	6	12.0	7.9	-	+ +	17.0	12.9	-	+ +
85	1	17.5	14.0	-	-	18.0	15.9	-	-
	2	17.0	13.0	-	-	19.0	15.4	-	-
	3	14.0	11.0	-	+	18.0	13.9	-	+
	4	12.5	12.0	-	+	18.0	13.9	-	+ +
	5	12.5	10.4	+	+ +	17.0	11.9	+	+ +
	6	10.0	6.5	++	+ +	16.0	10.9	++	+ +
90	1	17.0	14.9	-	-	17.0	15.9	-	-
	2	17.0	13.9	-	-	17.0	14.9	-	-
	3	lost				16.0	14.9	-	-
	4	13.5	9.9	-	-	16.5	13.9	-	-
	5	10.5	6.9	-	-	16.0	13.9	-	+ ?
	6	8.5	4.9	-	-	15.5	11.4	-	+ + ?

*Key: 1. Control
2. 13 Mg %
3. 26 "
4. 39 Mg. %
5. 52 "
6. 65 "

Table 11. The difference in ascorbic acid content between control sample and samples to which 52 mg. % copper was added at different temperatures, for the first and second day of storage.

Sample	65	70	75	80	85	90
	Ascorbic acid 1st day (Mg/L)					
	Temperature (° C. - 10 min.)					
	Cu added before heating					
Control	19.0	19.0	19.0	19.5	17.4	17.0
52 mg.%	5.4	4.0	11.4	14.9	10.5	7.0
Diff.	13.6	15.0	7.6	4.6	6.9	10.0
	Cu added after heating					
Control	18.0	19.0	19.0	18.5	18.0	17.0
52 mg.%	7.5	9.0	14.0	17.4	17.0	16.0
Diff.	10.5	10.0	5.0	1.1	1.0	1.0
	Ascorbic acid 2nd day (Mg/L)					
	Temperature (° C. - 10 min.)					
	Cu added before heating					
Control	15.9	15.9	17.9	17.9	17.0	15.0
52 mg.%	1.5	1.8	4.9	11.9	10.4	6.9
Diff.	14.4	14.1	13.0	6.0	6.6	9.1
	Cu added after heating					
Control	14.9	16.6	17.5	17.9	15.9	15.8
52 mg.%	1.9	1.9	6.9	14.8	13.8	13.9
Diff.	13.0	14.7	10.6	2.2	2.1	1.9

Table 12. <u>The difference in ascorbic acid content between control sample and sample to which 65 mg % copper was added at different temperatures for the first and second day of storage.</u>

Sample	Ascorbic acid 1st day (Mg/L)					
	Temperature (°C. - 10 min.)					
	65	70	75	80	85	90
	Cu added before heating					
Control	19.0	19.0	19.0	19.5	17.4	17.0
65 mg.%		2.5	7.0	12.0	10.0	9.5
Diff.		16.5	12.0	7.5	7.4	7.5
	Cu added after heating					
Control	18.0	19.0	19.0	18.5	18.0	17.0
65 mg.%	6.0	6.5	9.0	17.0	16.0	15.4
Diff.	12.0	12.5	10.0	1.5	2.0	1.6
	Ascorbic acid 2nd day (Mg./L)					
	Cu added before heating					
Control	15.9	15.9	17.9	17.9	17.0	15.0
65 mg.%		2.5	1.9	7.9	6.5	4.9
Diff.		13.4	16.0	10.0	10.5	10.1
	Cu added after heating					
Control	14.9	16.6	17.5	17.0	15.9	15.8
65 mg.%	1.9	1.9	1.9	12.9	10.9	11.4
Diff.	13.0	14.7	15.6	4.1	5.0	4.4

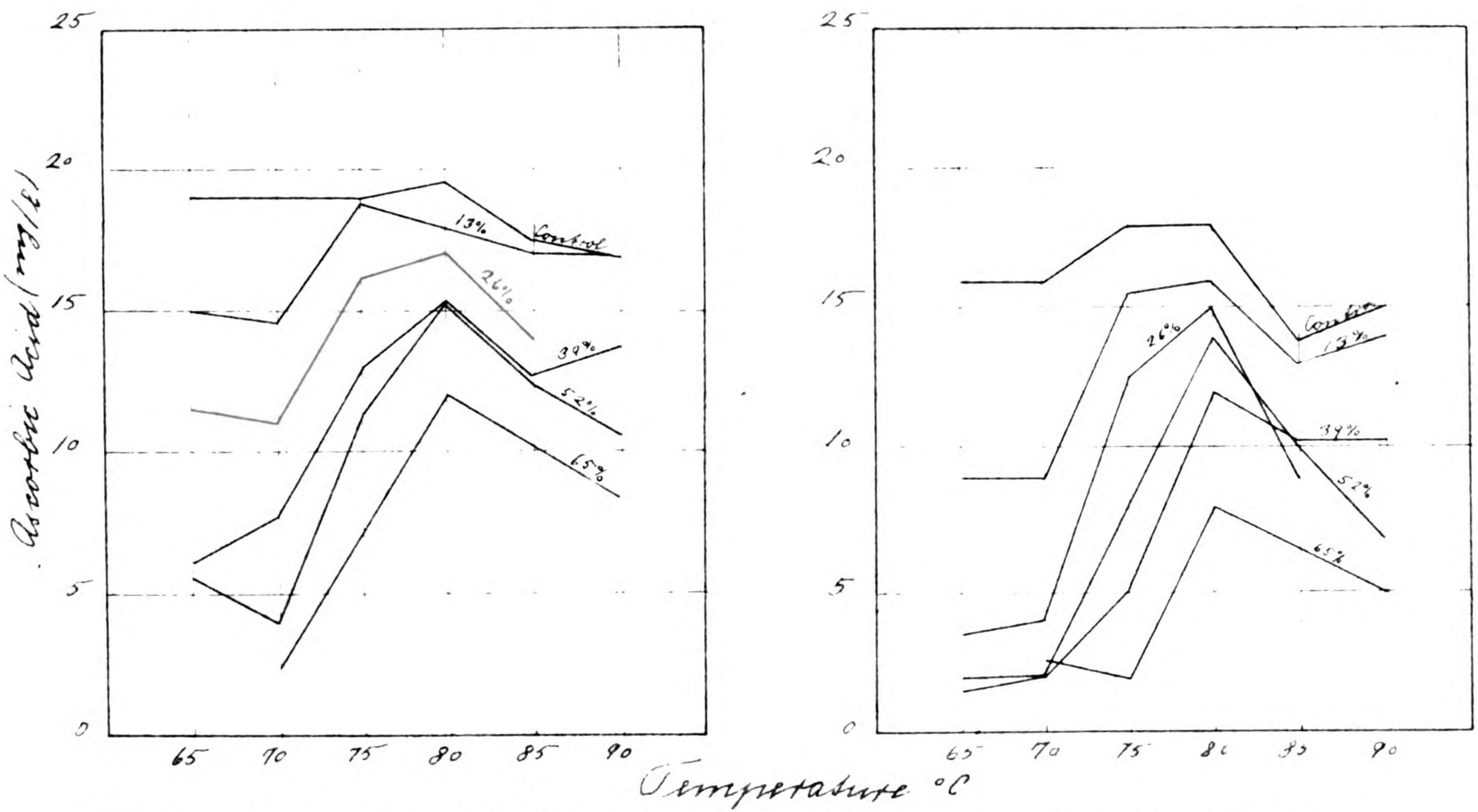

Figure 7. Showing the stability of ascorbic acid in milk when heated to various temperatures before copper had been added. Titration for the first and second day, respectively.

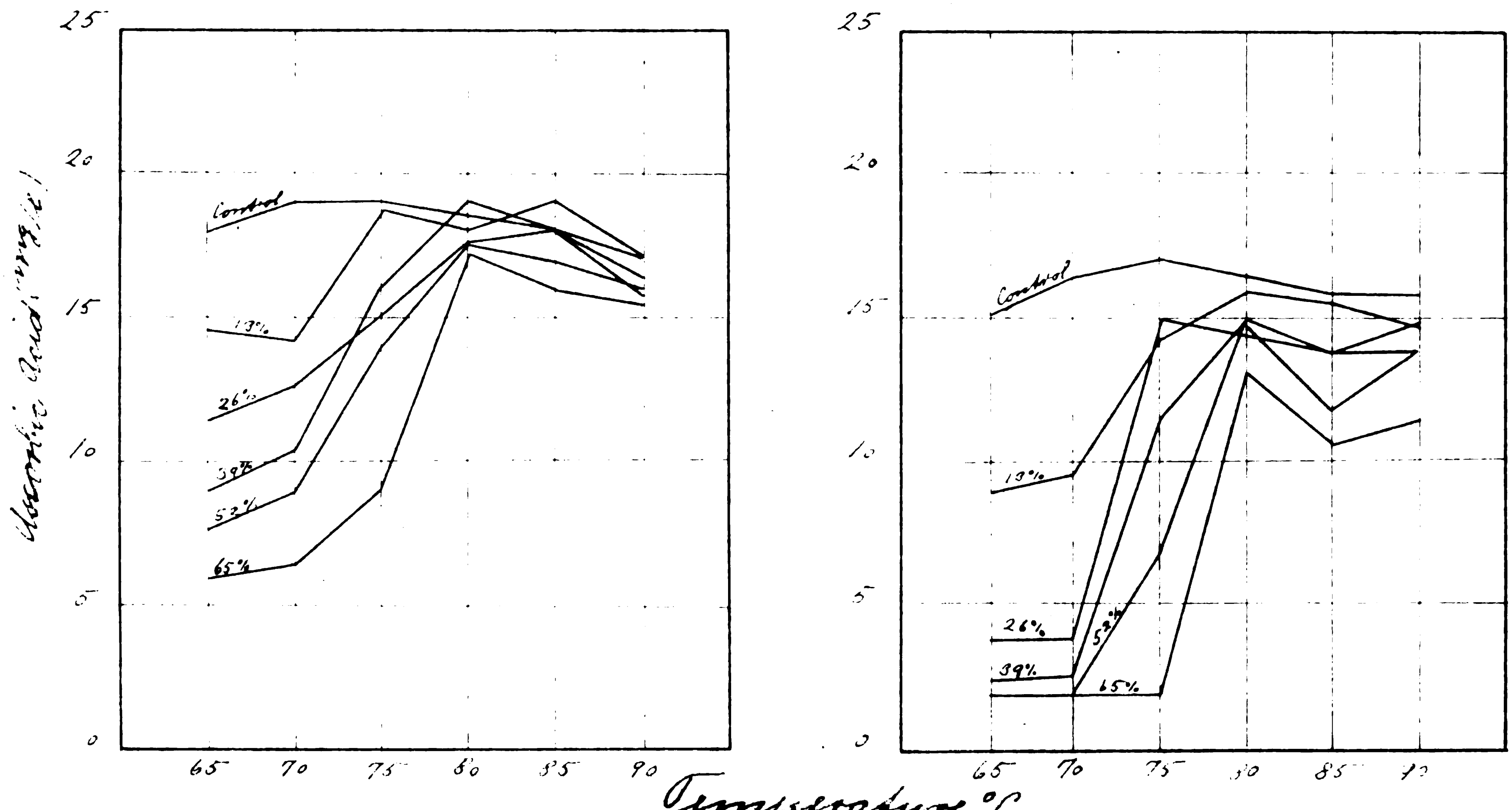

Figure 8. Showing the stability of ascorbic acid in milk when heated to various temperatures after which copper had been added. Titration for the first and second day, respectively.

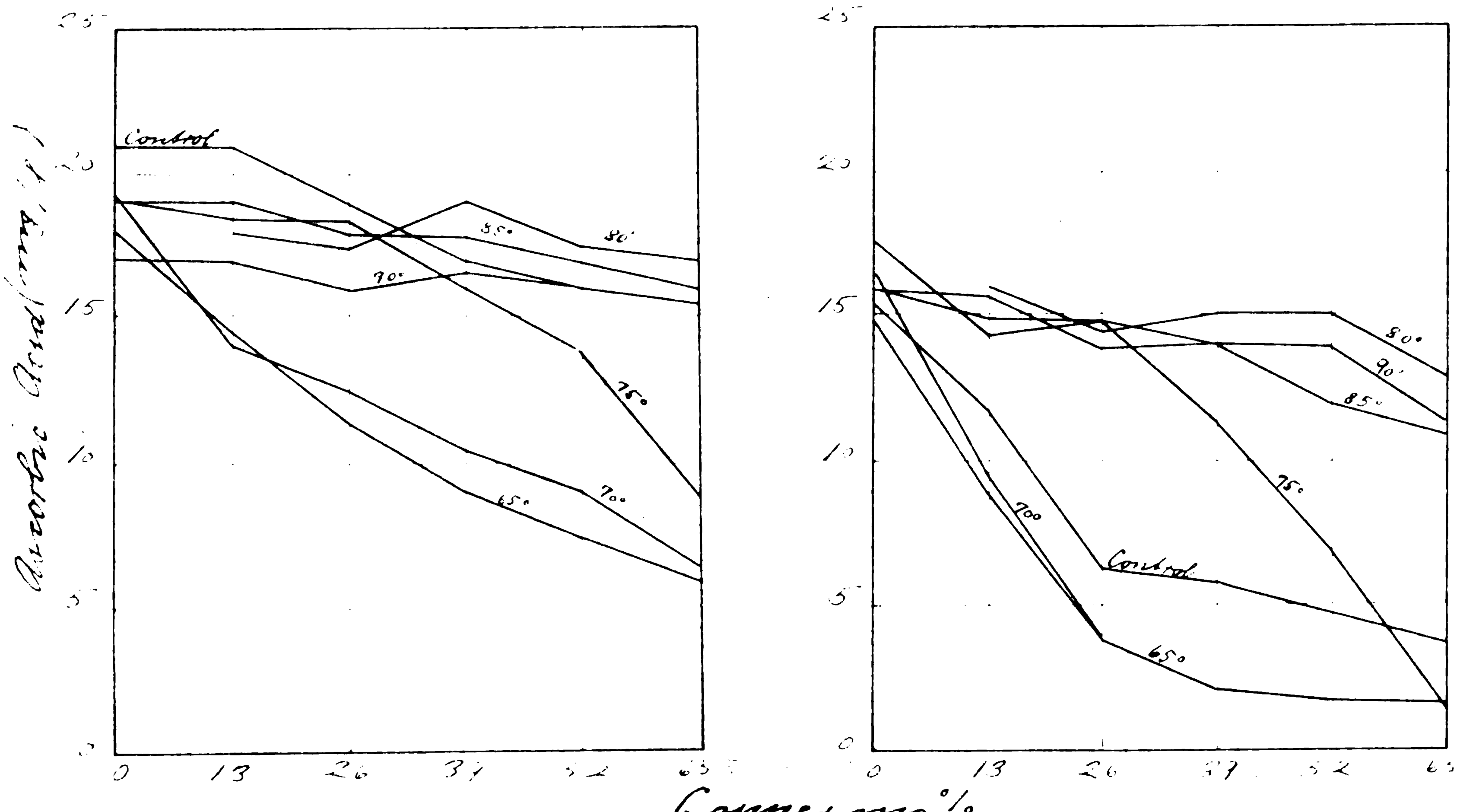

Figure 9. Showing the stability of ascorbic acid in milk when various amounts of copper were added after heat treatment. Titrations for the first and second day, respectively.

