THE EFFECT OF HYDROGEN ION CONCENTRATION ON THE BACTERIAL CONTENT OF MILK DURING PASTEURIZATION

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Submitted to the Faculty of the Michigan State College in partial fulfillment of the requirements for the degree of Master of Science.

by

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INTRODUCTION

Milk, one of the most indispensable foods in most of the civilized countries, presents also some very indispensable problems in relation to the health or to the physical well-being of the consumer. Such problems involve not only the passing of ordinances and laws (10) concerning its sale but also present various phases of investigational nature. Prominent among these phases are those concerning the safeguarding of public health. The fact that milk is a good food, containing the necessary elements for growth, makes it also a favorable medium in which to grow micro-organisms. Improperly handled, it becomes a source and a disseminator of a number of diseases and may be, in itself a menace instead of a benefit to the public. For this reason, researches have been made and have prompted many literary discussions so that to-day the literature, concerning the various phases of investigational nature, of milk and its product, is voluminous.

When it became known that milk was a source and a carrier of the germs of many diseases such as tuberculosis, diphtheria, typhoid fever, septic sore throat, scarlet fever, dysentery, malta fever, and recently Alkaligenes abortus (Bang) (This latter only recently found to be caused through infected milks from infected cows), the elimination of such diseases became the subject

of much investigation by workers in Public Health Laboratories and hospitals, and others interested in safeguarding the health of the public. To-day, except in rare cases, the public is well assured of a milk supply that is both sanitary and free from disease producing organisms.

Pasteurization of milk has been the subject of much investigation and researches have been justified by the resultant increase in public safety. Any work, therefore, in this field may be considered as contributing something in the production of a better and safer commodity. The following is a contribution to the process of pasteurization showing the effect of H-ion concentration on the bacterial content of milk during the process.

HISTORICAL

Perhaps there is not a subject in the dairy industry which has received greater attention than that which deals with the quality of the milk received and dispensed. The fact that milk harbors a great number of bacteria which are either pathogenic or non-pathogenic leads the pioneers of the dairy industry to subject their product to the process of pasteurization to eliminate all possible chances of infection from the use of their products. The early beginnings of pasteurization (17) may be traced back to Spallanzani who in 1765, disproved the theory of spontaneous generation by boiling meat extract in sealed flasks and finding that the contents did not spoil. In 1782, Scheele advised the use of this process in the preservation of vinegar by exposing the sealed bottles to boiling water. Appert, in 1804, used the process in canning, and in 1810, Durand, taking advantage of the opportunities offered by this method of preserving foods, obtained a patent for preserving foods in tin cans and glass jars.

Pasteur, (1860-1864) after revealing to the world the fact that fermentation was due to living cells and not to chemical reactions, next focused his attention on the diseases of wine. He found that spoilage was due to the presence of certain bacteria and he suggested that this could be eliminated by heating the grape juice at a temperature between 122° F. and 140° F. Since then this process of

partial sterilization has been called pasteurization.

Pasteurization, however, was not used so extensively until the medical profession recognized the value of heated milk in the elimination of certain intestinal troubles. We find that, in 1886, Soxhlet first proposed the use of heated milk for infant feeding. Other doctors and pediatricians seeing the possibilities of heated milk as a safeguard against intestinal troubles of their patients also adopted its use, and in 1889, Jacobi, a pediatrician, gave heated milk to his patients. In 1889, Dr. Henry Koplik was made director of the first pasteurized milk depot in New York City. The milk he distributed was pasteurized at 185° F. to 195° F. The temperature was made high because he found that milk pasteurized at that temperature kept well in a moderately cool place for 24 hours. In 1893, Nathan Straus, established his first milk depot where he sold milk pasteurized by the holder method for 20 minutes at 167° F.

From this brief summary of the use of pasteurization in the dairy industry may be traced the efforts of men who pioneered in bringing about a modern industry, the products of which are daily necessities in every man's home. The bacteriology of this industry is quite extensive and well-explored and workers in this field have endeavored to help the industry to maintain the highest quality of product and to safeguard the public. This present research is only part of the work that is being carried on to throw more light on the subject of pasteurization.

It is well known in bacteriology that bacteria require a certain amount of acidity or alkalinity in a medium to bring about the best possible growth. It is well known also that above or below this optimum acidity or alkalinity, bacteria exhibit certain atypical characteristics or the so-called dissociations, or may be entirely inhibited in their growth.

