THE EFFECT OF HOMOGENIZATION UPON THE VITAMIN C CONTENT OF COW'S MILK

BY

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Introduction

Vitamin C, which is now definitely known to be ascorbic acid, not only prevents scurvy but is important in normal nutrition. Advanced cases of scurvy seldom appear now; however, there are many incipient cases, especially among children. Incidence of latent scurvy as indicated by weakened capillaries has been reported in from 35 to 66 per cent of the children received from economically poor homes into a New York hospital (1).

The use of homogenized milk is increasing steadily in this and in other countries (2). The beneficial effects, from a nutritional and dietetic standpoint, of homogenization center around two factors, the finer subdivision of the butter fat and the softer curd (3). Homogenized milk is especially suited for hospitals and other institutions where creaming of the milk is not desirable.

The market milk industry is turning to the use of homogenized milk to combat the inroads of evaporated milk in infant feeding. Thus, the need of investigation of the effects of homogenization upon the nutritive value of milk in all aspects becomes apparent. Much work has already been done upon the value of homogenization in increasing the digestibility of milk but the effects upon other nutritional factors of milk have not been thoroughly studied. The vitamin content of milk has received much attention from the dairy industry. Of the nutritive factors in milk, vitamin C is probably the most easily destroyed.

In this work, studies will be made of the effect of homogenization upon the vitamin C potency of milk as measured in milligrams of titratable ascorbic acid per liter of milk. Varying pressure and varying temperatures of processing will be used to determine if these factors have any bearing upon any possible changes that may occur in the Vitamin C content of the milk.

Review of Literature

Doan and Minster (4) found that homogenization of milk and cream increases the hydrogen ion concentration if the milk is not preheated to a temperature above 150 degrees Fahrenheit. Homogenization of milk preheated at low temperatures causes the surface tension to be decreased. The change in the hydrogen ion concentration and surface tension of homogenized milk appears to be due to the hydrolysis of the fat by lipolytic enzymes producing soluble acids which ionize and are surface tension active. The degree of fat clumping obtained in homogenized milk is influenced markedly by the temperature of preheating and its relation to the temperature of homogenization. Two-stage homogenization as compared with single stage processing greatly reduces or entirely eliminates fat clumping under all conditions, reduces surface tension, and increases the alcohol stability of milk.

Dahle (5) has shown that the development of oxidized flavors in dairy products may be prevented or delayed by homogenization, high temperature pasteurization, nitrogen replacement of free oxygen, increasing bacterial population, and use of anti-oxidants such as hydroquinone and vitamin C. The addition of vitamin C prevented the occurrence of the oxidized flavor, the samples showing presence of the oxidized flavor also showed loss of vitamin C but the loss was very slight in samples heated to 170 degrees F. Homogenization at 145 degrees F. prevented the development of off-flavors.

Schwartze, Murphy, and Hann (6) showed that the original potency of milk as regards anti-scorbutic vitamin was quite important in the amount of destruction caused by lightly boiling milk for five minutes in a glass beaker or aluminum pan. This treatment resulted in approximately a 20 per cent loss.

Thurston, Brown, and Dustman (7) found that homogenization, severe agitation, and partial freezing of milk all tended to reduce the tendency of milk to become oxidized in flavor.

Sherman states that the greater part of the destruction of vitamin C which takes place upon heating is probably of an oxidative nature. The temperature and hydrogen ion concentration of the medium containing the vitamin C show a definite relation to the rate of destruction. The vitamin is comparatively stable in an acid medium but may be readily destroyed by heating.

Whitnah, et al, found that copper acts as a catalytic agent in accelerating the reduction of vitamin C (8).

Investigation

A. Object of the Investigation:

The object of this experimental work was to determine if the process of homogenization affected the vitamin C potency of milk and, if so, in what manner.

B. Method of Determining Vitamin C Potency of Milk:

It appeared from the literature (9) (10) that the method used by Bessey and King (11) was the best to use for the chemical titration of the vitamin C. Lemon juice has been used with more accuracy than other fruit juices to standardize the dye, although Birch, Harris, and Ray say that either lemon juice or orange juice may be used with equal success (12).

The dye was prepared just before it was to be used, and was prepared by dissolving 100 milligrams of powdered sodium 2,6 dichlorobenzenoneindophenol (EKC) in successive portions of warm water, diluted to 200 milliliters of solution. It was cooled and filtered and a small quantity of phosphate buffer with a pH of 6.8 was added. A 5 milliliter portion of freshly strained lemon juice to which starch indicator had been added was titrated to a permanent blue with 0.0098 normal iodine solution. The iodine solution was made up with 1.3 grams of resublimed iodine and 1.7 grams of potassium iodide to the liter and standardized against a 0.0102 normal sodium thiosulfate solution which had been standardized against a 0.05 normal potassium iodate (KIO_3). A second 5 milliliter portion of the lemon juice, to which 10 milliliters of 8 per cent acetic acid had been added, was titrated with dye solution which was run in from a microburette until a permanent pink color resulted.

The solution of iodine used in this work was 0.0098 normal, therefore one milliliter of the iodine was equal to 0.862 milligrams of ascorbic acid (11).

Twenty-five milliliters of milk was titrated in the presence of 5 milliliters of 8 per cent trichloroacetic acid, to precipitate the casein present. Sharp reports that he finds no mention in the literature of any other substance in milk other than the ascorbic acid which might be reduced by the dye. The presence of the proteins apparently did not affect the reaction (13). For convenience in reading the results of the chemical titration the amounts of ascorbic acid have been presented in milligrams per liter of milk.