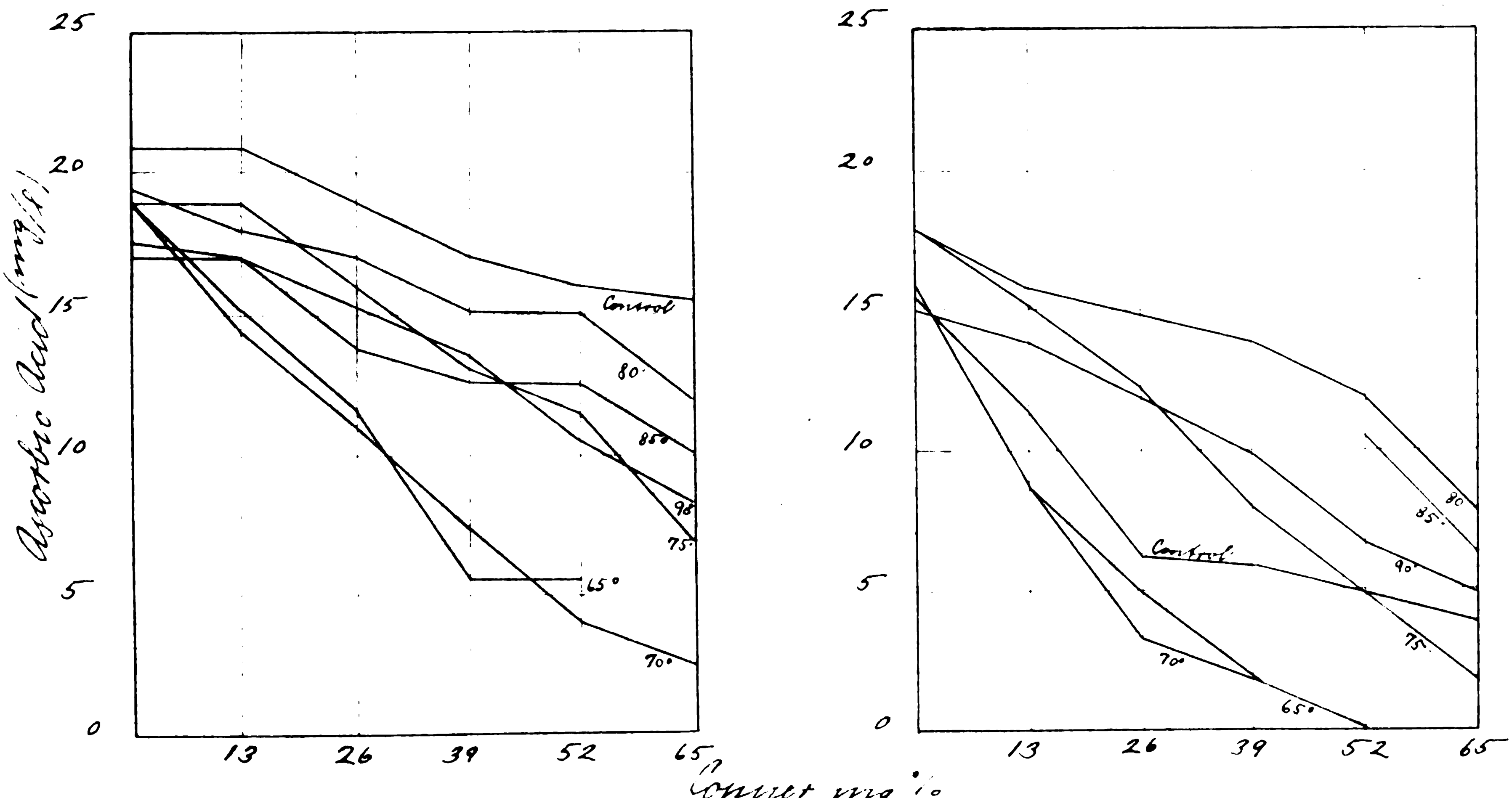

Figure 10. Showing the stability of ascorbic acid in milk when various amounts of copper were added before heat treatment. Titrations for the first and second day, respectively.

<u>The effect of copper and irradiation upon the ascorbic acid content of raw milk when the cold milk was passed over a surface cooler.</u>

Milk used in this experiment was secured from individual cows at the College Dairy Barn during the month of August. Immediately after sampling, the milk was put into a refrigerator at 4° C. where it was held until treated the next day. The ascorbic acid content was determined daily thereafter for four days. Likewise, the milk was tested organoleptically to note the development of the oxidized flavor. The data secured are presented in Tables 13, 14, 15, 16 and 17, inclusive, and are summarized in Table 18 and are shown graphically in Figure 11.

A comparison of the data in Table 14 indicates that aeration of the cold milk alone was sufficient to cause a considerable reduction in the ascorbic acid content of the milk. Not only was this effect true as an average, but also of each lot of milk from the 17 different cows. The addition of copper prior to aeration further reduced the ascorbic acid content of the milk, as shown in Table 15. However, as shown in Table 16 irradiation of the milk had little effect upon the ascorbic acid content of the milk beyond that resulting from aeration. Likewise, as shown in Table 17, irradiation of copper contaminated milk had little effect beyond that resulting from the copper alone. These comparisons can be made more readily from the summaries presented in Table 18. In these experiments irradiation was carried out similarly to that employed in some commercial plants, namely, irradiation of the milk raw, cold and pasteurizing afterwards. These data indicate that irradiation, in itself, as it is performed under practical conditions, has no deleterious effect upon the ascorbic acid present. However, the addition of copper to the milk followed by aeration resulted in a drop in the ascorbic acid considerably below that of the control samples and that of the samples irradiated. In the case where the milk had copper added to it and then irradiated, about the same drop in ascorbic acid was noted as in the case where copper alone was added. This is another indication that irradiation itself has little effect, if any, upon the ascorbic acid of milk.

In these experiments the samples were scored for oxidized flavor also. However, it was rarely possible to detect any oxidized flavor, although the milk was irradiated, copper contaminated, and was held as long as six days.

Despite the drastic reduction of ascorbic acid values resulting from aeration and from the addition of copper, oxidized flavors in milk were noted rarely, another indication that the development of oxidized flavor and instability of ascorbic acid are not so closely related as might be expected.

In this experiment the same interesting peculiarity regarding different milk's stability in respect to ascorbic acid was noted. Cows number 25, 30, 111 and 116 seemed to produce a milk which was markedly stable in respect to ascorbic acid despite the different treatments.

Table 13. The ascorbic acid content of raw milk from individual cows when stored at 4° C. for several days.

Cow (No.)	Ascorbic acid (Mg/L) First day	Second day	Third day	Fourth day
14	18.0	15.0	10.6	3.2
23	16.0	12.5	7.4	2.1
25	26.0	24.0	21.0	12.7
30	25.0	24.5	22.3	19.1
37	21.0	19.0	15.9	9.0
97	20.9	15.0	9.0	2.1
111	24.0	21.5	18.1	12.2
116	23.5	22.0	19.6	14.9
117	22.0	19.0	15.4	19.1
134	20.5	17.0	12.2	6.4
144	18.5	16.0	11.1	6.4
190	20.0	16.0	13.3	6.9
226	23.0	20.0	16.9	10.6
246	23.0	19.0	14.9	6.4
252	23.0	19.0	19.1	12.7
261	20.5	20.5	16.0	10.1
300	23.0	19.0	15.4	5.3
Ave.	21.5	18.8	14.4	9.4

Table 14. The ascorbic acid content of raw milk from individual cows when cooled over a surface cooler and stored.

Cow (No.)	Ascorbic acid (Mg/L) First day	Second day	Third day
14	13.5	7.9	2.7
23	13.0	7.9	1.1
25	22.5	18.1	9.6
30	22.0	19.6	15.4
37	14.5	7.9	3.7
97	16.0	9.6	2.1
111	18.0	13.3	4.2
116	16.5	11.7	2.1
117	17.5	12.7	5.3
134	16.5	12.7	5.8
144	15.5	9.6	4.2
190	14.5	9.6	3.7
226	14.0	6.4	1.6
246	16.5	9.6	3.2
252	19.5	14.9	12.2
261	17.5	13.8	6.4
300	19.0	12.7	4.2
Ave.	16.9	11.6	5.1

Table 15. The ascorbic acid content of raw milk from individual cows when cooled over a surface cooler, copper contaminated and stored.

Cow (No.)	Ascorbic acid (Mg/L) First day	Second day	Third day
14	8.0	3.2	0.0
23	5.5	2.1	0.0
25	14.0	4.8	0.0
30	16.5	10.6	3.7
37	8.0	2.7	0.0
97	5.5	1.6	0.0
111	11.5	3.7	0.0
116	13.0	5.3	3.7
117	9.5	2.1	0.0
134	5.5	2.1	0.0
144	6.0	1.6	0.0
190	4.5	1.6	0.0
226	8.0	1.6	0.0
246	7.5	2.1	0.0
252	10.5	3.7	0.0
261	9.0	2.7	0.0
300	10.5	2.7	0.0
Ave.	9.0	3.2	0.4

Table 16. The ascorbic acid content of raw milk from individual cows when cooled over a surface cooler, irradiated and stored.

Cow (No.)	Ascorbic acid (Mg/L) First day	Second day	Third day
14	11.5	5.3	2.1
23	10.5	5.8	2.1
25	18.5	15.4	7.4
30	20.5	19.6	17.0
37	15.0	10.1	5.3
97	11.5	4.2	1.6
111	19.5	14.1	10.1
116	18.0	15.4	9.6
117	15.0	10.1	4.7
134	13.5	8.5	2.7
144	12.5	7.4	2.7
190	15.5	9.0	3.7
226	8.5	1.1	1.1
246	16.5	9.0	2.7
252	17.5	14.9	9.6
261	16.5	14.3	1.9
300	16.5	14.3	5.3
Ave.	15.1	10.5	5.5

Table 17. The ascorbic acid content of raw milk from individual cows when cooled over a surface cooler, copper contamined, irradiated, and stored.

Cow (No.)	Ascorbic acid (Mg/L)		
	First day	Second day	Third day
14	6.0	3.7	0.0
23	4.5	2.1	0.0
25	14.0	3.7	0.0
30	16.0	7.4	2.1
37	6.5	2.7	0.0
97	6.5	2.1	0.0
111	10.5	3.2	0.0
116	12.5	3.2	0.0
117	9.5	2.1	0.0
134	6.5	2.7	0.0
144	6.5	2.1	0.0
190	5.0	2.1	0.0
226	9.5	2.7	0.0
246	9.5	2.1	0.0
252	10.0	2.7	0.0
261	8.5	2.0	0.0
300	11.0	3.0	0.0
Ave.	8.9	3.0	0.1

Table 18. The effect of irradiation upon ascorbic acid in raw milk with and without the addition of copper.

Treatment	Ascorbic acid (Mg/L)			
	1st day	2nd day	3rd day	4th day
Control	21.5	18.8	14.4	9.4
Cooled over surface cooler	-	16.9	11.6	5.1
Cooled and copper added	-	9.0	3.2	0.0
Cooled and irradiated	-	15.1	10.5	5.5
Cooled, copper added and irradiated	-	8.9	3.0	0.0

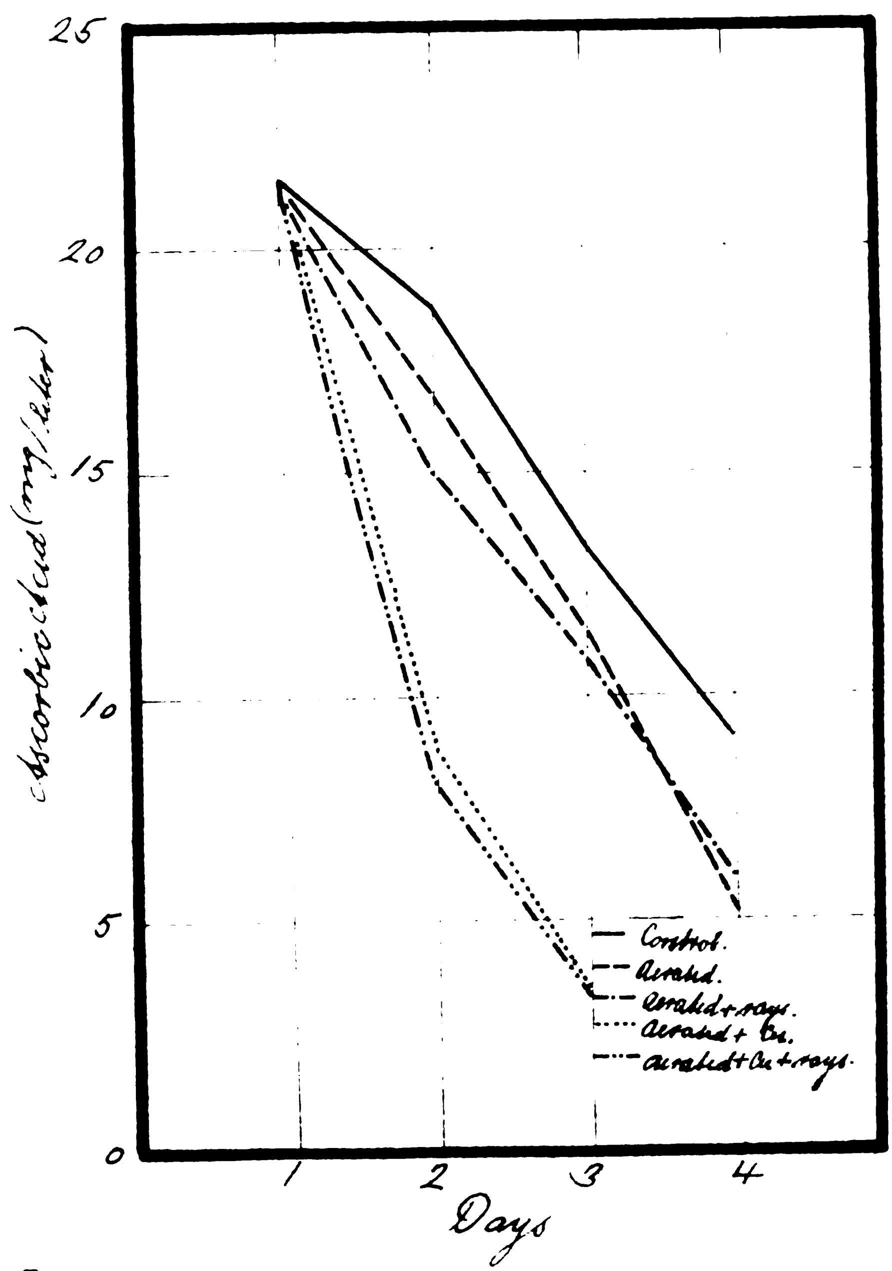

Figure 11. Showing the effect of aeration, irradiation and addition of copper upon the ascorbic acid content of milk after several days of storage.

Relationship between the oxidized flavor and the ascorbic acid of milk.

The data reported herein are from three different experiments carried out respectively in spring, summer and fall. Milk was secured from individual cows from the College Dairy Barn. An attempt was made to secure milk from ten cows whose milk showed no susceptibility to the development of the oxidized flavor under various treatments and from ten cows whose milk did show this characteristic.

In each experiment the milk samples were subjected to different treatments, such as pasteurization, the addition of copper, aeration, and so forth.

The data are classified into two groups, those cows whose milk, as a result of the various treatments, showed little, if any, susceptibility to develop oxidized flavor, and those whose milk had a pronounced susceptibility. These are shown in Tables 19 and 20.

The average values show that milk with a tendency to develop the oxidized flavor contained a higher initial ascorbic acid content than did that of the other group.

Tables 21 and 22 include data obtained from the respective milks of Tables 19 and 20 when both groups were pasteurized at 63° C. for one-half hour, with the addition of copper. Again, the non-susceptible group of milk showed a lower ascorbic acid content than did that of the other group. The data were treated statistically with the results summarized in Table 23. Very high standard errors, indicating very high significance of the different average values, were found. These results indicate a relationship between the susceptibility to the development of the oxidized flavor and the initial ascorbic acid content in milk. Data of Tables 19 and 20 show that milk testing high in ascorbic acid most easily became oxidized. Data of Tables 21 and 22 indicate also that as a result of heat treatment, even with such an oxidized flavor promoter as copper, the oxidized flavor was on the average most apt to develop in those samples having the highest initial ascorbic acid content.

Data of Tables 24 and 25 show the treatments to which the milk was subjected to determine its susceptibility to the development of the oxidized flavor. The data furnish sufficient proof as to the different susceptibilities of the milk toward oxidation.

Data in Tables 26 and 27 indicate that not only was the percentage drop largest in the group which showed no susceptibility to oxidized flavor, but also the actual drop. It must be noted that this drop was calculated also on the samples which were pasteurized and to which copper was added. Furthermore, the ascorbic acid values were determined before the samples were scored for oxidized flavor which was usually on the third day. Throughout the experiment it has been noticed, with a few exceptions, that milk which has any oxidized flavor of note usually has no ascorbic acid.