In looking over the literature on this subject, it was found that the determinations on the acidity of milk have not been as extensive as might be expected. Foà (11) was the first to report the H-ion concentration of cow's milk which fact he determined by the electrometric method. He found that fresh cow's-milk has a pH value between 6.6885 and 7.6209. Baker and Breed (2) tried to add the indicator directly to milk contained in test tubes and to compare unknown samples with those obtained with standard samples of normal milk. They came to the conclusion that this method was impracticable due to the lack of definite color standards and the opacity of the milk. To these difficulties may be added the fact that some milks contain higher amounts of phosphates, citrates, and other salts which increase the buffer action. (Another factor which can not be very well overlooked is that of the absorption of the color due to the presence of casein as reported by Sammis and Santschi (19) . These investigators (19) suggested the use of fresh whey as the "whey is said to have the same H-ion concentration as the milk". However,

they did not state the authority for above conclusion. Sharp and McInerney (21) indicated that it might or might not be true depending upon the H-ion concentration of the milk. They further said, "Also it is known that whey shows indicator errors....," and for this reason was of no value. These two workers (21) diluted milk 1-20, and using Brom Cresol Purple and Phenol Red as indicators, found that at 1-20 dilution, the error was very small. By means of a correction factor, they were able to transpose the colorimetric pH into the corresponding pH taken electrometrically. This factor which they found corresponded to a decrease of the H-ion concentration equivalent to 0.54 of a pH when the milk was diluted to 1-20 and compared to the same sample the pH of which was taken electrometrically. By using this correction factor, in the present work, the actual pH or true pH mentioned was determined.

Frei and Lienhard (12), using colorimetric method by adding the indicator directly into fresh cow's milk, with Brom Cresol Purple and Brom Thymol Blue as indicators, found that fresh, normal cow's milk has a pH range between 5.9 and 6.1.

Van Slyke and Baker (27) dialized 300 samples of milk and found that the pH values ranged from 6.4 to 6.7 and that 83 per cent of these have a range between pH 6.5 and 6.7. Schultz and Chandler (23) dialized 1 cc. of milk against 2 cc. of neutral distilled water for 7 1/2 minutes and determined the H-ion concentration of the diffusate colorimetrically, using Brom Thymol Blue as

indicator and found that the pH values ranged from 6.4 to 6.7. They found that distilled water gave the same results as physiological salt solution provided equal volumes were used.

Rice and Markley (18) diluted milk with water and found that a dilution of 1-10 did not further reduce the acidity appreciably when titrated with N/10 NaOH using phenolphthalein as indicator. They stated, "Dilution affects the titration values of high acid milk more than those of low acidity, which would indicate that phosphates have much to do with the effect." Lisk (14) analized 77 samples of sterile milk diluted 1-10 and found that the pH value varies from 6.4 to 6.6. She did not state whether the colorimetric or electrometric method was used. Clark (4) stated that a well buffered solution may often be diluted without seriously altering the pH.

The writer found that as long as the pH of the milk used for analysis did not reach beyond 6.6, diluted 1-20 or even 1-100, the pH value did not change appreciably provided the diluting water was made neutral. Using physiological salt solution or neutral distilled water in 1-20 or 1-100 dilution, there was a tendency for the diluted milk to approach the pH value of the diluting solution, i.e., instead of having the pH of 7.3 (pH 6.8) in a dilution of 1-20 or 1-100, the H-ion concentration decreased and there was an appreciable change toward the acid side. The same result was also obtained if the milk was diluted 1-20 or 1-100 and the pH value was brought to

6.5 (pH6.0), it had a tendency to go toward the alkaline side, even in the 1-20 dilution where there was some buffer action left to take care of the change. In both cases the pH determination was not accurate at the above pH ranges. In lower dilutions, there was buffer effect which could not be avoided and in higher dilutions, there was the pH decrease or increase due to the diluting water. However, the change effected was so small as to be almost negligible provided the H-ion concentration of the milk being tested was not carried far beyond the pH values 6.6 to 6.0 undiluted. These results agreed in substance with those of Baker and Van Slyke (28) who stated that the H-ion concentration of fresh milk decreased when water was added. Clark also stated, "Diluting milk with distilled water lowered the H-ion concentration appreciably".

Cullen (7) in determining the H-ion concentration of blood plasma, diluted the serum with 0.9 per cent NaCl and found it to increase in alkalinity up to dilution of 15 to 20-fold, beyond the 20-fold the change upon further dilution became so small as to be practically negligible. The writer also found this to be true in connection with fresh cow's milk.

Sharp and McInerney (22) titrated milk samples and also determined the pH value electrometrically. The titration values were expressed in terms of per cent lactic acid using N/10 NaOH solution. These workers found that there was a direct relationship between the titrable acidity and the pH.

Table I.

The pH of Fresh Milk and Corresponding Titrable Acidity, Expressed as Lactic Acid, Determined from Curve. (From Sharp and McInerney)

pH	Titrable Acidity	pH	Titrable Acidity
6.0	0.50	6.7	0.145
6.1	0.43	6.8	0.125
6.2	0.36	6.9	0.115
6.3	0.30	7.0	0.105
6.4	0.25	7.1	0.095
6.5	0.21	7.2	0.090
6.6	0.165	7.3	0.085

From the above discussion of the literature, one can see that in spite of the differences of opinion as to the methods of determining the pH values colorimetrically, each investigator believed that his work had advantages over that of the others.