C. Milk and Its Treatment:

The milk used for this work was supplied by the Virginia Polytechnic Institute college mixed herd of Jerseys, Guernseys, and Holsteins. These cows were on pasture during the months of April and May when the experiments were carried out. The raw milk was obtained from the cans in the creamery cold storage room and was never more than 24 hours old. Supplementary experimental work was carried out at the Evergreen Dairy in Covington, Virginia.

The homogenizer used for the experimental work was a laboratory model furnished through the courtesy of the Manton-Gaulin Manufacturing Company of Everett, Massachusetts. The machine was of stainless steel throughout the working parts.

Before homogenization, the milk was preheated by means of a water bath to the desired temperature in a 10 quart tinned milk can with the top partially open. Immediately preceding homogenization, the homogenizer was flushed out with water slightly warmer than the temperature at which the milk was to be homogenized in order to insure processing at the desired temperature. The temperature of the milk just prior to processing and immediately after processing was checked. Then the batch of heated milk, about 2 gallons, was placed in the supply tank of the homogenizer, any water remaining in the machine was flushed out, and two samples of the milk in the supply tank secured. Two samples of the homogenized milk was secured as it came from the delivery pipe. Within 5 minutes after each sample had been taken, triplicate twenty-five milliliter portions of the milk were pipetted into beakers and 5 milliliters of 8 per cent trichloroacetic acid added to precipitate the proteins present. After standing for 30 minutes, the solutions were filtered for 2 hours, and the filtrate was titrated with the indophenol. The triplicate determinations were averaged for each sample. Thus with two samples from each batch of milk, a total of six determinations was made on each batch.

D. Results:

The following data was obtained by experimental laboratory methods at the Virginia Polytechnic Institute. The experimental work performed at the Virginia Polytechnic Institute was done with a Manton-Gaulin homogenizer. The data obtained in this work is presented in Table 1 and in Figures 1, 2, 3, and 4.

The work done with the Manton-Gaulin homogenizer included homogenization at three pressures, 1000, 1750, and 2500 pounds. At each of these pressures observations were made at the following temperatures: 100, 110, 120, 130, 140, 150, 160, and 170 degrees Fahrenheit. Each series consisted of the processing of a single batch at the three different pressures under the same temperature conditions. Each series was repeated until triplicate determinations were secured from each sample. Two samples were secured from each batch and triplicate determinations were made on each sample. In a few cases data was secured from only one or two determinations. In these cases the results were not tabulated but were used in computing the percentage losses of ascorbic acid since they substantially increased the number of determinations upon which calculations could be based.

The series at 100° F., 110° F., 120° F., 130° F., 140° F., 150° F., 160° F., and 170° F. were processed on April 9, 10, 14, 15, 28, 29, 30, and May 2, 1939, respectively. The series at 100° F. and 160° F. were processed in the morning. All

Table 1.—Results of homogenization, including treatment of milk, changes in ascorbic acid content of milk, and percentage loss of ascorbic acid.

Sample No.	Treatment	Pressure lbs.	Ascorbic acid Milligrams per liter (a)	(b)	average	% loss
Preheated to 100° F.						
3	h *	----	10.1	9.9	10.0	(10)***
3-A	h & h **	1000	8.4	8.4	8.4	14.0
4	h	----	8.5	8.1	8.3	(11)
4-A	h & h	1750	7.5	7.5	7.5	9.8
5	h	----	8.3	8.3	8.3	(12)
5-A	h & h	2500	7.4	7.4	7.4	10.5
Preheated to 110° F.						
6	h	----	2.0	2.2	2.1	(10)
6-A	h & h	1000	1.9	1.9	1.9	10.0
7	h	----	2.0	2.0	2.0	(10)
7-A	h & h	1750	1.9	1.7	1.8	11.3
8	h	----	2.3	1.8	2.05	(10)
8-A	h & h	2500	1.9	1.7	1.8	11.7
Preheated to 120° F.						
9	h	----	2.0	2.0	2.0	(11)
9-A	h & h	1000	1.8	1.7	1.75	12.6
10	h	----	2.1	1.9	2.0	(10)
10-A	h & h	1750	1.6	1.8	1.7	14.3
11	h	----	2.0	2.0	2.0	(11)
11-A	h & h	2500	1.7	1.7	1.7	12.8
Preheated to 130° F.						
12	h	----	2.5	2.3	2.4	(10)
12-A	h & h	1000	2.1	2.0	2.05	11.7
13	h	----	2.3	2.3	2.3	(11)
13-A	h & h	1750	2.1	1.9	2.0	9.7
14	h	----	2.4	2.1	2.3	(12)
14-A	h & h	2500	2.1	2.1	2.1	11.3

* h indicates preheating.
** h & h indicates preheating and homogenizing.
*** number in parentheses indicates number of determinations used in computing percentage loss.

Table 1 continued

Sample No.	Treatment	Pressure lbs.	Ascorbic acid Milligrams per liter (a)	(b)	average	% loss
Preheated to 140° F.						
15	h	----	2.8	2.8	2.8	(11)
15-A	h & h	1000	2.4	2.6	2.5	11.8
16	h	----	2.4	2.2	2.3	(10)
16-A	h & h	1750	2.3	2.0	2.15	8.3
17	h	----	2.3	2.3	2.3	(11)
17-A	h & h	2500	2.1	2.3	2.2	7.4
Preheated to 150° F.						
18	h	----	2.9	2.7	2.8	(11)
18-A	h & h	1000	2.6	2.6	2.6	9.0
19	h	----	2.8	2.8	2.8	(12)
19-A	h & h	1750	2.7	2.4	2.55	9.4
20	h	----	2.8	2.7	2.75	(10)
20-A	h & h	2500	2.6	2.4	2.5	9.7
Preheated to 160° F.						
21	h	----	6.1	6.0	6.05	(10)
21-A	h & h	1000	5.1	5.3	5.2	12.2
22	h	----	4.2	4.2	4.2	(10)
22-A	h & h	1750	3.8	3.8	3.8	11.0
23	h	----	3.0	3.2	3.1	(10)
23-A	h & h	2500	2.6	2.7	2.65	14.0
Preheated to 170° F.						
24	h	----	3.8	3.8	3.8	(12)
24-A	h & h	1000	3.5	3.3	3.4	12.0
25	h	----	3.0	3.2	3.1	(10)
25-A	h & h	1750	2.6	2.8	2.7	12.0
26	h	----	3.0	2.8	2.9	(11)
26-A	h & h	2500	2.7	2.7	2.7	9.4