In the first series of these three experiments temperatures of heating were in one case as high as 75° C. and this temperature was employed as long as one-half hour. The data in Tables 28 show the comparative amounts of ascorbic acid in non-susceptible and susceptible milk under various heat treatments. These average values are illustrated in Figure 12. Heating at 75° C. for one-half hour increased the stability of the ascorbic acid content tremendously for the samples that showed no susceptibility to the development of the oxidized flavor, but had little influence upon the ascorbic acid of the susceptible samples.

Table 19. The ascorbic acid content of different cows' milk showing pronounced susceptibility to the development of the oxidized flavor.

Cow (No.)	Ascorbic acid (Mg/L) First day	Second day	Third day	Fourth day
50	20.2	16.3	14.4	
79	17.3	6.3	1.5	
146	23.5	24.0	16.3	
156	18.3	13.0	10.6	
197	20.2	15.4	12.5	
226	20.6	15.4	13.0	
240	26.4	22.1	20.2	
248	29.7	21.1	20.1	
254	17.8	16.8	13.0	
259	20.6	14.4	12.5	
261	21.1	16.3	16.0	
23	15.8	10.4	6.9	
25	27.2	19.8	14.8	
30	21.7	18.3	16.8	13.8
111	20.7	16.8	14.3	9.8
116	19.8	17.3	12.4	8.4
117	17.8	14.8	11.4	7.9
144	16.8	12.8	8.4	5.9
252	21.7	16.8	14.8	9.8
261	19.3	16.3	10.4	7.9
300	19.3	16.3	10.9	7.4
30	23.6	22.6	19.6	
41	19.3	18.5	13.7	
42	21.7	18.1	6.8	
111	24.5	23.5	23.1	
117	21.2	19.5	12.8	
134	20.2	17.0	10.8	
142	18.8	16.0	8.8	
180	20.7	16.0	10.8	
190	21.2	17.5	12.7	
197	22.2	17.5	11.8	
238	22.2	17.5	13.7	
300	18.8	16.5	13.7	
Ave.	21.0	17.0	13.0	7.1

Table 20. The ascorbic acid content of different cows' milk showing no susceptibility to the development of the oxidized flavor.

Cow (No.)	Ascorbic acid (Mg/L) First day	Second day	Third day	Fourth day
25	16.3	7.7	6.3	
28	16.3	7.7	3.9	
73	2.4	0.9	0.9	
100	7.3	1.8	1.5	
108	21.1	13.0	11.0	
180	15.9	6.8	7.7	
252	23.5	18.3	17.3	
14	16.3	8.4	4.4	3.4
37	18.3	14.3	8.9	5.9
47	18.3	14.8	10.9	6.9
97	20.7	15.3	13.3	10.9
134	17.8	13.8	12.4	7.4
190	20.7	15.3	9.9	5.9
226	17.8	13.3	8.4	4.9
240	16.3	12.8	7.9	4.4
246	18.3	9.8	5.9	1.7
13	16.4	12.5	6.8	
36	18.3	11.5	2.5	
37	18.3	10.5	3.4	
100	24.1	20.1	12.8	
174	16.4	12.5	7.8	
237	19.7	13.0	2.5	
239	21.7	-	6.8	
Ave.	17.4	11.5	7.4	5.7

Table 21. Ascorbic acid content of individual cow's milk showing susceptibility to oxidized flavor development when holder pasteurized, copper contaminated and stored.

Cow (No.)	Ascorbic acid (Mg/L)			
	First day	Second day	Third day	Fourth day
50		9.2		
79		5.4		
146		13.0		
156		8.7		
197		5.8		
226		5.8		
240		16.3		
248		14.9		
254		3.9		
259		7.7		
261		12.0		
23	12.4	5.9	1.4	
25	20.7	10.3	3.4	
30	16.8	12.4	6.4	3.4
111	16.8	8.9	2.9	
116	16.3	10.9	3.9	2.9
117	13.3	5.4	1.4	
144	10.9	4.4	0.0	
252	14.3	6.4	1.9	
261	12.9	5.9	1.9	
300	14.8	8.4	2.9	
30		16.0	6.8	
41		11.5	2.5'	
42		7.5	1.9	
111		16.0	5.9	
117		12.0	2.9	
134		5.5	1.5	
142		5.5	1.9	
180		4.5	1.9	
190		8.0	0.9	
197		4.5	0.9	
238		7.0	1.9	
300		9.0	0.9	
Ave.	14.9	8.6	2.7	

Table 22. Ascorbic acid content in samples of individual cows' milk showing susceptibility to oxidized flavor development when holder pasteurized, copper contaminated and stored.

Cow (No.)	Ascorbic acid (Mg/L)		
	First day	Second day	Third day
25		5.8	
28		5.4	
73		1.5	
100		0.9	
108		9.2	
180		4.4	
252		11.6	
14	8.4	4.9	2.4
37	11.4	4.9	1.9
47	11.4	4.4	1.4
97	14.3	7.4	1.9
134	10.3	2.9	1.9
190	8.4	3.4	0.0
226	8.9	2.9	0.0
240	6.9	2.9	0.0
246	8.4	1.8	0.0
13		2.0	1.9
36		3.8	2.5
37		3.5	3.4
100		10.0	1.9
174		2.5	1.9
237		3.0	0.9
239		5.0	0.9
Ave.	9.8	4.6	1.9

Table 23. Statistical treatment of data from Tables 19 and 20 and from Tables 21 and 22.

Treatment	Calculations	Ascorbic acid (Mg/L) Non-susceptible	Susceptible
None		First day	
Control	Calculated ave.	17.46 ± 4.55	19.92 ± 3.04
	Standard errors	3.23	
		Second day	
	Calculated ave.	11.45 ± 4.72	17.11 ± 3.5
	Standard errors	4.80	
		Third day	
	Calculated ave.	7.41 ± 4.16	14.05 ± 4.3
	Standard errors	5.77	
		First day	
Past 63° C. plus copper	Calculated ave.	4.46 ± 2.71	8.84 ± 3.77
	Standard errors	5.10	

Table 24. <u>Samples of individual cows' milk showing susceptibility to oxidized flavor development when subjected to several treatments.</u>

Cow (No.)	Oxidized flavor development when the milk was				
	Raw plus copper	Pasteurized (63° C - 30 min.) and			
		Control	Copper added	Aeration	Copper and aeration
50	+	+ + +			
79	+ + +	+			
146	+ + +	+ + +			
159	+ + +	+ + +			
197	+	+ + +			
226	+ + +	+ + +			
240	+ + +	+ + +			
248	+ +	+ +			
254	+ + +	+ + +			
259	+ + +	+ +			
261	+ + +	+ + +			
23		+	+ +	+ +	+
25		+	+	-	-
30		+	-	-	+ +
111		-	+ + +	+ +	+ + +
116		+	+	?	+
117		+	-	-	+
144		+	+ + +	+ +	+ +
252		-	+ + +	-	+
261		-	+ + +	+	+ + +
300		-	+ +	+	+ +
30			+ +		
41			+ +		
42			+ + +		
111			+ + +		
117			+ +		
134			+ +		
142			+ +		
180			+ + +		
190			+ + +		
197			+ + +		
238			+ +		
300			+ + +		

Table 25. Samples of individual cows' milk showing no susceptibility to oxidized flavor development when subjected to several treatments.

Cow (No.)	Oxidized flavor development when the milk was				
	Raw plus copper	Pasteurized (63° C. - 30 min.) and			
		Control	Copper added	Aeration	Copper added aeration
25	-	+			
28	-	+			
73	-	-			
100	-	-			
108	-	-			
180	-	-			
252	-	-			
14		-	-	-	-
37		-	-	-	-
47		-	-	-	-
97		-	-	-	-
134		-	-	-	-
190		-	-	-	-
226		-	-	-	-
240		+	-	-	-
246		-	-	-	-
13			-		
36			+		
37			-		
100			-		
174			+		
237			+		
239					

Table 26. The decrease in ascorbic acid of non-susceptible and susceptible milk from individual cows during the month of May.

Cow (No.)	Decrease in ascorbic acid from raw milk: Per Cent: Past. (63° C. 30 min.) Stored 2 da.	Per Cent: Past. plus copper Stored 3 da.	Mg. per liter: Past. (63° C. (30 min. Stored 2 da.	Mg. per liter: Past. plus copper Stored 3 da.
		Nonsusceptible		
13	64	88	10.5	14.4
36	90	81	16.4	14.8
37	90	81	16.4	14.8
100	47	59	11.3	14.1
171	85	85	14.0	13.9
237	85	85	16.8	16.7
239	82	75	17.8	16.2
Ave.	77.6	79.1	14.7	15.0
		Susceptible		
30	46	32	10.9	7.6
41	44	40	8.5	7.8
42	72	65	15.8	14.2
111	24	35	5.8	8.5
117	58	43	12.4	9.2
134	66	73	13.2	14.5
142	69	71	12.9	13.3
180	53	79	10.9	16.3
190	47	62	9.9	13.2
197	51	79	11.4	17.7
238	51	70	11.4	14.5
300	37	52	7.0	9.8
Ave.	51.5	58.4	10.9	12.2

Table 27. <u>The decrease in the ascorbic acid of non-susceptible and susceptible milk from individual cows during the month of July.</u>

Cow (No.)	Decrease in ascorbic acid from raw milk			
	Per Cent		Mg. per liter	
	Past. (63° C. 30 min.) Stored 2 da.	Past. plus copper Stored 3 da.	Past. Stored 2 days	Past. plus copper Stored 2 da.
		Non-susceptible		
14	57.7	70.0	9.4	11.4
37	41.0	73.2	7.5	13.4
47	37.7	75.9	6.9	13.9
97	28.5	64.3	5.9	13.3
134	38.7	83.7	6.9	14.9
190	44.9	83.6	9.3	17.3
226	44.9	83.7	8.0	14.9
240	39.8	82.2	6.5	13.4
246	56.8	89.6	10.4	16.4
Ave.	43.3	78.5	7.9	14.3
		Susceptible		
23	38.0	62.7	6.6	9.9
25	34.6	62.1	9.4	16.9
30	24.9	42.9	5.4	9.3
111	26.1	61.8	5.4	11.8
116	32.8	44.9	6.5	8.9
117	30.3	69.7	5.4	12.4
144	35.4	73.8	5.9	12.4
252	34.1	70.5	7.4	15.3
261	33.7	69.4	6.5	13.4
300	38.3	56.4	7.4	10.9
Ave.	32.8	61.4	6.6	12.1

Table 28. The ascorbic acid values of non-susceptible and susceptible milk on the third day of storage.

Cow (No.)	Ascorbic acid (Mg/L) Control	Pasteurized (63° C. - 30 min.)	Pasteurized (75° C. - 30 min.)
		Non-susceptible	
13	6.8	9.3	11.3
36	2.5	2.9	9.3
37	3.4	3.9	10.8
100	12.8	14.7	16.2
174	7.8	5.4	10.8
237	2.5	2.9	11.8
239	6.8	7.8	14.3
Ave.	6.1	6.7	12.1
		Susceptible	
30	19.6	19.7	18.2
41	13.7	14.7	12.8
42	6.8	8.8	13.7
111	23.1	18.7	18.7
117	12.8	16.7	13.7
134	10.8	10.8	12.8
142	8.8	10.3	10.3
180	10.8	12.3	14.3
190	12.7	13.3	14.3
197	11.8	13.7	14.3
238	13.7	12.9	14.3
300	13.7	14.7	12.8
Ave.	13.2	13.9	14.2

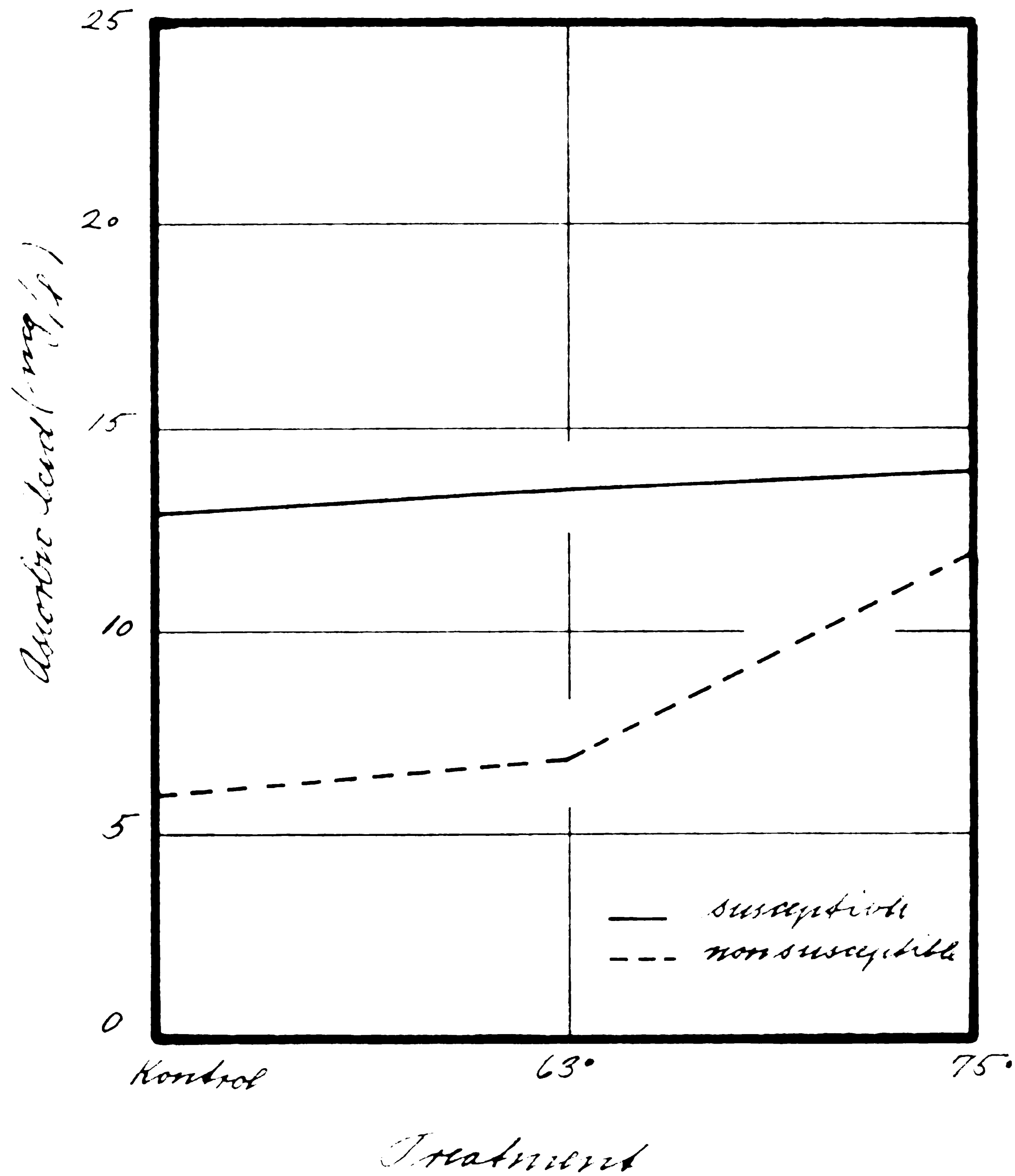

Figure 12. Showing the ascorbic acid values of milk susceptible and non-susceptible to the development of the oxidized flavor following different heat treatments.

Part II

ASCORBIC ACID OCCURRING NATURALLY IN MILK UNDER VARIOUS CONDITIONS

The effect of season upon the ascorbic acid content of streptococcic and non-streptococcic milk.

The time of the year in which the milk from streptococcic and from non-streptococcic cows was examined for ascorbic acid is given in Table 29. The ascorbic acid data obtained are presented in Table 30, and are shown graphically in Figure 13.

The data in Table 30 are treated statistically according to the law of variance and the values obtained are tabulated in Table 31. Data in this table indicate, by the large value of F, for monthly means, that there are significant differences between the monthly averages of ascorbic acid; and also, the size of F, for replications of cows, indicate significance between replications, or cows.

On examining the monthly means of ascorbic acid for the first day, it is seen that the means for December, January, February and March were significantly smaller than that for the rest of the months. This was done for the fourth day also with the same conclusions.