Dializing the milk, and determining the pH of the dialysate, eliminates some of the errors due to the absorption of the color by the casein, and also eliminates opacity which by any other method, except by the electrometric method, is an unavoidable factor of error. Dilution of the dialysate does not affect the H-ion concentration except when carried to a high dilution in which case the pH value becomes greater and has a tendency to go toward the pH of the diluting water or solution. Dilution with water causes a sudden change in the pH of milk but with any subsequent addition of the diluting solution the pH changes are not as sharp as at first. If these changes were plotted, there would be at first a sudden drop flattening out slowly as the dilution increases, arriving finally at the pH of the diluting solution.

Diluting milk does not change the H-ion concentration appreciably as indicated by Rice and Markley (18), Lisk, (14), Clark (4), Van Slyke and Baker (28), Sharp and McInerney (21), so that the pH can be determined to within a very small degree of error when compared with that taken electrometrically. Although the determination of pH values colorimetrically is very susceptible to various factors (20) which are slightly conducive to error in reading, the small variations in pH values in the determination which deviates from the true pH may be considered negligible for practical purposes in the experiments that follow.

Some of the pH values of milk as determined by different investigators are given in the following table by Frei and Lindhard (12).

Table II

pH of Normal Cow Milk as Reported in Literature
by Various Investigators.
(From Frei and Leinhard.)

1.	Courant (zit. nach Foa)	pH 6.6885 - 7.6209
2.	Davidsohn	pH 6.75 im Mittel
3.	Van Dam	pH 6.74
4.	Alleman	pH 6.61
5.	Milroy	pH 6.64 - 6.8
6.	Clark	pH 6.6
7.	Taylor (zit. nach Schults und Chandler	pH 6.8
8.	Obermeier	pH 6.3 - 6.6
9.	Kramer and Green	pH 6.5 - 6.7
10.	Slyke und Baker (300 Prob.)	pH 6.5 - 7.2
	About 83 per cent	pH 6.5 - 6.76
11.	Schultz und Chandler (160 samples)	pH 6.4 - 6.7

Method - 1 to 8, Electrometric
9 to 11, Dialysis
10, Colorimetric

EXPERIMENTAL

General Considerations.

This work was undertaken to determine if there was some practical significance of the H-ion concentration in the pasteurization of milk. It was hoped that it might aid in answering some of the questions that arise such as: Does the H-ion concentration of milk have any effect on the bacterial content during pasteurization? Does it affect the keeping quality after pasteurization?

Rice and Markley (18) concluded that normal milk with a high acidity contained more of all nutrients and was particularly high in phosphates and casein. They gound that, "High acid milks under the same conditions of storage, reached the coagulating point later than milks of lower acidity."

Milk, being a highly buffered substance has the property of resisting the changes of pH upon the addition of acid or alkali up to a certain point, and also, milk has the property of being amphoteric, i. e., it reacts acid to blue litmus paper, and alkali to red. It has been shown by Rice and Markley (18) that milk having a higher acidity than normal generally shows higher buffer action than milk of low acidity.

Milk heated for sterilization has a tendency to show decrease in pH. The work of Whittier and Benton (30) showed that the pH value of heated milk decreased and the titrable acidity in terms of N/10 lactic acid increased,

this increase being due to lactic acid. The percentage of increase was proportional to the temperature, time, and the concentration of lactose present in the milk.

Freshly drawn milk absorbs carbon dioxide from the air, and this is responsible for the decrease in the pH value aside from the effect produced from the milk sugar as it is acted upon by acid-producing bacteria. It is estimated by Van Slyke and Baker (27), (28), that normal milk contains about 10 per cent by volume of CO_2 and that carbon dioxide absorption is not so marked between the pH values of 6.50 and 6.65, as it is above pH 6.65. Rice and Markley (18) concluded that CO_2 accounted for 0.01 to 0.02 per cent of the acidity calculated as lactic acid.

Whittier and Benton (29) found that heating milk increased the acidity equivalent to a concentration of 0.02 mol. and lactose decreased in amount equivalent to 0.085 mol. In another experiment these two authors added lactose to the amount of 5 per cent, and found that the acidity was markedly greater than that of the normal sample. They concluded that the loss of lactose content in heated milk was more than sufficient to account for the acid production even on a mol to mol basis.

The facts, then, are that milk absorbs carbon dioxide from the air and that heating produces a decrease in the H-ion concentration due to breaking down of the lactose molecule and the subsequent oxidation of the glucose forming into molecules of lactic acid. This acid will cause some

trouble in the adjusted pH values of the samples to be pasteurized. However, Van Slyke and Baker (28), on their observations on this point say, "We had noticed that pasteurization, if properly performed, is without observable effect in changing the H-ion concentration of milk." They also concluded from an experiment as shown in Table III that in milk in which carbon dioxide was not removed before heating, the pH value remained the same before and after heating, even though the percentage of CO_2 was decreased by the heating from 10 to 2 per cent.