Figure 1.--Difference in milligrams of ascorbic acid per liter of milk in milks preheated and the same milks preheated and homogenized at 1000 pounds pressure at various temperatures.

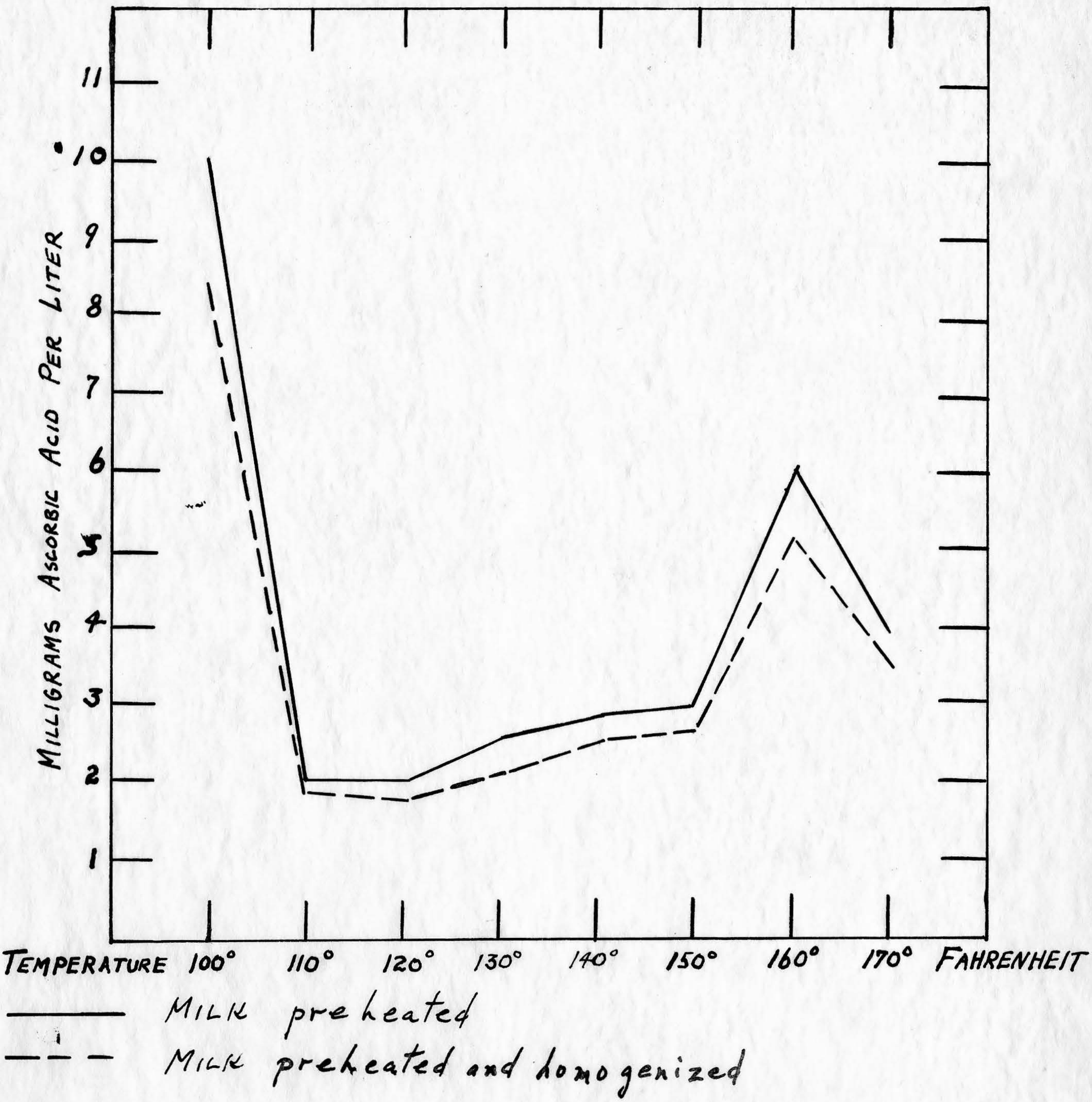


Figure 2.—Difference in ascorbic acid content of milks preheated and the same milks preheated and homogenized at 1750 pounds pressure at the various temperatures.