Ascorbic acid values of milk from normal and from mastitic cows respectively are presented in Tables 32 and 33. The average ascorbic acid value of the milk from each cow was calculated and are assembled in Tables 34 and 35. The data demonstrate clearly that the mastitic cows have a decidedly lower ascorbic acid content than that of the normal cows.

The ascorbic acid values of the milk from non-mastitic and from mastitic cows per month are given in Tables 36 and 37 and are shown graphically in Figure 14. These data indicate clearly that the mastitic milk show the greatest variation in ascorbic acid throughout the year.

Table 29. The different periods in which the cows' milk were used for ascorbic acid determinations.

Cow (No.)	Normal		
	Breed	Started	Ended
230	Brown Swiss	1st December	2nd July
150	Ayrshire	2nd April	24th November
146	Ayrshire	1st December	2nd April
116	Jersey	7th July	15th October
37	Guernsey	20th October	24th November
Cow (No.)	**Mastitic**		
	Breed	**Started**	**Ended**
239	Brown Swiss	4th March	24th November
207	Holstein	9th December	26th April
174	Holstein	29th April	24th November
63	Jersey	9th December	19th March

Table 30. The average daily values of ascorbic acid (Mg/L) in milk for both normal and mastitis cows throughout the year when stored for several days.

Month	First day	Second day	Third day	Fourth day
January	16.1	11.6	8.9	7.5
February	12.9	10.3	9.2	9.2
March	15.4	11.1	7.6	5.4
April	17.3	12.5	8.9	8.3
May	18.5	15.1	11.2	7.9
June	19.8	16.4	13.2	10.7
July	18.3	15.4	12.5	11.2
August	20.5	17.9	15.4	13.7
September	18.3	16.6	13.4	12.0
October	21.0	17.9	15.5	12.0
November	19.1	16.2	13.0	11.3
December	15.6	11.7	8.9	7.3

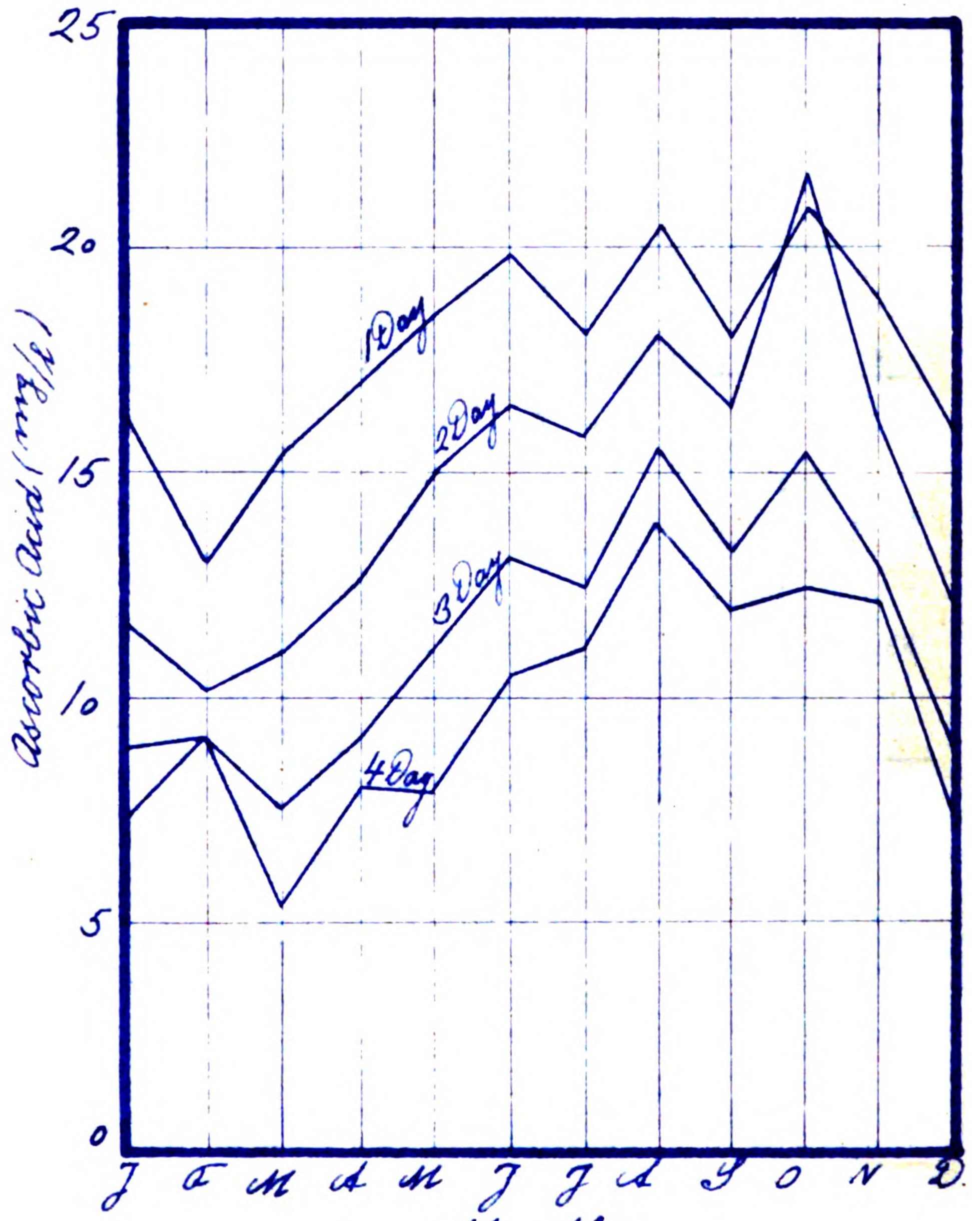

Figure 13. Showing average values of ascorbic acid in milk for both normal and mastitis cows throughout the year.

Table 31. Analyses of variance pertaining to seasonal variations of ascorbic acid in raw milk from both normal and mastitis cows (1st day titrations).

Source of variation	Degrees of freedom	Sums of squares	Variance	F
Total	47	394.5		
Between month means	11	233.5	21.2	6.23
Between replication means	3	47.4	15.8	4.64
Error	33	113.6	3.4	

Table 32. The average ascorbic acid content (Mg/L) of normal cows' milk throughout the year (874 determinations).

Month	First day	Second day	Third day	Fourth day
	No. 37 - Guernsey			
October	20.5	17.4	13.8	9.6
November	19.9	16.4	12.2	11.6
Average	20.1	16.7	12.6	11.3
	No. 16, Jersey			
July	19.1	16.3	13.4	11.8
August	20.8	18.3	15.9	13.9
September	19.2	17.6	16.7	12.9
October	22.5	19.2	15.2	13.4
Average	20.0	17.5	15.0	12.6
	No. 146 - Ayrshire			
December	16.8	12.9	12.1	11.4
January	17.2	12.9	12.9	13.2
February	16.0	14.7	14.9	15.6
March	17.8	15.6	10.3	10.3
Average	17.2	14.2	12.4	12.5
	No. 150 - Ayshire			
April	17.3	13.2	10.8	8.7
May	19.6	17.4	14.9	13.6
June	18.5	16.5	13.8	10.9
July	17.6	15.4	13.3	12.4
August	19.8	17.4	15.4	15.0
September	15.0	13.4	11.4	9.1
October	18.6	15.4	14.2	9.8
November	19.7	16.2	13.1	10.2
Average	18.4	15.7	13.7	11.1
	No. 230 - Brown Swiss			
December	18.0	14.0	10.7	9.4
January	16.7	13.1	9.8	8.0
February	14.2	11.3	9.9	9.1
March	15.9	12.9	10.0	6.6
April	17.4	13.5	11.1	11.6
May	16.9	13.6	11.6	8.5
June	19.8	14.4	12.0	10.5
Average	17.0	13.3	10.7	9.1

Table 33. The average ascorbic acid content (Mg/L) of mastitis cows' milk throughout the year (922 determinations).

Month	First day	Second day	Third day	Fourth day
		No. 63 - Jersey		
December	15.1	10.9	5.4	4.4
January	14.4	9.3	5.4	3.3
February	10.4	6.7	3.9	5.1
Average	13.3	9.0	4.9	4.3
		No. 174 - Holstein		
May	16.7	13.4	8.3	4.4
June	18.5	15.7	11.9	9.0
July	17.1	14.0	11.4	10.1
August	19.4	17.2	15.0	12.8
September	18.6	17.1	14.2	12.7
October	21.5	18.8	17.3	15.8
November	18.0	15.3	12.2	12.2
Average	18.6	15.8	12.9	10.7
		No. 207 - Holstein		
December	12.3	9.3	7.4	4.1
January	15.9	10.9	7.6	5.4
February	10.9	8.5	8.2	6.9
March	12.5	8.2	6.2	3.8
April	13.3	9.0	5.3	6.7
Average	12.9	8.8	6.7	5.5
		No. 239, Brown Swiss		
March	15.4	8.0	3.7	0.9
April	21.2	14.4	8.2	6.0
May	20.8	15.8	10.0	5.4
June	22.2	18.8	15.2	12.5
July	19.5	15.8	11.7	10.6
August	22.0	18.6	15.3	13.2
September	21.1	18.4	11.4	13.1
October	22.0	18.7	16.8	14.1
November	18.6	16.8	14.5	11.4
Average	20.4	16.0	11.9	10.0

Table 34. The average ascorbic acid (Mg/L) content of raw milk from normal cows.

Cow (No.)	Breed	First day	Second day	Third day	Fourth day
37	Guernsey	20.1	16.7	12.6	11.3
116	Jersey	20.1	17.5	15.0	12.6
146	Ayrshire	17.2	14.2	12.4	12.5
150	Ayrshire	18.4	15.7	13.7	11.1
230	Brown Swiss	17.0	13.3	10.7	9.1
Average		18.6	15.5	12.9	11.3

Table 35. The average ascorbic acid (Mg/L) content of raw milk from mastitis cows.

Cow (No.)	Breed	First day	Second day	Third day	Fourth day
63	Jersey	13.3	9.0	4.9	4.3
174	Holstein	18.6	15.8	12.9	10.7
207	Holstein	12.9	8.8	6.7	5.5
239	Brown Swiss	20.4	16.0	11.9	10.0
Average		16.3	12.4	9.1	7.6

Table 36. The average values of ascorbic acid (Mg/L) in milk from normal cows throughout the year.

Month	First day	Second day	Third day	Fourth day	Average of all days
January	16.9	13.0	11.3	10.6	13.0
February	15.1	13.0	12.4	12.3	13.2
March	16.8	14.2	10.1	8.4	12.4
April	17.3	14.2	10.9	10.1	13.1
May	18.2	13.3	13.2	11.1	14.0
June	19.1	15.5	12.9	10.7	14.6
July	18.3	15.4	13.3	12.1	14.8
August	20.3	15.9	15.7	14.4	16.6
September	17.1	17.8	14.1	11.0	15.0
October	20.5	17.5	14.4	10.7	15.8
November	19.8	16.3	12.7	10.9	15.0
December	17.4	13.4	11.4	10.4	13.2

Table 37. The average value of ascorbic acid (Mg/L) in milk from mastitis cows throughout the year.

Month	First day	Second day	Third day	Fourth day	Average for day
January	15.1	10.1	6.5	4.4	9.0
February	10.7	7.6	6.1	6.0	7.6
March	13.9	8.1	4.9	2.3	7.3
April	17.2	11.7	6.7	6.3	10.5
May	18.7	14.6	9.1	4.9	11.8
June	20.3	17.2	13.5	10.7	15.4
July	18.3	14.9	11.5	10.3	13.8
August	20.7	17.9	15.1	13.0	16.7
September	19.8	17.2	12.8	12.9	15.7
October	21.7	18.7	17.1	14.9	18.1
November	18.3	16.1	13.3	11.8	14.9
December	13.7	10.1	6.4	4.2	8.6

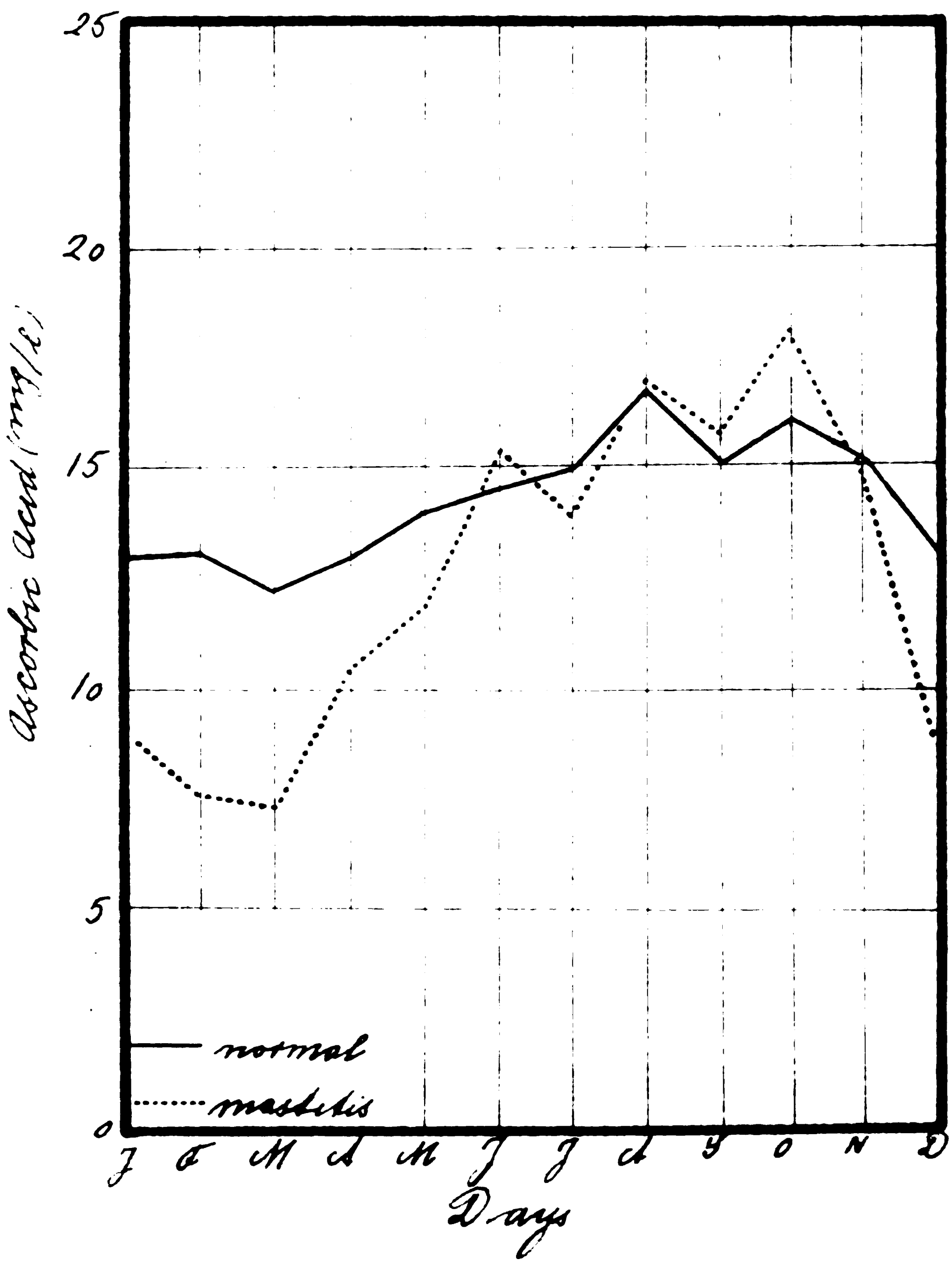

Figure 14. **Showing monthly average values of ascorbic acid in milk throughout the year for normal and for mastitic cows. Each month's value represents the average found each day for four days average.**

The ascorbic acid content of commercial pasteurized milk, irradiated milk, and raw milk throughout the year.

Samples of Grade A raw, of regular pasteurized and of irradiated milk were titrated for ascorbic acid daily throughout the year. The Grade A raw milk was produced at the College Dairy Barn. The irradiated milk was high grade, high testing milk produced at the College Dairy Barn and was irradiated in the CP unit at the College Creamery. The regular pasteurized milk was mixed milk testing approximately 3.7 per cent fat from approximately thirty patrons delivering milk to the College Creamery. The milk was pasteurized by the holder process in Pfaudler glass-lined and in Allegheny metal pasteurizers. The percentages distribution of the ascorbic acid contents of these milks are given in Tables 38, 39 and 40. The average seasonal values are given in Table 41 and from this Table the graphs of Figures 15, 16 and 17 are constructed.