Table III

Results Showing Effect of Removal of CO_2 on Reaction of Pasteurized Milk.

(From Van Slyke and Baker)

Before Removal of CO_2				After Removal of CO_2			
Before Heating		After Heating		Before Heating		After Heating	
pH Value	Per ct. of CO_2 by Vol.	pH Value	Per ct. of CO_2 by Vol.	pH Value	Per ct. of CO_2 by Vol.	pH Value	Per ct. of CO_2 by Vol.
6.54	10	6.54	2	6.60	0.0	6.56	0.0

The above results by Van Slyke and Baker (28) were obtained by heating milk at a temperature of 63° C. for 15 minutes.

Since heating milk without CO_2 decreases the pH value by only 0.04, it is quite possible that such a small amount of change is quite negligible considering the H-ion concentration by colorimetric measurements, inasmuch as the lowest change that could possibly be detected under ordinary conditions using a comparator block and blanks, is around 0.1 of a pH. In this work, it was found that milk under the ordinary method of laboratory pasteurization using test tubes plugged with cotton, a majority of the samples tested did not show any change in H-ion concentration and some showed a decrease in their pH values. The greatest change in some cases was not enough so that even approximate values could be designated, and, therefore, signs were used to express greater than (+) or less than (-) to designate the change. For experimental purposes, some of the samples were acidified by adding N/10 lactic acid until the desired pH was obtained, while in others N/10 NaOH was used to make them more alkaline. There was a tendency for the milk to show a brownish color if adjusted to a concentration higher than pH 7.2 (diluted 100 columes) or equivalent to pH 6.7 in the actual pH using the correction factors as given by Sharp and Mc Inerney (21). This borwnish coloration was probably due to the action of the alkali upon the protein material in the milk or due to the alteration of the sugar content. As it was more frequently found in samples heated in flowing steam, one might believe that this change was due to the caramelization of the sugar.

Table IV gives the result of some experiments on samples of milk having known pH values and pasteurized at a temperature of 62.5° C. for thirty minutes, and the pH value taken after cooling to 20° C.

Table IV.

The Effect of Pasteurizing Temperature Upon the H-ion Concentration of Milk of Known pH Value.

Before Heating pH	After Heating pH	Before Heating pH	After Heating pH
6.8	6.8	7.2	7.1
6.8	6.8	6.8	6.9
6.9	6.9	6.7	-6.7
6.9	6.9	6.6	6.6
6.8	6.8	6.6	+6.5
6.5	6.4		

The above pH values were obtained by diluting the milk sample 1-100 both before and after heating. Phenol red and Brom cresol purple were used as indicators.

As shown by the above data, the change is very slight and checks closely with that of Table III given by Van Slyke and Baker (27).

The Determination of the pH at Which Bacteria in Milk are Killed Most Rapidly.

Collection of Samples.

The samples of milk used in this work were obtained from the college creamery, placed in sterile flasks and taken to the laboratory and pasteurized in test tubes of the same size and thickness. Some of the samples were stored in the ice-box, the temperature of which varied only from 4° C. to 6° C. Four days storage was allowed before pasteurizing. This was done to increase the bacterial count of some of the samples taken during the winter months. Changes in the H-ion concentration during storage were also determined. Table V shows the result of the changes in pH of ten samples of fresh milk that were thus stored.

It will be seen in the following table that there were no appreciable changes in the pH values during the four days of storage, except in the last sample which was made alkaline by the addition of N/10 NaOH. This sample showed a change of 0.2 of a pH after four days storage. It will also be noted that samples No. 6 and No. 9 showed changes of 0.1 and less than 0.2 of a pH respectively.

The Effect of Storage Upon the H-ion Concentration of Fresh Cow's Milk at Ice-Box Temperature.

Sample No.	Diluted 100 volumes		
	Initial pH	After 48 hrs. pH	After 4 das. pH
1	6.8	6.8	6.8
2	6.8	6.8	6.8
3	6.9	6.9	6.9
4	6.9	6.9	6.9
5	6.8	6.8	6.8
6	7.0	7.0	6.9
7	6.8	6.8	6.8
8	6.9	6.9	6.9
9	7.0	7.0	+6.8
*10	7.1	7.1	6.9

* This sample was made alkaline by the addition of N/10 NaOH.

Method of pH Determination.

The pH determination was made by diluting 1 cc. of milk to 100 volumes and the desired amount of indicator was added to 10 cc. of this dilution. Sharp and McInerney (21) used Phenol red and Brom cresol purple as their indicators in a dilution of 1-20 which proved by experimental evidence that the changes in pH produced by dilution were gradual. Checking their results by using a 1-100 dilution, it was found that this latter dilution agreed with their results from pH 7.2 to pH 6.4. Slight deviations occur outside these values, especially if Brom thymol blue is used as an indicator. This deviation was much more noticeable in 1-20 dilution if Brom thymol blue is used as an indicator. Table VI shows the relationship between the three indicators, Phenol red, Brom cresol purple, and Brom thymol blue in 1-20 and 1-100 dilutions.