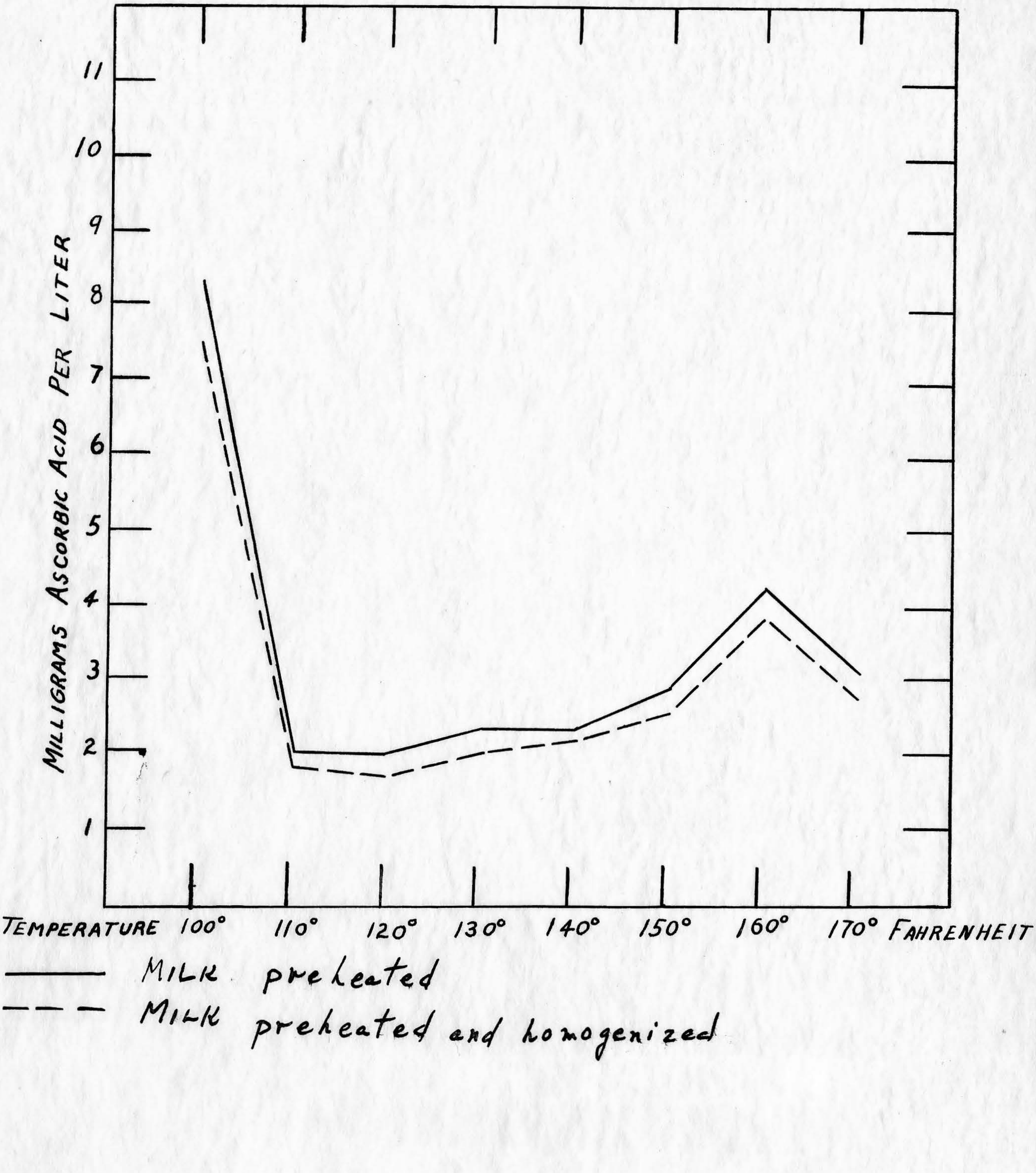


Figure 3.—Difference in ascorbic acid content of milks preheated and the same milks preheated and homogenized at 2500 pounds pressure at the various temperatures.