On the basis of these findings it can be stated that as far as the irradiated and Grade A milk were concerned there was a very definite correlation between the season and the ascorbic acid content of the milk. This was very pronounced in the irradiated milk in connection with which it must not be overlooked that the oxidized flavor was not noticed from the first of April to the first of November.

The seasonal variation did not show up very well in the pasteurized milk. However, this milk was mixed milk from thirty different patrons, of various quality, and was at least 24 hours old before titration.

Table 38. **Percentage distribution of ascorbic acid in irradiated milk throughout the year, by seasons.**

Ascorbic acid (Mg/L)	Distribution of ascorbic acid per day when milk was held four days at 5° C. (%). Winter				Spring				Summer				Fall			
	1st day	2nd day	3rd day	4th day	1st day	2nd day	3rd day	4th day	1st day	2nd day	3rd day	4th day	1st day	2nd day	3rd day	4th day
0 - 1		18.0	46.7	100.0		4.8	11.4	71.5			2.9	14.3		10.7	25.0	52.6
1 - 2	2.3	25.6	43.3				14.3	14.3		2.5		25.7			10.0	26.3
2 - 3	4.5	10.3	10.0			4.8	31.4	7.1			20.0	37.1		3.6	15.0	10.5
3 - 4		28.2				7.1	17.1	4.8		2.5	20.0	14.3		3.6	25.0	10.5
4 - 5	4.5	17.9				9.5	11.4	2.4			14.3	5.7		10.7	15.0	
5 - 6	18.2					14.3	5.7			2.5	17.1	2.8	2.9	10.7	5.0	
6 - 7	9.1					23.8	5.7			10.0	11.4			28.6		
7 - 8	25.0					19.0				15.0	5.7		2.9	14.3		
8 - 9	18.2				2.0	9.5	2.9			17.5	2.9		11.7	3.6	5.0	
9 - 10	11.4				2.0	2.4				17.5	2.9		14.7	3.6		
10 - 11	6.8				16.3	4.8				17.5	2.9		23.5	3.6		
11 - 12					18.4				8.7	5.0			11.7	7.2		
12 - 13					28.6				16.7	5.0			17.6			
13 - 14					18.4				21.7	2.5			2.8			
14 - 15					12.2				15.2				5.9			
15 - 16					2.0				19.4	2.5			5.9			
16 - 17									13.0							
17 - 18									4.3							
19 - 20																

Table 39. Percentage distribution of ascorbic acid in regular pasteurized milk throughout the year by seasons.

Ascorbic acid (Mg/L)	Distribution of ascorbic acid per day when milk was held four days at 5° C. (%)															
	Winter				Spring				Summer				Fall			
	1st day	2nd day	3rd day	4th day	1st day	2nd day	3rd day	4th day	1st day	2nd day	3rd day	4th day	1st day	2nd day	3rd day	4th day
0 - 1		2.6	14.2	63.6			9.4	15.0				2.9				29.1
1 - 2		5.1	14.3	22.7		6.8	9.4	36.4			3.2	5.7			5.3	11.8
2 - 3		10.3	25.0	4.5			6.3	12.1		2.6	6.6	31.4		3.2	15.8	11.8
3 - 4	2.3	7.7	17.9	4.5	2.1	4.5	18.8	30.3			3.2	22.9			10.5	
4 - 5		10.3	7.2	4.5		2.3	9.4				19.4	25.7		9.4	10.5	11.8
5 - 6	4.5	10.3	7.2			4.5	18.8			2.6	16.1	8.6		3.2	5.3	5.9
6 - 7	9.1	15.4	10.7			9.1	15.6	3.0	2.3	2.6	12.9	2.9	2.6	9.4		
7 - 8	9.1	17.9	3.6		8.3	25.0	9.4	3.0		7.7	19.4		5.3	3.2	21.1	11.8
8 - 9	2.3	2.6			12.5	22.7				15.4	16.1			9.4		11.8
9 - 10	18.2	2.6			4.2	11.4	3.1			28.2			15.8	16.1	5.3	
10 - 11	15.9				10.4	6.8				20.5	3.2		2.6	3.2	21.1	
11 - 12	13.6				14.6	4.5			6.8	12.8			5.3	9.4	5.3	
12 - 13	20.5				25.0	2.3			9.1	5.1			7.9	19.4		
13 - 14	4.5				12.5				20.5	2.6			18.4	12.9		
14 - 15					6.3				43.2				10.5			
15 - 16					4.2				13.6				18.4			
16 - 17									4.5				7.9			
17 - 18									2.3				5.3			

Table 40. Percentage destribution of ascorbic acid in Grade A milk throughout the year by seasons.

Ascorbic acid (Mg/L)	Distribution of ascorbic acid per day when milk was held four days at 5° C. (%).															
	Winter				Spring				Summer				Fall			
	1st day	2nd day	3rd day	4th day	1st day	2nd day	3rd day	4th day	1st day	2nd day	3rd day	4th day	1st day	2nd day	3rd day	4th day
0 - 1		7.1	60.1	75.1		8.6	18.5	36.7			3.3	12.9	2.9	3.2	22.3	54.6
1 - 2	3.2	21.4	16.7	18.8	3.2	14.3	22.2	33.3			13.3	19.4	5.9	19.4	33.3	27.3
2 - 3		7.2	5.6			8.6	3.7	10.0	4.4	15.8	16.6	25.8	11.7	22.6	22.2	9.1
3 - 4	3.2	14.3	35.6	6.3	4.7		11.1	6.7	8.9	6.3	13.3	6.6		16.1	5.6	9.1
4 - 5	12.9	7.2	5.6		7.0	14.3	14.8	10.0	8.9	13.2	6.7	9.7	5.9	12.9	5.6	
5 - 6	3.2	10.7	5.6		2.3	8.6	14.8		2.2	15.8	10.0	12.9	5.9	3.2		
6 - 7	9.7	17.9			2.3	2.9	7.4		11.1	15.8	20.0	3.2	5.9	3.2		
7 - 8	12.9				4.7	8.6	3.7	3.3	6.8	2.6	3.3	9.7	8.8	6.6	5.6	
8 - 9	9.7	7.2			7.0	5.7	3.7		4.4	13.2	6.7		20.6		5.6	
9 - 10	12.9	3.6			9.3	8.6			11.1	5.3	3.3		2.9	3.2		
10 - 11	9.7	3.6			9.3	8.6			6.8	2.6	3.3		2.9	3.2		
11 - 12	3.2				14.0	8.6			11.1	7.9			8.8	3.2		
12 - 13	6.6				2.3	5.7			2.2	2.6			5.9	3.2		
13 - 14	9.7				11.6				2.2				5.9			
14 - 15	3.2				11.6				13.3				2.9			
15 - 16					7.0				4.4							
16 - 17					2.3				2.2				2.9			
17 - 18					2.3											

Table 41. Average seasonal values of ascorbic acid (Mg/L) in the three types of milk used in this experiment (1594 determinations).

Seasons	First day	Second day	Third day	Fourth day
		Irradiated milk		
Winter	7.2	2.5	0.4	0.0
Spring	11.1	6.1	2.0	0.7
Summer	14.2	9.1	4.7	2.2
Fall	10.9	6.1	2.8	1.2
Average	10.9	6.0	2.5	1.0
		Grade A milk		
Winter	6.0	4.8	1.3	0.6
Spring	10.5	6.0	3.4	1.8
Summer	8.8	6.4	4.7	3.3
Fall	7.7	4.2	2.5	1.2
Average	8.3	5.3	3.0	1.7
		Pasteurized milk		
Winter	9.9	5.8	3.1	0.8
Spring	12.9	7.6	4.0	2.2
Summer	13.0	9.5	5.8	3.3
Fall	13.0	9.4	6.8	3.4
Average	12.2	8.1	4.9	2.4

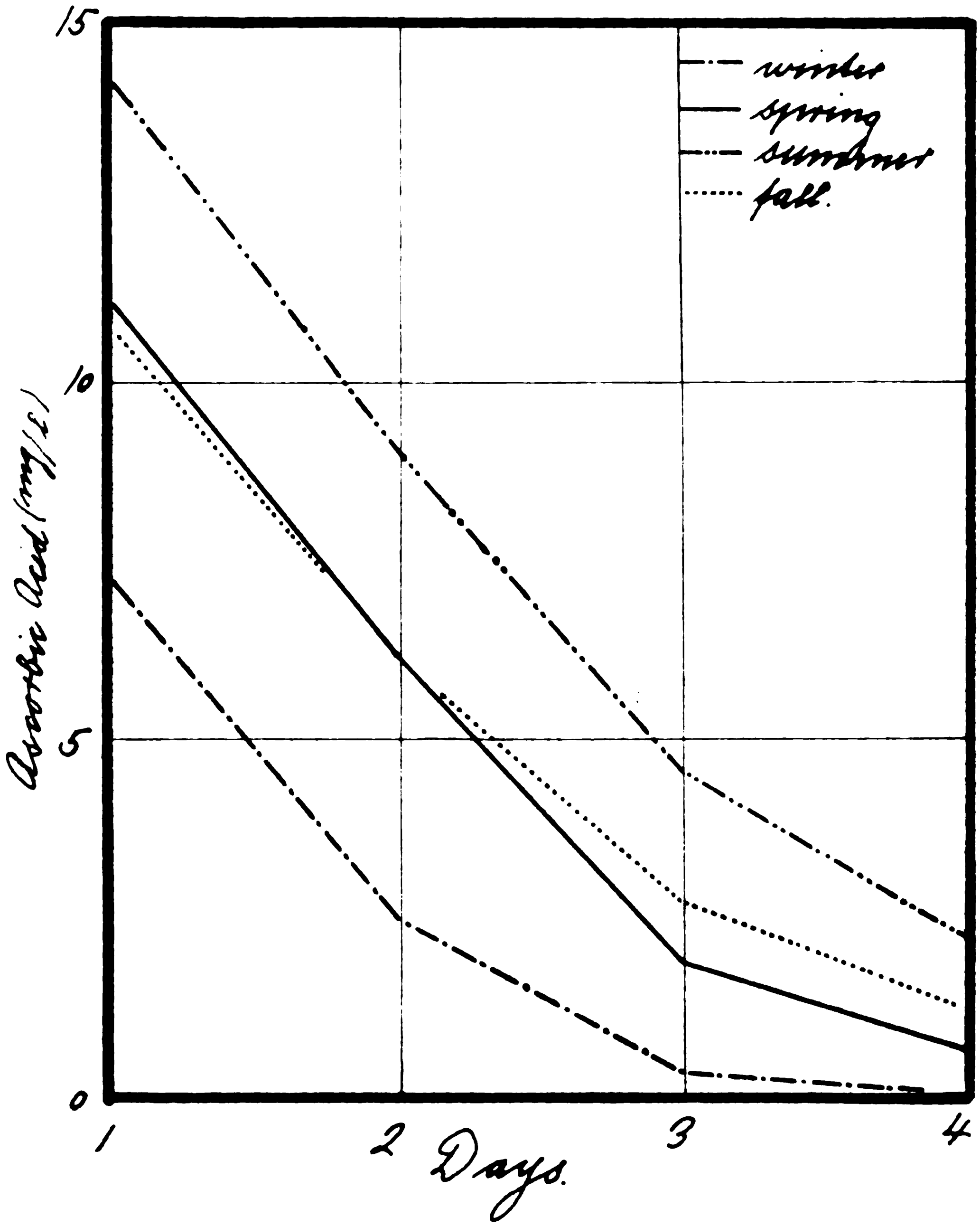

Figure 15. Showing the seasonal values of ascorbic acid in irradiated milk after different periods of storage.

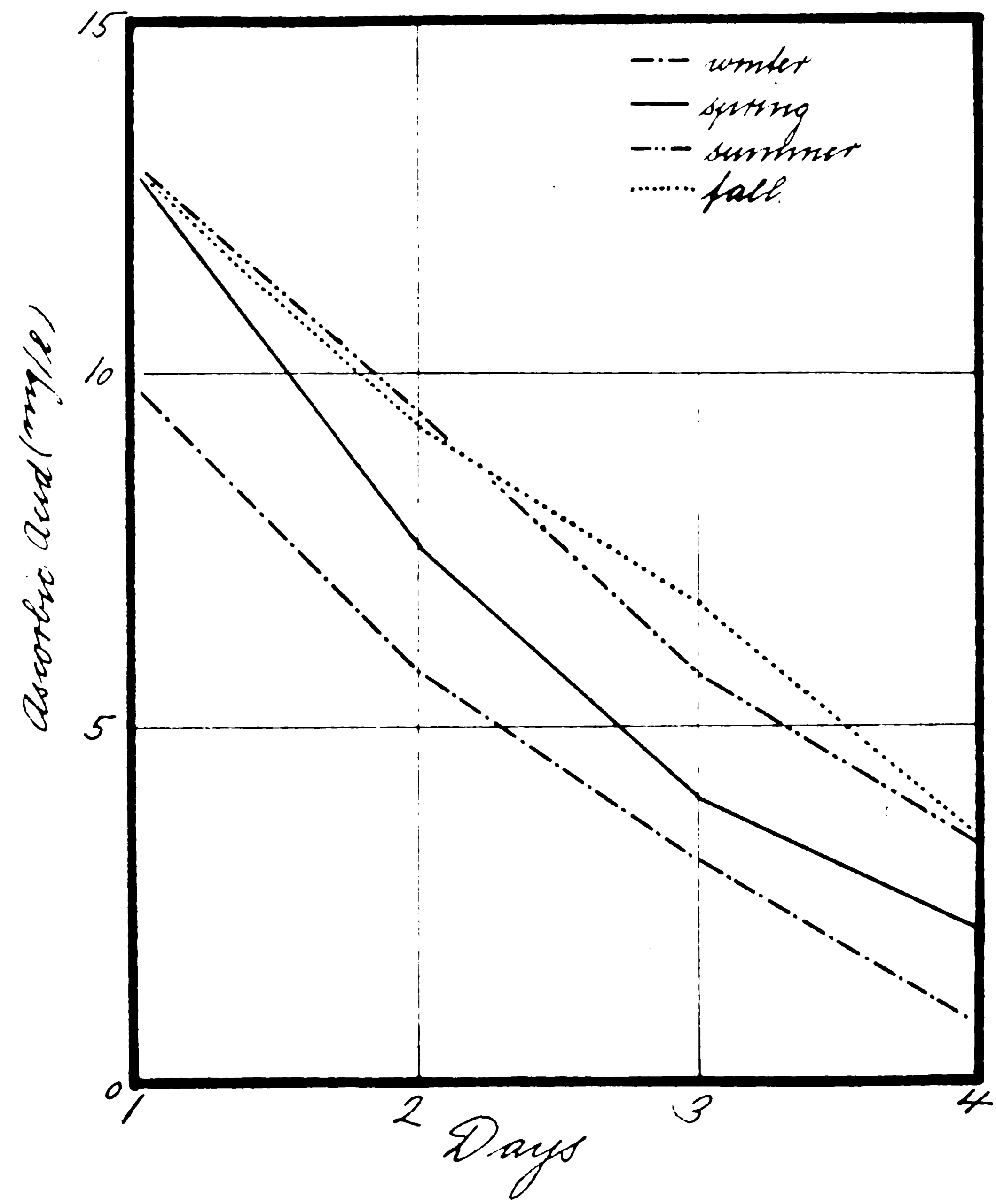

Figure 16. Showing the seasonal values of ascorbic acid in pasteurized milk after different periods of storage.