It will be noted that no values were given for the undiluted milk having the adjusted pH 7.5 (diluted 1-20) due to the fact that no correcting factor was given for that pH. The reason for this was that Sharp and McInerney (21) were dealing only with fresh, normal cow's milk.

Table VI

The Effect of Dilution Upon the Hydrogen Ion Concentration of Milk of Increasing Acidity.

Sample No.	Undiluted Milk	Indicators: Phenol Red		Brom thymol blue	Brom cresol purple		Error	Correcting factor
		1:20	1:100	1:100	1:20	1:100		
	pH	pH	pH	pH	pH	pH		
1		7.5	7.4	7.3			+0.1,+0.2	0.54
2		7.3	+7.3	7.2			1.0,+0.1	0.54
3		7.2	7.2	+7.2			....	0.54
4	6.56	7.1	7.1	7.1			....	0.54
5	6.46	7.0	7.0	7.0			....	0.54
6	6.36	6.9	6.9	6.9			....	0.54
7	6.36	6.9	6.9	6.9			....	0.54
8	6.36	6.9	6.9	6.9			....	0.54
9	6.26	6.8	6.8	6.8			....	0.54
10	6.26	6.8	6.8	6.8			....	0.54
11	6.16	6.7	6.7	6.7			....	0.54
12	6.06	6.6	6.6	+6.6			....	0.54
13	5.96			6.6	+6.6	6.6	....	0.54
14	5.96			6.6	6.5	6.6	+0.1,0.0	0.54
15	5.86			6.5	6.4	6.5	+0.1,...	0.54
16	+5.76			6.5	+6.3	6.5	+0.2,...	0.54
17	+5.76			6.5	+6.3	6.5	+0.2,...	0.54
18	5.66			-6.5	6.2	6.4	+0.2,...	0.54
19	5.48			6.4	6.0	6.4	+0.4,...	0.52

Samples 1, 2, and 3 are the same milk but made alkaline to give the desired pH, and samples 10to 19 are made acid.

Pasteurization.

Pasteurization of the samples was made in a water bath heated with flowing steam and the amount of steam was regulated by means of a valve. The temperature was kept constant by means of melted paraffin on the surface of the bath to prevent radiation.

After the pH values were adjusted, the tubes containing the samples were immersed in the bath. Pasteurization time started as soon as the temperature of the milk reached that of the water bath. This was determined by placing a thermometer in a tube containing the same amount of milk. The samples were pasteurized for 30 minutes at 62.5° C.

Plating and Counting.

After pasteurization and when the tubes were sufficiently cooled, dilutions were made and 1 cc. of the desired dilution was plated. Duplicate plates were used. The plates were incubated at 37° C. for 48 hours and counted at the end of the incubation period. The plates showing the average growth of the different dilutions made, were counted.

The medium used for plating has the following composition.

Agar (market, not oven dried).......... 1.5%

Beef extract (Bacto) 0.3%

Peptone (Bacto) 0.5%

Distilled water.

Table VII.

The Effect of Different pH Values on the Bacterial Content of Milk During Pasteurization.

Sample No.	Original pH 1-100	Original Count	Count on Adjusted pH Values					
			7.4	7.2	7.0	6.8	6.6	6.4
1	6.8	53,000	500	700	400	600	400	400
2	6.8	52,000	1,200	1,200	1,300	400	500	200
3	6.9	910,000	5,400	500	4,000	300	200	3,900
4	6.8	170,000	1,700	67,000	22,000	12,000	500	1,000
5	6.8	120,000	3,900	2,300	2,500	2,000	2,400	700
6	6.8	350,000	2,900	2,900	2,600	3,800	200	200
7	6.7	7,100,000	200	200	200	300	100	100
8	6.8	340,000	11,000	11,000	7,900	7,000	2,400	1,800
9	6.8	12,000,000	1,300	2,000	2,000	2,000	1,300	500
10	6.9	4,300,000	58,000	69,000	50,000	7,000	39,000	1,100
11	6.8	3,400,000	4,100	11,000	7,200	8,400	3,000	2,500
12	6.8	1,700,000	2,500	1,500	1,500	1,300	1,500	800
13	6.8	210,000	8,700	7,300	7,700	6,800	6,500	6,900
14	6.9	180,000	1,000	1,000	500	600	400	300
15	6.9	2,220,000	800	4,500	1,700	400	600	200
16	6.8	4,900,000	21,000	20,000	4,400	4,800	4,600	1,000
17	6.9	37,000	600	1,500	600	400	400	200
18	6.8	240,000	4,400	1,200	900	700	500	300

Table VII. (Con.)

The Effect of Different pH Values on the Bacterial Content of Milk During Pasteurization.