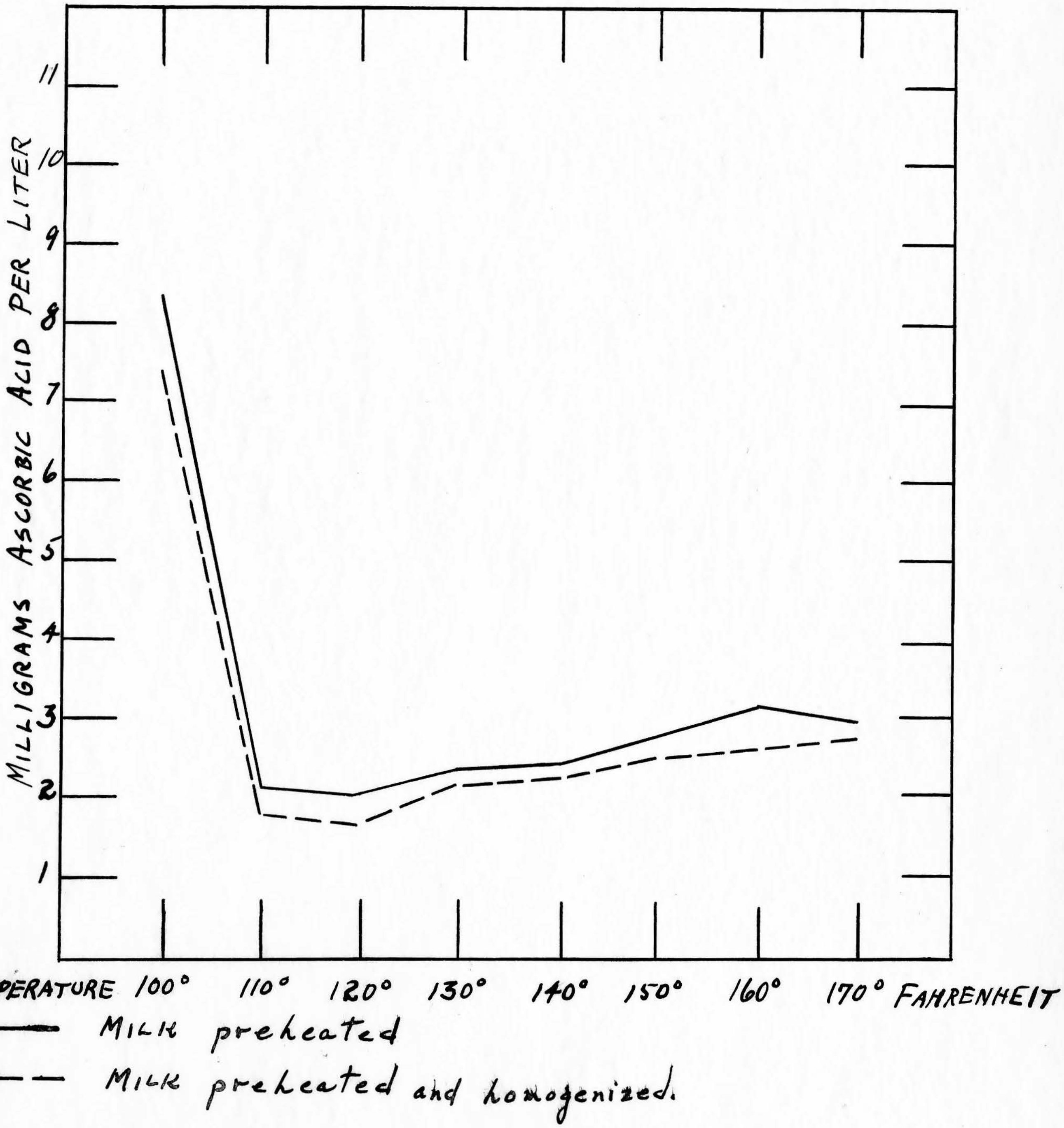
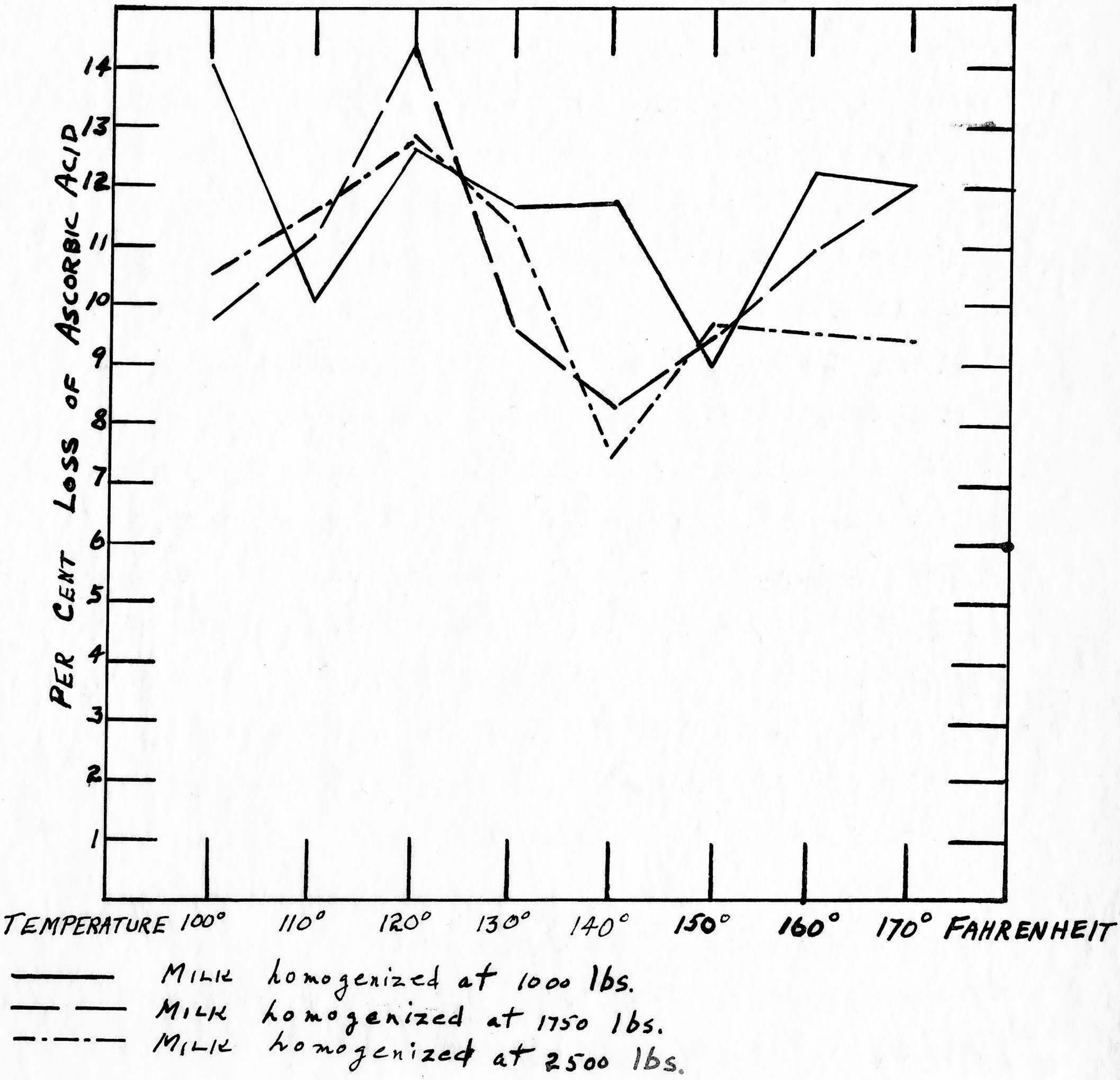


Figure 4.--Percentage loss of ascorbic acid caused by homogenization at the varying temperatures used.



other series were processed in the evening.