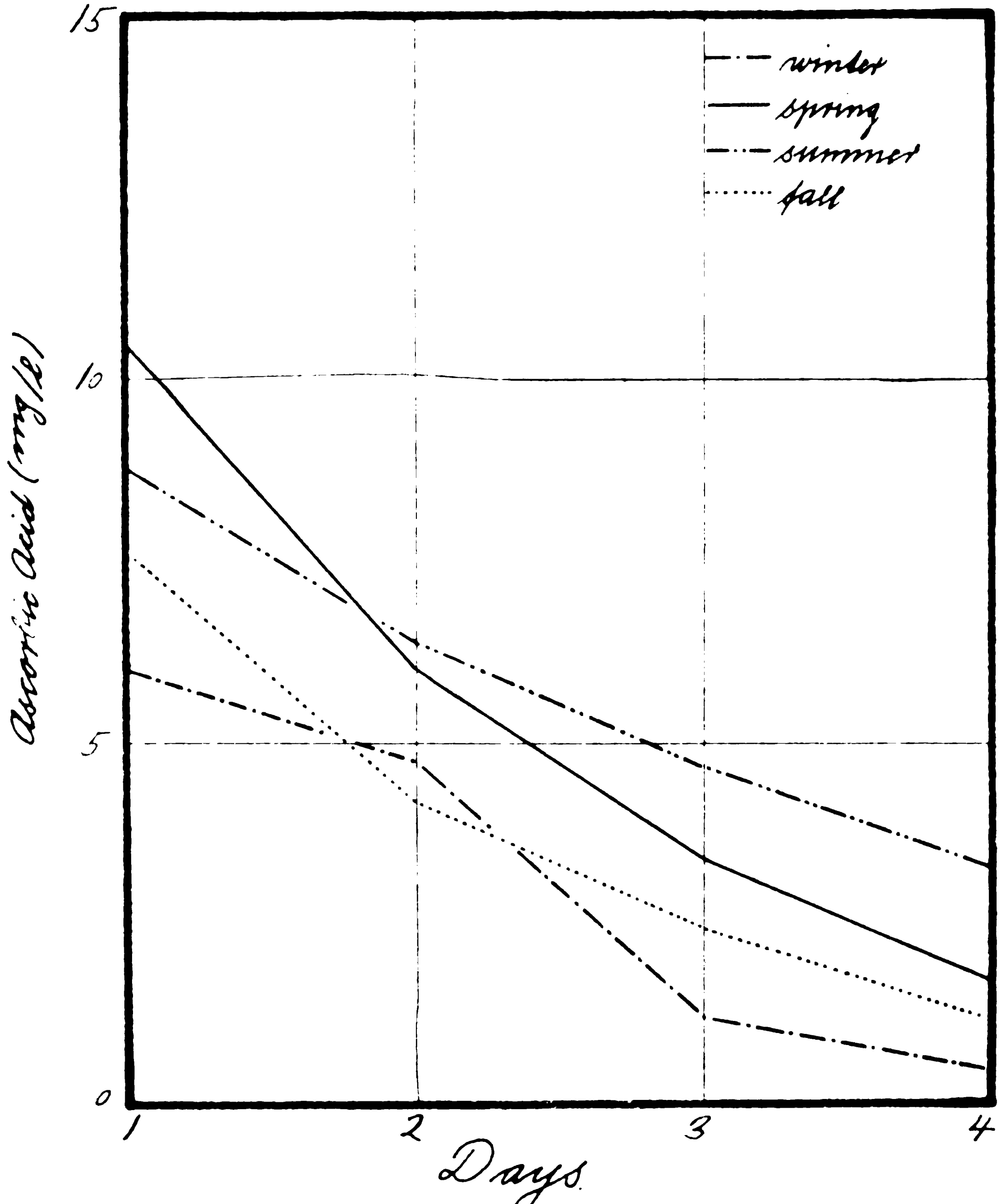

Figure 17. Showing the seasonal values of ascorbic acid in Grade A raw milk after different periods of storage.

Influence of the leucocyte count of the milk upon the ascorbic acid content.

Different experiments were carried out during the spring of 1937 to study the effects of leucocytes on the ascorbic acid content of milk.

Mixed milk, both from mastitis and from non-mastitis infected animals, from the different breeds of cattle were secured. The milk studied was obtained from 91 cows of which 28 were Guernsey; 17, Jersey; 8, Ayrshire; 18, Holstein; and 20, Brown Swiss.

The data secured are tabulated in Tables 42 and 43, rearranged in another grouping in Table 44, treated statistically in Table 45, and presented graphically in Figure 18. Data secured on the milk having a leucocyte count below 500,000 per ml. are included in Table 43 while those from milk having a count of above 1,000,000 per ml. are included in Table 42. From these tables, as well as Figure 18, it can be seen that the higher the leucocyte count the lower the ascorbic acid value. The rate of oxidation of ascorbic acid in the two groups of milk upon storage appeared to be parallel, as noted in Figure 18.

The standard errors between the means of ascorbic acid values for the first day was found to be extremely high, indicating great significance, as shown in Table 41. As the milk was held in storage, the difference in the means showed a lower standard of error, but the value for the fourth day was yet significant.

In Table 44 the data are grouped differently from those in Tables 42 and 43. In this case, five different classes were established as follows: leucocyte count below 0.5 million per ml. (151 determinations);

between 0.5 and 2 million (88 determinations); from 2 to 4 million (36 determinations); from 4 to 6 million (17 determinations) and over 6 millions (30 determinations).

The results indicate that a relationship exists between the leucocyte count and the ascorbic acid values of the milk. The higher the leucocyte count the lower the ascorbic acid value and vice versa. However, the differences in the ascorbic acid values for milk of different groups, as indicated by the leucocyte count, diminished from the first to the fourth day. As pointed out in Table 45, the standard error decreased from the first to the fourth day, indicating the decrease in the significance of the difference. The graph illustrates this difference in another way. On the first day the difference in the ascorbic acid values between the two extreme groups, below 0.5 million and above 6 million leucocytes per ml. was large, being approximately 6 mg., while on the fourth day the difference was small, being approximately 2.5 mg.

Table 42. <u>The ascorbic acid content (Mg) of individual cows' milk having leucocyte count over one million per ml. after different periods of storage.</u>

Cow (No.)	First day	Second day	Third day	Fourth day	Fifth day
2	13.9		11.9	8.1	7.6
14	11.2		9.5	9.0	7.6
25	25.2		12.8	3.8	1.9
63	17.2		10.9	5.4	2.9
174	17.2		13.9	9.0	8.5
180	19.1		10.7	7.6	5.3
207	18.6		15.7	14.8	13.3
2	16.2	12.4	7.6	6.2	4.3
13	19.5	17.1	12.8	8.1	6.2
28	16.6	13.3	7.1	6.2	1.4
42	20.5	15.7	10.4	8.5	6.2
63	4.3	2.8	0.0	0.0	0.0
174	20.0	13.3	7.6	2.4	0.9
180	21.0	15.3	10.5	5.7	1.4
220	23.8	20.9	14.3		3.8
239	23.3	17.6	4.7	0.9	0.0
2	14.8	14.3		11.4	8.1
13	18.6	14.8		8.5	6.7
28	18.6	15.3		10.5	6.7
63	14.3	8.6		2.4	0.0
174	17.6	11.9		5.2	2.8
220	23.8	19.1		12.4	10.0
237	13.4	4.7		2.6	0.9
248	20.4	17.2		9.5	8.6
2	16.0	13.4	9.5	5.7	
13	19.4	14.4	8.1	4.3	
28	17.5	17.9	8.6	8.0	
41	15.0	10.0	7.2	4.3	
174	18.4	9.5	2.9	0.9	
180	19.4	13.4	5.3	1.9	
220	15.5	14.8	17.2	16.0	
13	16.4	12.5	6.8		2.9
142	18.8	16.0	8.8		2.9
174	16.4	12.5	10.8		1.9
197	22.2	17.5	11.8		5.9
239	21.7		6.8		1.4
Average	17.94	13.61	9.44	6.31	4.49

Table 43. The ascorbic acid content (Mg/L) of individual cows' milk having a leucocyte count below 0.5 million per ml. after different periods of storage.

Cow (No.)	First day	Second day	Third day	Fourth day	Fifth day
111	20.9		19.6	13.8	16.2
146	22.4		19.1	15.3	12.4
41	20.0	17.1	14.3	8.5	6.2
111	25.7	23.3	18.6	15.7	
115	20.0	17.1	11.4	6.6	5.2
190	21.9	16.6	15.2	8.5	6.2
248		18.1	14.3		7.6
300	24.8	11.4	16.2	15.2	14.3
48	20.9	20.9		14.8	11.4
42	21.5	7.6		6.6	1.9
111	25.3	14.3		10.9	6.7
115	20.1	17.6		15.7	12.4
190	20.1	16.2		10.9	6.7
239	23.8	16.2		3.8	1.4
300	25.3	20.4		12.8	10.4
146	20.9	18.6		12.4	11.4
30	25.2	20.6	17.2	15.6	
37	19.9	9.1	2.9	0.9	
42	19.4	13.6	7.6	2.4	
100	25.7	17.7	12.5	8.5	
111	27.6	20.1	12.5	7.1	
113	22.3	17.3	11.0	5.2	
117	25.2	16.8	9.5	3.3	
146	21.8	16.8	13.4	9.5	
190	22.3	17.7	13.8	9.5	
197	23.3	14.8	11.0	6.2	
237					
248	20.4	14.8	13.4	9.5	
300	24.2	18.7	14.4	9.5	
30	23.6	22.6	19.6	16.7	
37	18.3	10.5	3.4	2.9	
41	19.3	18.5	13.7	8.8	
42	21.7	18.1	6.8	2.5	
111	24.5	23.5	23.1	17.7	
116	25.5	24.1	10.8	14.7	
117	21.2	19.5	12.8	7.8	
134	20.2	17.0	10.8	4.9	
190	21.2	17.5	12.7	8.8	
237	19.7	13.0	2.5	1.9	
300	18.8	16.5	13.7	3.9	
Average	22.1	17.1	12.8	9.2	8.7

Table 44. The ascorbic acid content (Mg/L) of milk having various leucocyte counts after different periods of storage (322 determinations).

Storage (days)	Ascorbic acid value (Mg/L) when leucocyte count was in millions per ml.				
	Below 0.5	Between 0.5 - 2	Between 3 - 4	Between 4 - 6	Over 6
1	22.1	21.2	18.6	16.8	16.2
2	16.8	16.0	15.1	13.2	11.2
3	12.5	10.0	8.2	10.8	7.9
4	9.0	7.5	6.9	6.5	6.8

Table 45. Statistical treatment of data from Tables 38 and 39

Calculations	Ascorbic acid values in low and high leucocyte count milk after various periods of storage (Mg/L)	
	Below 0.5 million leucocytes per ml.	Over 1.0 million leucocytes per ml.
	First Day	
Calc. mean	22.26 ± 2.36	18.41 ± 3.15
Standard errors	5.21	
	Second Day	
Calc. mean	17.20 ± 3.70	13.64 ± 3.95
Standard errors	3.96	
	Third Day	
Calc. mean	12.79 ± 4.89	9.48 ± 3.69
Standard errors	3.10	
	Fourth Day	
Calc. mean	9.06 ± 4.5	6.27 ± 3.71
Standard errors	2.87	

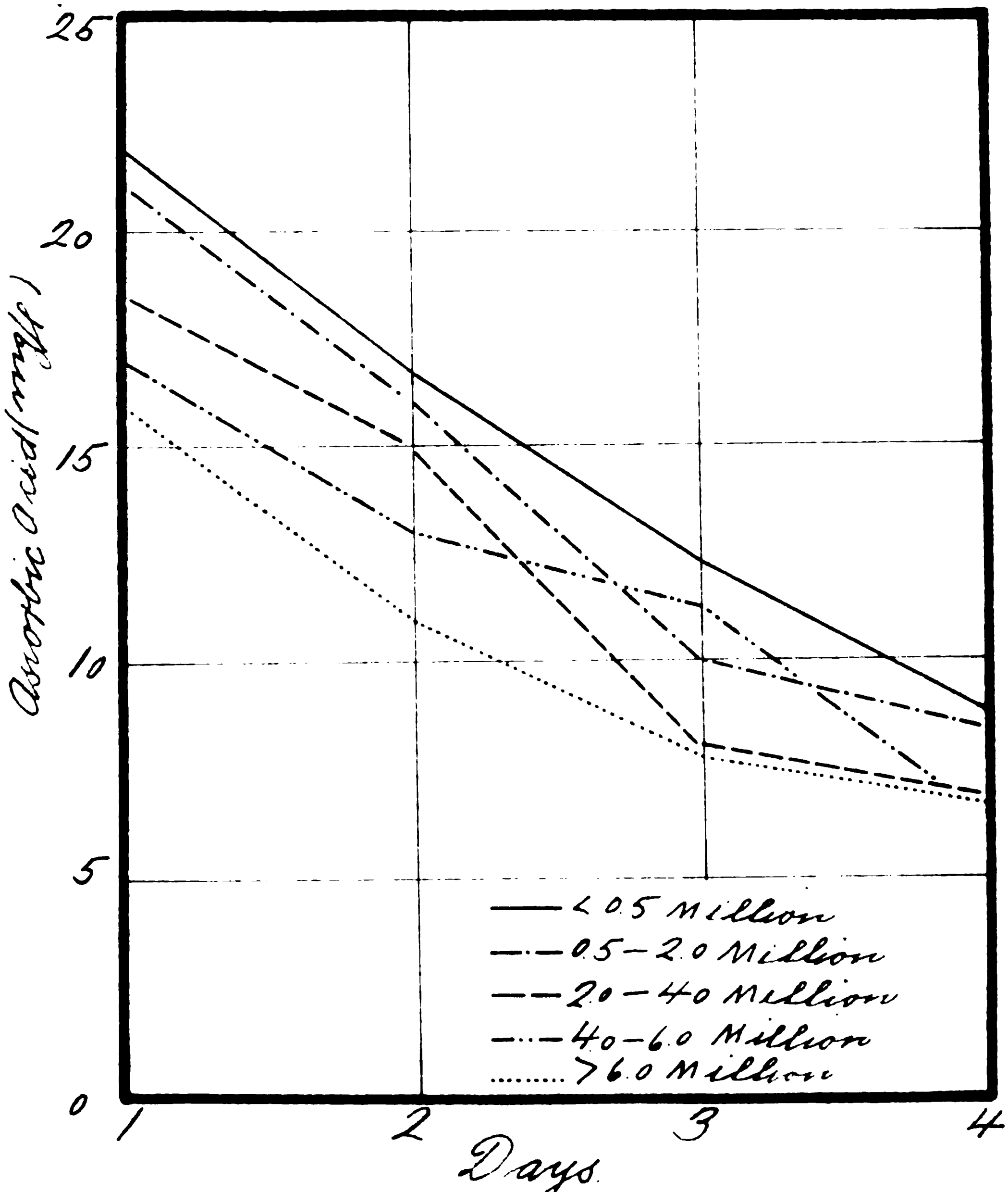

Figure 18. Showing the average values of ascorbic acid (Mg/L) in milk having various leucocyte counts after different periods of storage (322 determinations).

Correlation between the bacteria count, the leucocytes, the reduction time and the stability of ascorbic acid in milk.

Studies were made on individual samples of milk from 19 cows of the college herd and upon 38 lots of mixed milk delivered at the College Creamery. The methylene blue reduction periods, the bacterial counts, the leucocyte counts and the daily ascorbic acid values are presented in Tables 46, 47 and 48.

The data in Table 46 were obtained from studies on individual cows' milk from the College Herd. These milks were obtained in glass and cooled immediately. Consequently, the bacterial counts were extremely low. Likewise, the methylene blue reduction periods were high, averaging 6.5 hours for the 19 samples. The ascorbic acid values ranged from an average of 20.3 mg.per liter on the first day to 9.8 mg. per liter on the fourth day, indicating an inverse relationship between the reduction time and the oxidation of the ascorbic acid.

The data in Tables 47 and 48 pertain to studies on mixed milk delivered at the College Creamery. Here the relationship between the amount of ascorbic acid present, its drop, the bacterial counts, and the leucocyte counts of mixed milk of average quality will be noted.

The large number of bacteria seemed tohave a protective effect upon the stability of ascorbic acid while a high leucocyte count had a harmful effect. High bacteria and leucocyte counts in the same sample seemed to have a counteracting effect upon the ascorbic acid, therefore, resulting in its greater stability. In several cases in which the bacteria count was high, the milk not only had a high initial ascorbic acid content, but in some cases the ascorbic acid value, instead of decreasing from day to day, remained constant, or actually increased. Conversely, if the milk had a high leucocyte count, the ascorbic acid value was likely to decrease to a greater extent than in those samples showing a low leucocyte count.