Sample No.	Original pH 1-100	Original Count	Count on Adjusted pH Values 7.4	7.2	7.0	6.8	6.6	6.4
19	6.9	14,000	1,100	1,300	300	1,000	500	200
20	6.8	470,000	2,200	1,600	1,900	1,800	1,700	1,800
21	6.9	270,000	1,300	1,300	1,600	1,100	1,200	1,200
22	6.9	67,000	1,100	1,200	1,200	1,100	1,500	1,200
23	6.8	64,000	200	200	1,000	1,400	1,700	200
24	6.8	240,000	1,600	1,000	800	1,100	800	800
Ave. count		1,575,472	5,468	8,456	4,964	4,252	3,036	1,104
Per cent reduction			99.65%	99.46%	99.69%	99.73%	99.81%	99.93%

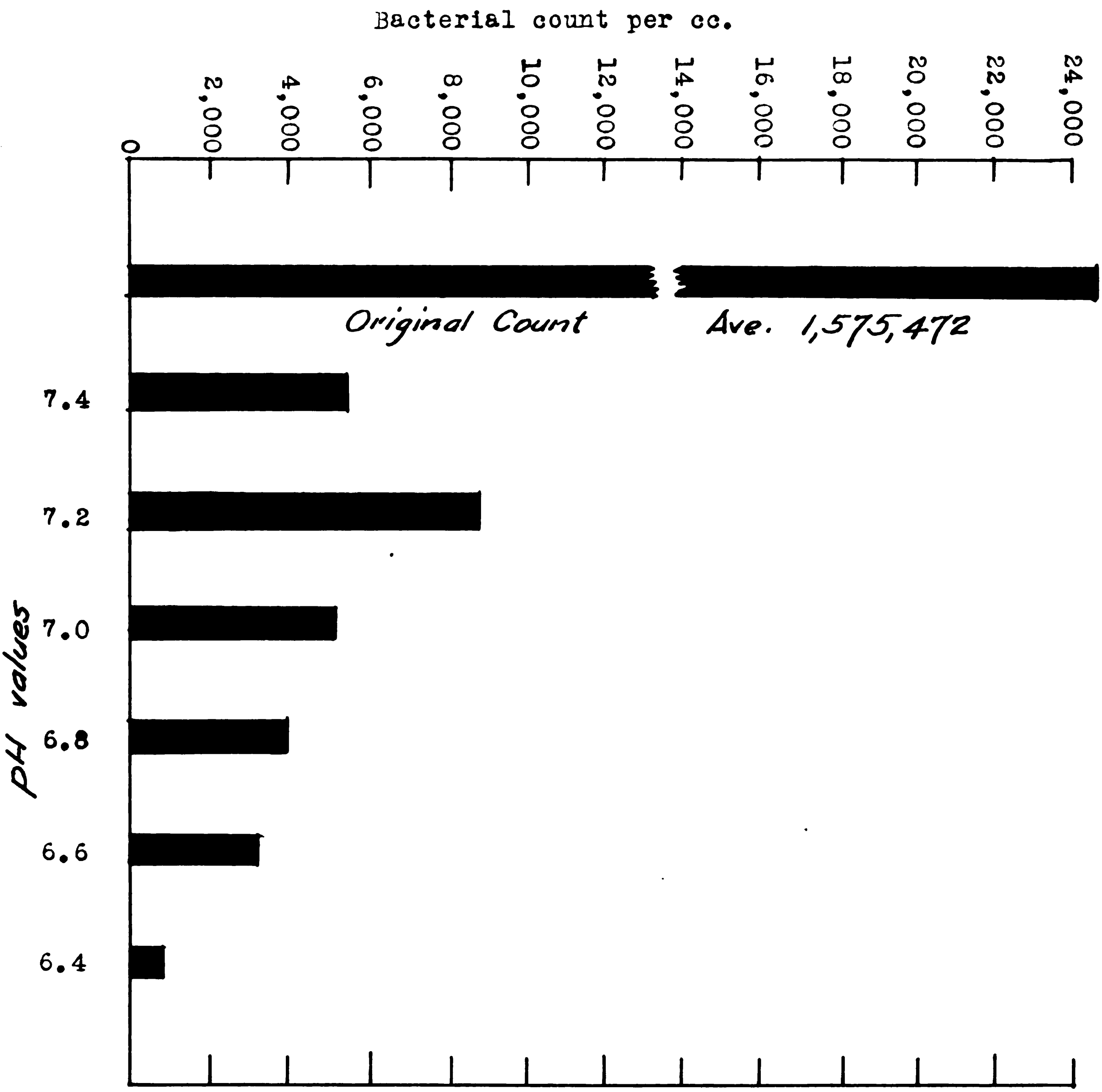

Fig. 1. Bacterial count on milk at different pH values.