Table 1 shows the treatment the milk received such as preheating, homogenization after preheating, temperature of preheating and processing, and pressure of homogenization. The same table shows the ascorbic acid content in milligrams of ascorbic acid per liter of milk, averaged from triplicate determinations of each of the two samples taken from each batch of milk, and the per cent loss of ascorbic acid.

As seen in Table 1, the series at 100° F. showed a reduction in the amount of ascorbic acid which varied considerably with the different pressures. The low pressure showed the greatest amount of loss and the high pressure showed the next greatest loss. The series at 110° F. showed an increasing percentage of reduction as the pressure was increased. The series at 120° F. showed increasing losses as the pressure was increased. However, the next two series, 130° F. and 140° F. showed decreasing losses as the pressure was increased. The series at 150° F. showed increasing losses as the pressure was increased. The series at 160° F. showed the greatest loss at the highest pressure and the next greatest loss at the low pressure. The series at 170° F. showed decreasing losses as the pressure was increased.

The foregoing data shows that the process of homogenization causes a reduction in the amount of ascorbic acid at all temperatures and all pressures employed. The percentage of loss as shown in Table 1 varies from a minimum of 7.4 per cent to a maximum of 14.3 per cent with an average loss for all temperatures and all pressures of 11.34 per cent. As stated previously, these percentages are based on data additional to that shown in Table 1.

Figure 1 shows the difference in milligrams of ascorbic acid per liter of milk in milks preheated and the same milks preheated and homogenized at 1000 pounds pressure at the various temperatures. The line indicating the ascorbic acid content of the homogenized milk rather closely parallels the line indicating the ascorbic acid content of the preheated milk. This shows that there is a consistent reduction of ascorbic acid content of the milk at this pressure and that the loss does not vary a great deal with the temperature changes.

Figure 2 shows the difference in ascorbic acid content of milks preheated and the same milks preheated and homogenized at 1750 pounds pressure at the various temperatures. It can be readily seen that there is a consistent reduction of ascorbic acid at all temperatures. The two lines, indicating preheated milk and homogenized milks, very closely parallel each other, indicating that there is no great difference in the amount of reduction of ascorbic acid at any of the temperatures.

Figure 3 shows the difference in ascorbic acid content of milks preheated and the same milks preheated and homogenized at 2500 pounds pressure at the various temperatures.

Processing causes a steady reduction of the ascorbic acid content of the milk. The amount of reduction does not vary greatly between temperatures.

Figure 4 shows the percentage loss of ascorbic acid caused by homogenization at the varying temperatures used. There is loss of ascorbic acid throughout the entire temperature range of processing.

The following data was obtained by commercial methods of processing at the Evergreen Dairy, of Covington, Virginia. The supplementary data secured at the Evergreen Dairy are all based on commercial methods of processing. Fresh raw milk was used for each processing. Lot 1 was processed on July 14 and lot 2 on July 15.

Table 2 shows the results of three experiments conducted at this dairy where commercial methods were used. Experiment 1 is concerned with the reduction of ascorbic acid at all steps prior to and including homogenization. This was done in order to show the importance of homogenization in reducing the ascorbic acid content of milk. Experiment 2 shows the ascorbic acid content of the raw milk, the preheated milk, the milk homogenized at the three pressures, 800, 2500, 4500, and the homogenized milks after pasteurization. Experiment 3 shows the ascorbic acid content of milk pasteurized in a 300 gallon stainless steel pasteurizer and the ascorbic acid content of the same milk after homogenization at 2500 pounds pressure.

Table 2.—Results of three experiments conducted at Evergreen Dairy, Covington, Virginia.

Experiment	Treatment	Ascorbic acid milligrams per liter Lot 1	Lot 2	% loss average of 1 & 2
1 Milk pasteurized before homogenization	Raw milk	15.8	11.5	
	Milk heated to 143° F.	13.2	8.7	19.8
	Milk pasteurized at 143° F. for 30 minutes	10.6	8.5	11.9
	Pasteurized milk homogenized at 800 lbs.	9.5	7.5	11.9
	Pasteurized milk homogenized at 2500 lbs.	9.5	7.7	10.9
	Pasteurized milk homogenized at 4500 lbs.	9.5	7.5	11.9
2 Milk pasteurized after homogenzation	Raw milk	11.7	12.5	based on Lot 2
	Milk heated to 143° F.	8.0	9.9	20.8
	Milk homogenized at 800 lbs.	8.8	9.4	5.1
	Milk homogenized at 2500 lbs.	8.8	8.8	11.1
	Milk homogenized at 4500 lbs.	14.3	8.3	16.2
	Milk homogenized at 800 lbs. and pasteurized	11.4	2.4	74.5
	Milk homogenized at 2500 lbs. and pasteurized	6.6	2.7	69.3
	Milk homogenized at 4500 lbs. and pasteurized	9.1	2.4	71.7
3 Milk pasteurized in commercial pasteurizer	Milk pasteurized at 143° F. for 30 minutes	11.5		
	Pasteurized milk homogenized at 2500 lbs.	9.8		14.8

Figure 5 is a graphic illustration of the data in Experiment 1 and shows that there is a steady decrease of the ascorbic acid content of the milk during processing. Figure 6 is compiled from the data obtained in Experiment 2 and shows that there is a steady decrease of the ascorbic acid content of the milk during processing.

Figure 5.--Illustration of the data in Experiment 1 at Evergreen Dairy.