The data of Tables 47 and 48 are reclassified according to the decrease in ascorbic acid from first to third day and are presented in Tables 49 and 50. Two groups of milk, those which showed a decrease in ascorbic acid between 0 and 5 mg.per liter and those which exhibited a decrease between 6 and 13 mg. per liter, are listed.

The average results from these Tables show clearly that the group of samples exhibiting the largest decrease, 6 to 13 mg. per liter of ascorbic acid, had the longest reduction time and the lowest bacterial count, while those samples showing the smallest decrease, 0 to 5 mg. per liter, had the shortest reduction time and the highest bacterial count. The leucocyte count was about the same in each class, therefore, their effects upon the ascorbic acid might be considered to be similar.

The above data seem to indicate that the main factor affecting the stability of the ascorbic acid of the milk in this experiment was chiefly the bacteria. The data reported in Table 46 obtained from studies of milk in which the bacterial count was very low, showed that apparently no relationship existed between the long methylene blue reduction periods and the stability of the ascorbic acid, while data of the two other experiments, summarized in Tables 47 and 48, showed that a very high bacterial count, therefore, a short reduction period, resulted in the smallest decrease of ascorbic acid. The bacterial populations of milk were thus able to influence the ascorbic acid stability in milk to a marked degree.

Table 46. The ascorbic acid content of individual cows' having comparatively high methylene blue reduction periods.

Cow (No.)	Ascorbic acid (Mg/L) First day	Second day	Third day	Fourth day	Reduction time hours
7	25.5	18.0	14.0	9.9	6.00
13	17.0	13.0	9.5	5.9	4.25
25	29.5	26.0	18.0	12.9	8.45
30	27.0	23.5	22.5	20.9	7.45
37	19.5	16.0	16.0	11.9	7.15
41	19.0	16.5	15.0	13.4	7.45
42	18.5	16.0	14.0	9.9	7.15
101	19.5	15.0	11.5	5.4	8.45
108	14.5	3.5	2.0	2.4	6.00
111	23.5	19.0	20.5	15.4	6.30
116	23.0	21.0	20.0	15.4	7.45
117	23.5	21.0	18.0	15.9	7.30
134	18.5	17.5	15.0	10.9	6.45
150	17.5	16.5	12.0	10.4	7.45
190	21.0	20.0	14.0	7.9	4.25
232	17.0	11.0	4.0	1.9	5.20
237	20.0	17.5	13.0	8.4	6.15
239	21.0	16.5	9.0	5.4	6.30
300	10.0	7.0	4.0	1.9	5.0
Average	20.3			9.8	6.53

Table 47. <u>The ascorbic acid content of mixed milk from individual patrons compared with the methyleneblue test, with the bacteria count and with the leucocyte count.</u>

Patron (No.)	Ascorbic acid (Mg/L) First day	Second day	Third day	Reduction time hours	Bacteria count (Mill. per ml.	Leucocyte count (Mill per ml.
2	16.9	15.5	13.8	1.35	0.88	1.20
4	13.9	9.5	7.9	2.55	0.02	4.17
7	18.5	13.5	13.8	5.50	0.14	0.57
11	20.9	13.5	16.3	2.20	1.67	1.48
12	16.9	9.5	9.8	6.45	0.16	0.49
13	11.9	10.9	10.1	5.05	0.11	0.79
14	21.4	18.9	18.6	1.10	2.66	0.38
16	18.9	9.9	11.8	6.45	0.03	1.20
20	13.9	4.9	6.9	8.45	0.07	1.39
21	17.9	15.9	14.8	2.20	3.48	0.71
23	16.9	7.9	11.8	1.55	0.52	0.77
24	18.9	6.9	8.4	4.15	0.44	1.32
25	18.9	17.5	17.8	1.50	1.37	1.34
26	15.9	9.9	10.1	2.20	0.68	1.17
36	16.9	15.5	15.8	1.25	2.00	0.46
51	23.9	12.9	10.8	5.05	0.56	0.49
61	17.9	3.9	6.9	5.05	0.08	2.88

Table 48. The ascorbic acid content of mixed milk from individual patrons compared with the methylene blue test, with the bacteria count and with the leucocyte count.

Patron (No.)	Ascorbic acid (Mg/L)				Reduction time hours	Bacteria count (Mill. per ml)	Leucocyte count (Mill. per ml.)
	First day	Second day	Third day	Fourth day			
2	15.8	15.8	14.8	16.7	1.30	6.00	1.09
4	9.9	4.9	2.5	2.5	3.00	0.52	2.90
5	16.8	14.8	16.3	15.2	1.45	1.43	0.66
6	19.8	14.8	16.3	15.2	3.20	0.38	0.71
7	18.3	12.8	9.4	8.8	7.30	0.02	0.66
11	16.8	11.8	10.9	10.4	7.05	0.27	2.11
12	18.3	11.4	12.8	11.3	8.15	0.04	0.57
13	18.8	14.8	14.8	15.7	6.10	0.85	0.66
14	20.7	18.8	20.7	20.6	1.45	2.63	0.19
16	18.3	9.9	8.0	6.9	7.05	0.02	1.54
20	15.3	9.4	12.8	11.3	6.30	0.27	1.60
21	18.3	16.8	17.8	18.2	2.40	3.50	1.07
22	17.8	16.8	18.8		2.20	2.50	1.65
23	16.8	11.8	9.9		6.00	0.03	0.85
24	17.3	11.8	13.8		5.30	0.33	0.98
25	18.8	16.8	18.8		1.05	11.79	1.15
26	17.3	9.9	13.8		2.40	0.44	1.07
36	14.8	5.9	4.9		4.50	0.13	0.71
51	20.3	12.8	13.8		4.45	0.08	0.38
59	13.8	8.9	14.8		2.40	5.50	0.95
61	15.8	3.9	5.9		6.15	0.04	2.74

Table 49. The reduction time, the bacteria count and the leucocyte count of milk showing a decrease in ascorbic acid of less than 5.0 mg. per liter after three days' storage.

Decrease in ascorbic acid (Mg/L) after three days	Reduction time Hours	Bacteria count (Mill per ml.)	Leucocyte count (Mill per ml.)
3.1	1.35	0.08	1.2
1.8	5.05	0.10	0.8
2.8	1.10	2.70	0.4
3.1	2.20	3.50	0.7
1.1	1.50	1.40	1.3
1.1	1.27	2.00	0.5
1.0	1.30	6.00	1.1
0.5	1.45	1.40	0.7
4.0	6.10	0.90	0.7
0.0	1.45	2.60	0.2
2.5	6.30	0.30	1.6
0.5	2.40	3.50	1.1
- 1.0	2.20	2.50	1.7
4.0	5.35	0.30	1.0
0.0	1.05	11.80	1.2
3.5	1.40	0.40	1.7
- 1.0	2.40	5.50	1.0
4.7	5.50	0.10	0.6
4.6	2.20	1.70	1.5
Ave. 1.91	2.71	2.46	1.0

Table 50. The reduction time, the bacteria count, and the leucocyte count of milk showing a decrease in ascorbic acid of more than 5.0 mg. per liter after three days' storage.

Ascorbic acid (Mg/L) after three days	Reduction time hours	Bacteria count (Mill. per ml.)	Leucocyte count (Mill. per ml.)
6.0	2.55	0.02	4.20
5.1	1.55	0.50	0.80
5.8	2.20	0.70	1.20
5.9	7.05	0.30	2.10
5.5	8.15	0.04	0.60
7.1	6.45	0.20	0.50
7.1	6.45	0.03	1.20
7.0	8.45	0.07	1.40
10.5	4.15	0.40	1.30
13.1	5.05	0.60	0.50
11.0	5.05	0.08	2.90
7.4	3.00	0.50	2.90
8.9	7.30	0.02	0.70
10.3	7.05	0.02	1.50
6.9	6.00	0.03	0.90
9.9	4.50	0.13	0.70
6.5	4.45	0.08	0.40
9.9	6.15	0.04	2.70
7.57	5.30	0.21	1.5

<u>A study of the relationship between the per cent lactic acid, the flavor score and the ascorbic acid of milk.</u>

Individual samples of milk from approximately 30 cows of the College Herd were obtained and cooled immediately over a surface cooler. Samples for study were secured before and after cooling. The titratable acidity, expressed as per cent lactic acid, the ascorbic acid content and the flavor score were determined. The data are assembled in Tables 51 and 52.

The data indicated that a high flavor score was associated with a large amount of ascorbic acid; likewise, that a high per cent of lactic acid in milk was associated with a low ascorbic acid content.

Table 51. The relationship between the per cent of lactic acid and the ascorbic acid content of raw milk.

Lactic acid (%)	Number of samples	Ascorbic acid (Mg/L)			
		Before going over cooler		After going over cooler	
		First day	Second day	First day	Second day
Above 0.180	48	15.3	8.6	14.1	7.2
Below 0.180	67	17.6	10.0	15.9	7.6

Table 52. The relationship between the flavor score and ascorbic acid content of raw milk.

Flavor score	Number of samples	Ascorbic acid (Mg/L)			
		Before going over cooler		After going over cooler	
		First day	Second day	First day	Second day
Above 22.5	60	17.4	10.0	15.8	7.8
Below 22.5	65	15.9	9.0	14.9	7.4

Influence of sodium citrate and citric acid upon the ascorbic acid content of milk and upon the development of the oxidized flavor.

This experiment was conducted to determine what relationships, if any, existed between the citric acid or its salts and the ascorbic acid content of milk. The study was made also to note what effect the presence of citric acid or its citrates had upon the oxidized flavor of milk. However, in the latter study it was not possible to trace any relationship inasmuch as the oxidized flavor did not develop in the original milk, or to a very small extent, even by additions of large amounts of copper.

The data secured are presented in Tables 53 to 57 inclusive. The first three tables represent data secured on the influence of the addition of sodium citrate. The milk was obtained from the College Herd and was treated the same day. The data in Table 53 show the effect of the addition of sodium citrate upon the stability of ascorbic acid in milk, with and without the addition of copper. The milk was not heat treated in this experiment. The data indicated that the addition of sodium citrate did not have any appreciable effect upon the stability of ascorbic acid when copper was present. On the contrary, the data seemed to indicate, when sodium citrate was added to the milk without the addition of copper, that the sodium citrate contributed slightly to the destruction or loss of ascorbic acid. When copper was present also the same trend was noted. In the presence of copper the destruction of ascorbic acid was rapid and on the second day had disappeared.

The data in Tables 54 and 55 indicated that the same general re-

results were obtained on holder pasteurized milk as when obtained on raw milk. Here it will be noted that the addition of sodium citrate in increasing amounts, both with and without the presence of copper, increased the loss of ascorbic acid.

The data in Tables 56 and 57 were secured on similar milk, raw and pasteurized, to which citric acid rather than sodium citrate was added. Under these conditions a slightly different result than that noted above was obtained. There was a slight indication that citric acid might retard the destruction or loss of ascorbic acid. This can be explained as due partly to the change in the pH value or as due in part to the possibility that the citric acid actually did function as a hydrogen donator.

The data in both Tables 56 and 57 show that the addition of citric acid to milk, with or without copper present, was apt to give a slight increase in the ascorbic acid content. As the addition of citric acid increased, however, this increase was very small and undoubtedly without any significant value.

The conclusions from this experiment must be negative. The amounts of citric acid and sodium citrate added were far beyond the amounts present in milk even under abnormal conditions. Even with the addition of these excessive amounts, the stability of ascorbic acid was not maintained.

Table 53. <u>The ascorbic acid content of raw milk to which sodium citrate and copper were added.</u>

Additions	Ascorbic acid (Mg/L) First day	Second day	Third day
0.0: % Na citrate	19.5	14.0	12.0
0.025 " "	19.0	13.0	10.5
0.050 % Na. citrate	18.0	13.5	11.5
0.10 " " "	17.5	14.5	12.5
0.20 " " "	13.5	16.0	10.0
0.0 % Na citrate + 39 mg % Cu.	4.5	0.0	0.0
0.025 " " " " " "	2.5	0.0	0.0
0.050 " " " " "	3.0	0.0	0.0
0.10 " " " " "	2.0	0.0	0.0
0.20 " " " " "	1.5	0.0	0.0
0.0 % Na citrate + 52 mg % Cu.	3.5	0.0	0.0
0.025 " " " " " "	3.5	0.0	0.0
0.050 " " " " " "	3.0	0.0	0.0
0.10 " " " " " "	2.0	0.0	0.0
0.20 " " " " " "	2.0	0.0	0.0
0.0 % Na citrate + 65 mg % Cu.	3.0	0.0	0.0
0.025 " " " " " "	2.0	0.0	0.0
0.050 " " " " " "	2.5	0.0	0.0
0.10 " " " " " "	2.0	0.0	0.0
0.20 " " " " " "	2.0	0.0	0.0

Table 54. The ascorbic acid content of raw and of pasteurized milk to which sodium citrate and copper were added.

Heat treatment	Addition	Ascorbic acid (Mg/L) First day	Second day	Third day
None	0.0 % Na citrate	22.8	19.3	18.1
	0.1 " " "	22.5	19.8	16.7
	0.2 " " "	22.5	18.1	17.2
	0.3 " " "	19.8	16.3	14.2
	0.4 " " "	19.8	16.0	13.6
	0.0 " " " + 39 mg % Cu.	12.3	3.1	0.0
	0.1 " " " + " " " "	10.9	2.6	0.0
	0.2 " " " + " " " "	7.9	2.6	0.0
	0.3 " " " + " " " "	7.9	1.8	0.0
	0.4 " " " + " " " "	7.0	1.8	0.0
63° C. for 30 min.	0.0 % Na citrate	20.7	18.4	16.3
	0.1 " " "	19.3	16.3	lost
	0.2 " " "	19.3	16.7	14.1
	0.3 " " "	17.2	14.7	10.9
	0.4 " " "	16.7	14.1	10.0
	0.0 " " " + 39 mg % Cu.	6.2	1.8	0.0
	0.1 " " " + " " " "	4.8	1.8	0.0
	0.2 " " " + " " " "	4.8	1.3	0.0
	0.3 " " " + " " " "	3.5	0.8	0.0
	0.4 " " " + " " " "	3.1	1.3	0.0

Table 55. The ascorbic acid content of raw and of pasteurized milk to which sodium citrate and copper were added.

Heat treatment	Addition		Ascorbic acid (Mg/L) First day	Second day	Third day
None	0.0 % Na citrate		18.9	14.1	10.9
	0.1 " " "		18.9	14.1	10.5
	0.2 " " "		18.4	11.8	7.5
	0.3 " " "		16.3	11.4	6.1
	0.4 " " "		15.4	10.5	4.4
	0.0 " " "	+ 13 mg. % Cu.	12.7	3.5	0.0
	0.1 " " "	+ " " " "	10.5	3.9	0.0
	0.2 " " "	+ " " " "	9.2	1.7	0.0
	0.3 " " "	+ " " " "	8.4	1.8	0.0
	0.4 " " "	+ " " " "	7.9	1.8	0.0
63° C. for 30 minutes	0.0 % Na citrate		15.4	11.8	9.2
	0.1 " " "		11.8	10.5	7.4
	0.2 " " "		11.8	10.5	7.4
	0.3 " " "		13.6	8.8	6.1
	0.4 " " "		13.2	7.0	4.4
	0.0 " " "	+ 13 mg % Cu.	7.9	2.6	0.0
	0.2 " " "	" " " " "	5.3	2.2	0.0
	0.3 " " "	" " " " "	5.3	1.8	0.0
	0.4 " " "	" " " " "	1.8	0.0	0.0

Table 56. The ascorbic acid content of pasteurized milk to which copper and citric acid were added before pasteurization.