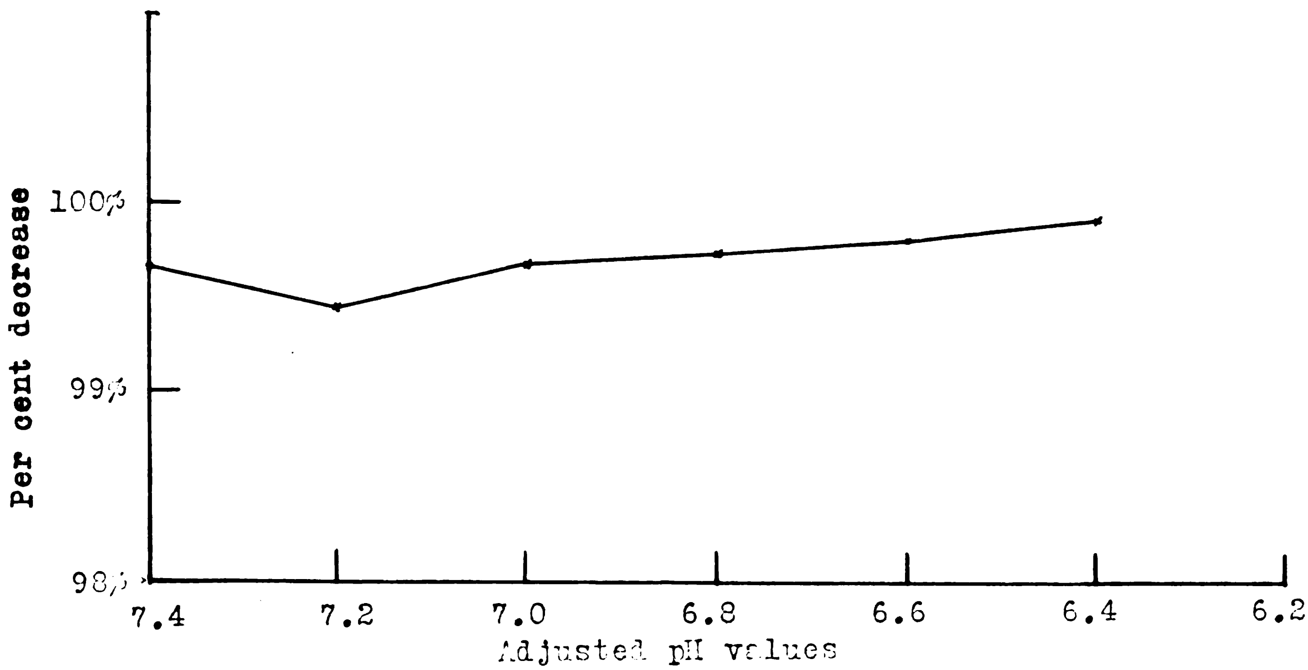

Fig. 2. The per cent decrease of bacteria in milk at different pH values, pasteurized at 62.5° C. for 30 minutes.

DISCUSSION OF DATA.

Studying carefully the data presented, it can be seen that the different counts for the corresponding pH values used, differ somewhat and indicate differences in degree of effectiveness of pasteurization. The pH on the adjusted values corresponding to the original pH value of the milk may be taken as a basis for comparison. It seems, as the data indicates, that samples having pH values nearest to the original have approximately the same number of colonies, except at pH 6.6 in which case there seems to be a noticeable decline in the bacterial count. For instance, in the sample having pH value 7.2, the bacterial count on the graph was higher but beyond pH 7.2, there was a sharp decline on the bacterial count. This then, seems to indicate that at pH 7.4 and at pH 6.6, there was a noticeable effectiveness of the pasteurizer. This efficiency was not so noticeable in some of the individual samples. This was due to the microflora of the milk itself. In most cases, where there was a high count, and the efficiency of the pasteurizer was low, the microflora of the milk was made up of organisms in which spore formers predominated and also some heat resisting organisms. Brannon and Prucha (3) isolated 70 different kinds of bacteria from milk, pasteurized them at 62.5° C. for 35 minutes, and found that 47 organisms were non-spore formers a and only two of these survived pasteurization. In two instances larger counts were obtained after pasteurization. They attributed this increase to the breaking up of the clumps

of spores, since none of the organisms growing at a temperature of 50° C., no thermophiles could be demonstrated.

In this work some of the pasteurized samples were placed at 37° C. for incubation to determine the presence of the organisms that survived pasteurization and develop at 37° C. In some instances no fermentation took place at the end of 24 to 48 hours, and some showed the presence of lactose-splitting organisms causing stormy fermentation with the evolution of gas. As far as it could be determined from the type of fermentation, the organisms causing it belong to the sporogenes group.

The data shows that there was a noticeable decrease in the bacterial count under pH values 7.4 and 6.6. This at first was thought to be due to the germicidal effect of the alkali or the acid added, but it was found that it was not the case. Plates were made before pasteurization of the samples adjusted to the above pH values and the count showed a greater number of colonies developing in 48 hours of incubation than the original sample at the original pH. This increase may be attributed to the shaking of the sample as the alkali or the acid was added, in which the bacterial clumps were broken thereby giving a higher bacterial count. Inasmuch as milk is coagulated at pH 5.6 (undiluted) when heated, it is possible then that at pH 6.4 (pH 5.96 undiluted) there is not enough acid present to destroy the organisms present in the milk. It is a well known fact that most of the organisms in milk survive the acidity produced by <u>Bact. lactis acidi</u> which is enough to coagulate the milk without heating. This source of error may then be neglected.