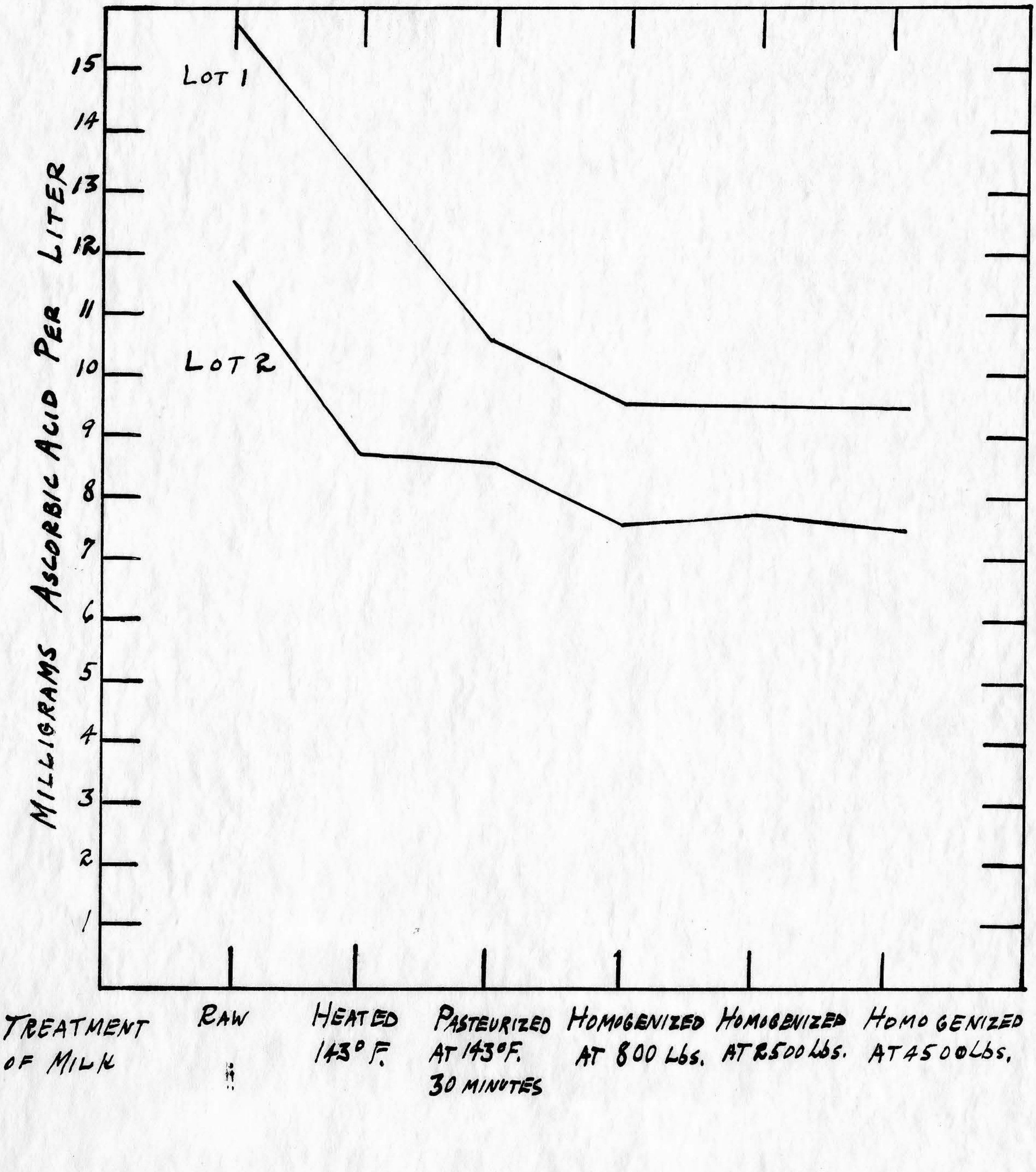
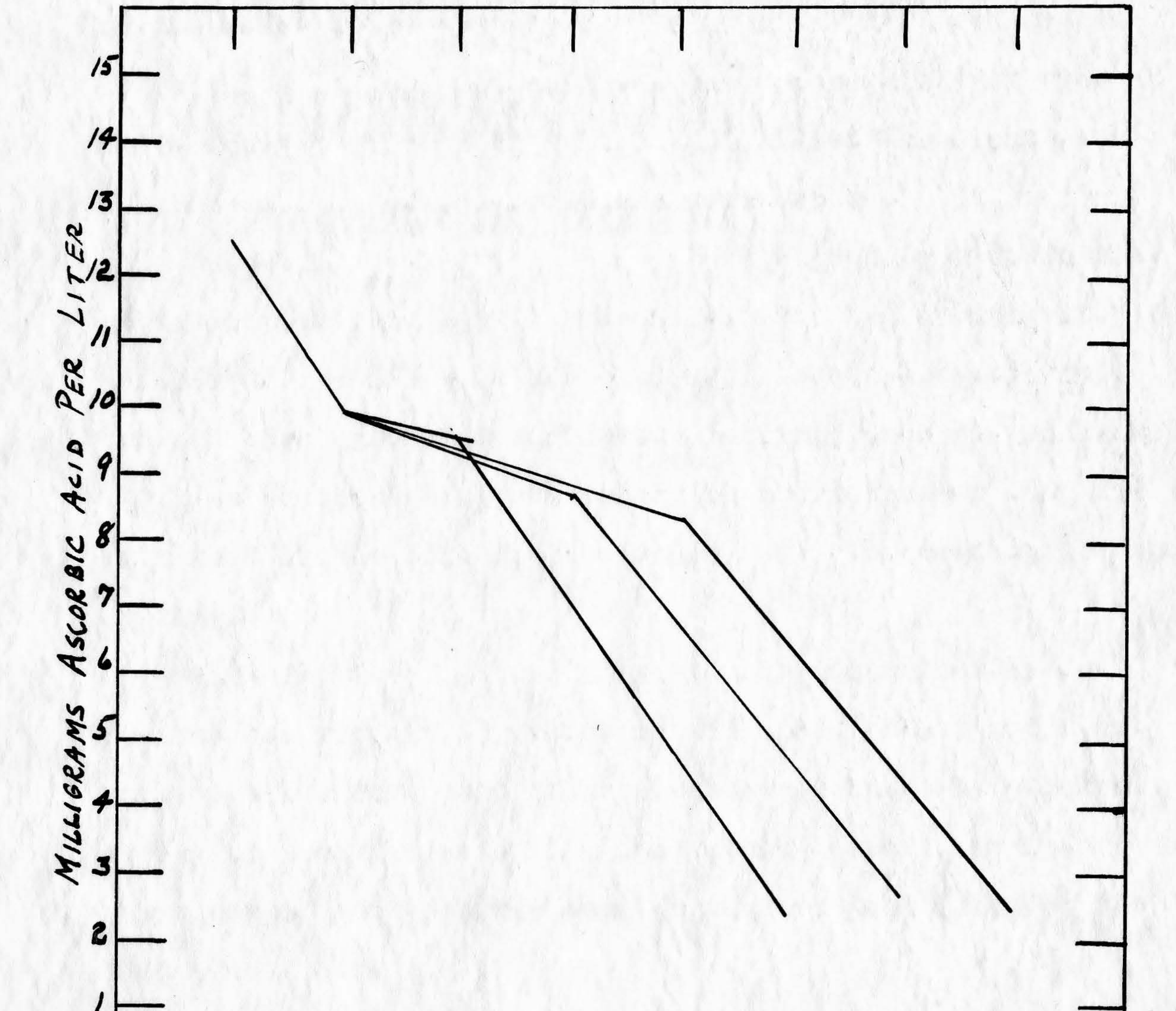