Additions	Ascorbic acid (Mg/L) First day	Second day	Third day
0.0 % citric acid	14.0	11.0	9.0
0.025 " " "	14.5	11.0	9.0
0.050 " " "	15.0	12.5	10.0
0.1 " " "	15.0	12.5	10.0
0.0 " " " + 13 mg. % Cu.	5.5	3.0	1.5
0.025 " " " + " " " "	9.5	5.0	3.0
0.050 " " " + " " " "	11.0	5.5	3.0
0.1 " " " + " " " "	10.0	5.0	3.0
0.0 " " " + 26 mg. % Cu.	lost		
0.025 " " " + " " " "	5.0	2.0	0.0
0.050 " " " + " " " "	7.5	1.5	0.0
0.1 " " " + " " " "	6.0	2.0	0.0

Table 57. <u>The ascorbic acid content of raw milk to which citric acid and copper were added.</u>

Additions	Ascorbic acid (Mg/L) First day	Second day	Third day
0.0 % citric acid	18.0	14.5	12.5
0.025 " " "	18.0	14.0	13.5
0.050 " " "	19.5	15.5	12.5
0.10 " " "	19.0	16.0	13.5
0.20 " " "	19.5	15.0	15.5
0.0 " " "	18.5	15.5	13.0
0.025 " " " + 39 mg. % Cu.	7.0	2.0	0.0
0.050 " " " + " " " "	8.5	2.0	0.0
0.10 " " " + " " " "	10.0	1.0	0.0
0.20 " " " + " " " "	9.5	2.0	0.0
0.0 " " " + 52 mg. % Cu.	4.0	2.0	0.0
0.025 " " " + " " " "	4.0	3.0	0.0
0.050 " " " + " " " "	5.0	2.0	0.0
0.10 " " " + " " " "	6.0	3.0	0.0
0.20 " " " + " " " "	7.0	3.0	0.0
0.0 " " " + 65 mg. % Cu.	2.5	0.0	0.0
0.025 " " " + " " " "	2.5	0.0	0.0
0.05 " " " + " " " "	2.5	0.0	0.0
0.10 " " " + " " " "	4.5	0.0	0.0
0.20 " " " + " " " "	7.0	1.5	0.0

Effect of the addition of copper and glutathione upon the ascorbic acid content of milk and upon the development of the oxidized flavor.

Recently much work has been done in respect to the presence of reduced sulphur containing compounds in living tissues and their possibility in biological oxidations. Of chief importance, because widely distributed throughout different tissues, is glutathione. Glutathione is a tripeptide, glutamyl-cysteinyl-glycine, having in its reduced form the following formula:

$$\underset{\displaystyle NH_2}{COOH.\overset{}{CH}}.CH_2CH_2CO\text{-}NH.\underset{\displaystyle CH_2SH}{CH}.CO\text{-}NH\ CH_2\ COOH$$

Glutathione has been attributed great importance as to its protective action against oxidation of ascorbic acid, not only in respect to its reducing capacity because of sulphur present in the molecules, but also due to its ability, as most disulphide systems, to form complexes with metal ions.

From the above discussion, the conclusion would seem logical that if glutathione were present in milk, it might affect not only the ascorbic acid present in milk, but also, the oxidation of the fats. Accordingly, pure glutathione was added to milk in an effort to determine its effect upon these constituents. To check the value of the method used, glutathione was added to distilled water in known amounts and then redetermined by titration as outlined in theprocedure. Ten trials were run and, in each case, the amount of glutathione checked within five per cent of the actual amount added.

Glutathione was then added to milk in known amounts and then redetermined. As much as 49.8 mg. glutathione per liter were added to milk. However, before glutathione was added to milk, titrations were run in order to determine the blank value. Fairly high titration values were obtained on these blanks, despite the fact that the milk was four days old, was pasteurized, irradiated and ascorbic acid was not present.

Immediately after the glutathione was added, the milk was titrated, and remarkably low values were obtained over the blank, considering the large amount of glutathione added. Still more surprising was the fact that, as time progressed, this value became smaller and smaller, and after 2 hours the titration value was the same as the blank. The phenomena was observed in several trials.

In another experiment the glutathione was added to fresh raw milk in which an appreciable amount of ascorbic acid was present. Two trials were run the results of which are tabulated in Tables 58 and 59. Data in Table 58 gave indication that the glutathione had little, if any, protective action on the ascorbic acid, both with and without copper present. Although the ascorbic acid value dropped rapidly, the milk did not develop an oxidized flavor. However, this milk was raw and was not susceptible to oxidation. From the data presented in Table 59 the same conclusions as above can be drawn. In addition, the data showed that glutathione had no effect upon the development of oxidized flavor in raw milk when copper was present.

It is fully appreciated that the conclusions which may be drawn from the above two sets of data are far from being final but are given to indicate the relationship found between added glutathione and the stability of ascorbic acid in milk.

Table 58. <u>The effect of the addition of copper and glutathione upon the ascorbic acid content of milk and upon the development of the oxidized flavor.</u>

Treatment	Ascorbic acid (Mg/L) First day	Ascorbic acid (Mg/L) Second day	Ascorbic acid (Mg/L) Third day	Oxidized flavor third day
Control	17.2	12.4	7.6	-
13 mg. % Cu. added	14.3	1.4	0.0	-
26 " " " "	10.9	1.0	0.0	-
52 " " " "	6.7	0.5	0.0	-
+ G S H	15.8	9.1	4.3	-
+ " " "	15.3	9.1	4.3	-
13 mg. % Cu. added	12.4	1.0	0.0	-
26 " " " " + G S H	11.9	0.5	0.0	-
39 " " " " + " " "	10.5	1.0	0.0	-
52 " " " " + " " "	7.2	1.0	0.0	-
65 " " " " + " " "	6.7	1.5	0.0	-
78 " " " " + " " "	3.8	2.0	1.4	-

Table 59. The effect of the addition of copper and glutathione upon the ascorbic acid content of milk and upon the development of the oxidized flavor.

Treatment	Ascorbic acid (Mg/L) First day	Second day	Third day	Oxidized flavor third day
Control no addition	14.8	10.9	4.5	-
G S H	13.4	10.9	5.3	-
" " "	12.4	10.5	4.8	-
" " " + 13 mg. % Cu.	10.5	4.8	1.9	-
" " " + 26 " " "	10.5	1.4	0.0	-
" " " + 39 " " "	10.0	1.4	0.0	+
" " " + 52 " " "	9.1	1.9	0.0	+ + ?
" " " + 65 " " "	8.1	1.0	0.0	+ +
" " " + 78 " " "	6.7	1.0	0.0	+ + +
13 " " "	12.4	3.9	0.0	-
26 " " "	10.0	1.9	0.0	?
39 " " "	7.6	1.4	0.0	?
52 " " "	7.6	1.4	0.0	+
65 " " "	8.6	1.4	0.0	+ + +

DISCUSSION

Milk from individual cows studied in this experiment showed a seasonal variation in the ascorbic acid content of the milk regardless of the presence of streptococci infection or otherwise. However, milk from the mastitic cows showed a greater variation in this respect than did that from the normal cows. The mastitic milk had on the average a lower ascorbic acid value. The ascorbic acid in milk seemed to reach a minimum in the months of December, January, February and March.

The ascorbic acid content of the raw milk decreased uniformaly upon storage and was, on the average, constant for each of the four days studied.

Grade A raw milk and irradiated milk from the College Creamery showed the same seasonal variation in ascorbic acid as did that studied from the individual cows. However, the pasteurized milk of the College Creamery failed to show such a pronounced variation. These variations were not shown, as in the case of the raw milk, by the different months, but by the four seasons. However, it must be remembered that this pasteurized milk was farmer-produced mixed milk, produced under different conditions as to sanitation and equipment and invariably was older milk at the time of processing. Of the Grade A raw milk, pasteurized milk, and irradiated milk, the irradiated milk showed the widest variations for the four seasons. The irradiated milk also had the highest ascorbic acid content during the summer months, but showed a considerably small amount during the spring and fall months. This milk was high quality milk produced at the College Dairy Barn and was irradiated in a CP unit at the

College Creamery. The irradiated milk from the fifteenth of October to the first of May consistently yielded upon storage the oxidized flavor, but such a flavor was not noted so extensively in the Grade A raw milk or in the regular pasteurized milk.

A high leucocyte count in milk was apparently correlated with the lower ascorbic acid content of the milk. If this were due to the leucocytes themselves or to certain abnormal conditions in the udder, resulting in abnormal milk, is still uncertain. The leucocytes do, however, contain enzymes, such as catalyse and peroxidase, which might have oxidized the ascorbic acid.

An abundance of bacteria seemed to have a preserving effect upon the ascorbic acid or to increase the stability of the ascorbic acid present in milk. Kende (1922) indicated that a large number of bacteria in milk prevented the development of oxidized flavor which was considered to be due to an oxidation of the fat. The above mentioned result of the bacteria might, therefore, be expected. However, this does not mean that the development of the oxidized flavor and the decrease in the ascorbic acid are due to the same cause, enzymes produced by bacteria, for instance. High leucocyte counts and high bacteria counts together seemed to have a counteracting effect upon each other, the ascorbic acid remaining fairly constant.

As shown by the methylene blue test, there was an indirect correlation between the time of reduction and the stability of the ascorbic acid. However, this was undoubtedly due to the enzymic action of the bacteria present since the reduction time is only an indication of the numbers of bacteria present.

A slight correlation was noted between the ascorbic acid content or its stability and the per cent acid, calculated as lactic acid, originally present. Likewise, a slight relationship was noted between the flavor score of the fresh raw milk and the original ascorbic acid content. On the other hand, neither the citrates nor the citric acid had little effect, if any, upon the stability of the ascorbic acid in milkor upon the development of the oxidized flavor. It is a well known fact that sodium citrate as well as formaldehyde can act as hydrogen donator in biological oxidations. Formaldehyde is not present in milk but citrates are to a varying extent. From the above conclusions it could rightly be assumed that the citric acid or its salts present in milk would affect the ascorbic acid. The citric acid would function by transforming its hydrogen to the ascorbic acid thereby reducing the oxidized form which is not determined in the titration method. As has already been demonstrated the ascorbic acid is subjected to considerable variations throughout the year and it could be assumed that the variations in the citric acid and its salts could possibly be the explanation of these variations.

Surface cooler aeration of milk always decreased its ascorbic acid content, although the extent varied with milk from the individual cows. Irradiation of the raw milk, as it was performed in the commercial plant, did not affect the stability of the ascorbic acid in milk to any appreciable extent.

Heating milk for one-half hour at 63° C. in glass containers caused a slight initial destruction in the ascorbic acid as compared with that present in similar milk raw, but the ascorbic acid was more stable upon storage. This effect showed up after the third or fourth day, as then the

heat treated milk had a larger ascorbic acid content than did the raw. Heating for one-half hour at 75° C. gave a still larger initial destruction than heating at 63° C., but the stability was increased still more, so that after three or four days' storage the ascorbic acid was present in considerably larger amounts than that present in the raw milk, or in the milk pasteurized by the holder method.

Heating milk, with and without additions of copper, for ten minutes from 65° C., with five degree intervals, up to 90° C. seemed to have different effects upon the ascorbic acid value, depending upon whether the copper was added before or after heating. In all cases the greatest stability of ascorbic acid was noted at the 80° C. exposure. If the copper were added before heating, a decreased stability of ascorbic acid at temperatures above 80° C. was noted. However, if copper were added after heating, an increase or decrease in the stability of the ascorbic acid beyond 80° C. did not occur. There was a very definite indication that a critical temperature in the stability of ascorbic acid existed between 70° C. and 80° C. Especially was this pronounced in the samples to which copper had been added after heating. The temperature of 75° C. seemed to be close to the border line of this critical temperature.

In the case of flash pasteurization similar results were noted. However, the critical temperature was found to increase slightly and lay between 75° C. and 85° C. The optimum temperature for ascorbic acid stability was apparently the highest temperature employed, 97° C. Flash pasteurization at temperatures below 75° C. decreased the stability of the ascorbic acid as compared to that of the raw milk.

Milk from different cows showed a wide variation in the effect of temperature treatment. Some showed a very pronounced increased stability of the ascorbic acid while others did not. Those that did show an increased stability were of lower initial ascorbic acid content. This phenomena might support the theory regarding the formation of reducing substances when milk is heated to certain temperatures.

The addition of copper under many conditions, particularly of low heat treatment, decreased the ascorbic acid content of milkin a relatively short time. However, this effect was not noted in the milk samples when they were heated as high as 75° C. When a ten minute exposure was employed, the temperature of 80° C. was found to have the most stabilizing effect upon the ascorbic acid. Irradiation had no further effect upon the ascorbic acid beyond that resulting from the presence of copper in the milk.

Addition of glutathione to milk did not have any effect either on the stability of ascorbic acid or on the development of the oxidized flavor.

Throughout these studies some cows' milk were observed to have responded differently to the various treatments to which they were subjected than that of others. Some showed a very pronounced stability in regard to ascorbic acid while others did not exhibit this stability. This variation was noted in the raw milk as well as in the milk to which copper had been added. Likewise, those samples showing much stability did not show any increased stability by heating to high temperatures, for example 75° C. for one-half hour. However, these samples under other conditions were readily susceptible to the development of the oxidized flavor.

The oxidized flavor was found to develop more readily in milk with a higher initial ascorbic acid content, but never developed until the ascorbic acid was gone. The samples which were most susceptible to oxidixed flavor development upon treatment, not only had the largest amount of ascorbic acid when raw, but also showed, on the average, the smallest percentage decrease and the smallest actual decrease during storage. This was also the case when the milk samples were pasteurized, with and without copper present, for one-half hour at 63° C. When copper was present in these samples a very intense oxidized flavor was usually noted after three days.

In one case in which a set of samples was treated at 75° C. for one-half hour, the samples having the most susceptibility to oxidized flavor development showed by far the greatest increased stability upon heating to such temperatures.

The observation was made on numerous occasions that the ascorbic acid, upon additions of copper to the milk, would decrease rapidly and would have disappeared entirely in the milk in the course of two or three days without any development of oxidized flavor. This was practically always the case in the summer months. It follows, therefore, that the rapid decrease of the ascorbic acid is no indication of the milk's susceptibility to oxidized flavor, even in the presence of copper. When the oxidized flavor did develop in the individual cow's milk, as was the case in the late fall, winter, and early spring, it could always be predicted that the samples with the highest ascorbic acid content would be the most susceptible to the oxidized flavor development. Neither must it be forgotten the suggestions already mentioned in the review of literature that the ascorbic acid (vitamin C) might function as a catalyst in biological oxidations.

SUMMARY

A seasonal variation in the average ascorbic acid content of the raw milk from individual cows was found. Likewise, a seasonal variation for the irradiated milk from the College Creamery was noted. This variation was less pronounced in the Grade A raw milk and was not observed in the mixed farmer-produced milk when pasteurized.

Streptococcic milk contained less ascorbic acid on the average, with wider variations, than nonstreptococcic milk.

Milk containing a high leucocyte count had less ascorbic acid than milk having a low leucocyte count. The ascorbic acid in milk of high bacterial count was more stable than that of low count milk.

Slight correlations between the titratable acidity and the flavor score of raw milk and the ascorbic acid were noted. However, no relationship was found between citric acid and its salts and the ascorbic acid.

Processing had diverse effects upon the ascorbic acid values of milk. Aeration decreased the ascorbic acid in milk. Irradiation of raw milk with and without copper had no effect upon the amount of ascorbic acid. Pasteurizing at 63° C. for one-half hour caused a slight destruction of ascorbic acid, but increased its stability upon storage. Pasteurizing at 75° C. for one-half hour caused a still greater initial destruction of ascorbic acid, but further increased its stability.

Heating for ten minutes at different temperatures showed a temperature of 80° C. as giving greatest stability to ascorbic acid. When the milk was heated for ten minutes, the critical temperature for the stability of ascorbic acid was found to be close to 75° C.

Flash pasteurization at different temperatures gave the critical temperature for ascorbic acid stability as being between 75° C. and 85° C. Different cows' milk showed varying effects upon the ascorbic acid as a result of flash pasteurization.

Usually milk with an initial low ascorbic acid content showed the greatest increased stability upon heat treatments. This might support the theory regarding the formation of reducing substances when milk is treated at certain temperatures.

Addition of glutathione to milk was found to have had no effect upon the ascorbic acid value or upon the development of the oxidized flavor.

A few cows' milk showed a very great stability in respect to the ascorbic acid. By different treatments such samples always maintained a higher ascorbic acid value.

Milk with relatively large amounts of ascorbic acid originally was found to be more susceptible to the development of the oxidized flavor under various processes. Copper added before and after heating had different effects upon the ascorbic acid. That added before had the most destructive effect. Samples showing the smallest decrease in ascorbic acid after various treatments were most susceptible to the development of the oxidized flavor upon the addition of copper.

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