Another source of error may arise in deciding whether the low count on pH 7.4 and pH 6.6 is due to the alkali agglutination or acid agglutination. The alkaline agglutination may be eliminated since the pH 7.4 taken after diluting the milk to 100 volumes, assuming that the error in the dilution, is about 0.56 of a pH according to Sharp and McInerney (21), would mean that the actual pH of the undiluted milk is only 6.86 which lies within the pH values of normal milks as obtained by various investigators as shown in Table II. Bacteria also agglutinate at certain points of acidity in a medium. Szent-Györgi (13) place the isoelectric point of agglutinins between pH 5.69 and 5.39. These values have a greater H-ion concentration than that of pH 6.6 (pH 6.04 undiluted) or pH 6.4 (pH 5.86 undiluted). Therefore, while there is no agglutination there is a probable tendency for agglutination.

By examining the curve on the percentage of reduction it is evident that there is a steady increase in the efficiency of the pasteurizer and a subsequent decrease of the bacterial count as the H-ion concentration of the milk increases except at pH 7.4. The difference in the percentage of reduction between the different pH values is very small and almost insignificant, but this difference even in so small a fraction may be enough to determine the

acceptability of the milk at dairy plants. According to Rice and Markley (18), milk with higher acidity contains more of all nutrients and is particularly high in phosphates and casein. They concluded, "High acid milks under the same condition of storage, reach the coagulating point later than milk of low acidity". For the same reason, milks during the summer months may owe part of their keeping quality to high acidity and that bacteria which otherwise survive the pasteurizing temperature may be destroyed. Therefore, the high acidity of the milk may serve as a safety factor.

SUMMARY

Pasteurization does not have any appreciable effect on the H-ion concentration of milk. That there is no evidence of appreciable increase in the H-ion concentration does not mean that there is no acid formed from the sugar in the milk. There is probably some increase in acidity due to the lactose being broken down but the buffer action prevents the change, but the colorimetric determinations using the comparator block is not delicate enough to show the change.

Carbon dioxide is driven off during pasteurization, but is a very small factor so that its absence does not have any appreciable effect on the pH value of the milk. As shown in Table III, a decrease of ten per cent in the carbon dioxide content of the milk after heating does not change the pH value of the milk.

Milk stored for four days at a temperature of 4° - 6° C. does not show enough change in the H-ion concentration to be of any practical experimental value. The fact that cold hinders to a certain degree the growth of bacteria, accounts for the very small changes in the pH values of the samples of milk.

Diluting milk to 100 volumes does not show any appreciable change in pH from that diluted 20 volumes, provided the values lie between the pH 6.56 and 6.06 true acidity, or pH 7.2 to pH 6.6 diluted to 100 volumes.

With any further decrease or increase in the H-ion concentration beyond these points, the indicators used begin to show slight deviations and as the acidity or alkalinity increases the greater is the deviation from the normal pH of the milk. For testing the pH of normal milk, it is possible to dilute the milk to 100 volumes with neutral distilled water and with the use of correction factors like those given by Sharp and McInerney (21), one is able to determine the approximate pH of fresh milks.

The bacterial count on the different pH values of increasing acidity shows a very marked decrease as the acidity increases if the normal pH is taken as a basis for comparison. A large percentage of the samples show this decrease while in a few samples in which spore forming bacteria predominate, the count does not show a marked decrease and the efficiency of the pasteurizer is low. The acid or the alkali added does not show any germicidal effect on the bacterial content even to pH 7.4 and 6.4, diluted. Instead, the count increases due to the breaking up of the clumps of bacteria during the shaking of the samples as the acid of the alkali is added.

The percentage of reduction of bacteria in the samples with increasing acidity is very small when compared to the per cent reduction of the milk sample at its normal pH after pasteurization. However, this small percentage of reduction in the bacterial count due to acidity may be considered as a safety factor especially during the

summer months when the bacterial content of milk is at its peak.

CONCLUSIONS.

1. According to the results obtained from the experiment, milks having a H-ion concentration higher than the normal, have a lower bacterial count than the normal after pasteurization at 62.5° C. for 30 minutes.
2. On the average, the per cent decrease of the bacterial count on the adjusted pH values is very small as shown by the graph. However, this small decrease may be an important factor during the summer months when the bacterial counts on milks are high.
3. The increase in the efficiency of the pasteurizer, as far as the adjusted pH values are concerned, is not enough to warrant any practicable application of it in a big scale inasmuch as the temperature used in pasteurization can still be maintained higher thereby increasing the efficiency of the pasteurizer with but a slight change in the constituents of the milk.

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