Figure 6.—Illustration of the data in Experiment 2 at at Evergreen Dairy.



Discussion

1. Data obtained by experimental laboratory methods at Virginia Polytechnic Institute: With the exception of two of the series, the preheated milk used in this work was extremely low in ascorbic acid content. This milk was from 12 to 24 hours old, some of it had been milked the night previous to processing, and all of it had been aerated. It is probable that this aeration and the storage greatly reduced the amount of ascorbic acid in the milk. The two series, 100° F. and 160° F., which were not extremely low in ascorbic acid content were processed during the morning, thus the milk used in these series was approximately 12 hours fresher than the milk used in the other series which were processed during the evening.

Whitnah, et al, found that storage progressively decreased the vitamin C potency of milk (8). Sharp and other workers are agreed that milk contains a relatively constant supply of ascorbic acid (13, 14) although there may be a variation in the ascorbic acid content of the milk from the individual cows.

It has been found that plant tissues containing ascorbic acid have an ascorbic acid oxidizing enzyme which is liberated when the plant is crushed (15).

The variation in the oxidation and reduction of ascorbic acid in milk may logically be explained by assuming that a similar enzyme is present in milk. Some of the observed differences in the rate of destruction of the ascorbic acid may be due to differences in the amount of the enzyme present (15). It seems reasonable in this work that there may be an oxidase present in the original raw milk as there is a gradual destruction of the ascorbic acid in the milk as it ages.

This milk which was probably of a high ascorbic acid content originally had the ascorbic acid reduced by the oxidase which acted during the aeration, storage, and heating of the milk. Any such work in the future dealing with vitamin C of milk should be conducted with milk freshly drawn and not aerated.

2. Data obtained by commercial methods at the Evergreen Dairy of Covington, Virginia: The work in which the processing was done according to commercial practice was carried out at the Evergreen Dairy using a Cherry-Burrell viscolizer. This machine had stainless steel working parts with no copper exposed.

The raw milk used in this work was obtained fresh each morning just prior to processing and was from 3 to 6 hours old. Thus there was very little opportunity for the ascorbic acid content of the milk to decrease with aging.

It will be seen in Table 2 that the results obtained from Lot 1 of Experiment 2 are very erratic. During this processing the viscolizer was mechanically defective in that valve packing in the machine had given way and the pressure was extremely variable. The machine was taken apart, new packing was inserted, and the mechanism readjusted. After this, the machine gave very satisfactory service.

The results obtained commercially checked quite closely with those obtained experimentally and verified the results obtained in the previous experimental work.

Summary

Series of experiments employing laboratory methods of homogenization were run at temperatures of 100° F., 110° F., 120° F., 130° F., 140° F., 150° F., 160° F., and 170° F. and with pressures of 1000, 1750, and 2500 pounds at each temperature.

In all series, at all temperatures and at all pressures, the results of homogenization indicated a reduction of the titratable ascorbic acid content of the milk processed.

The amount of destruction of ascorbic acid was variable, ranging from 7.4 per cent to 14.3 per cent with an average reduction of 11.34 per cent.

Supplementary experiments, employing commercial experiment and commercial methods corroborated the results obtained in the laboratory.

Conclusions

The data obtained indicates that homogenization reduces the vitamin C potency of milk by approximately 11 per cent.

Inasmuch as the vitamin C potency of milk, especially when heated as in pasteurization, is not considered to be entirely adequate for the daily requirements of the human, this comparatively small loss is not of great significance. Thus the supplementary value of the vitamin C in milk is not greatly diminished by homogenization.

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