STUDIES ON THE ELECTROPURE PROCESS OF MILK "PURIFICATION"

THESIS FOR THE DEGREE OF M. S.

A. J. Gelpi

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Milk - Sterilization

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TABLE OF CONTENTS

I. INTRODUCTION

The process of applying heat to human foodstuff in order to make it more palatable, digestible, and otherwise safe for human consumption most probably dates back to very early primeval times. It has only been within comparatively recent years, however, that heat has been applied to foods for the purpose of preservation. The discovery that animal and vegetable materials could be preserved by the application of heat began or resulted properly with the researches and subsequent discoveries of Anthony Leeuwenhoek in 1670, followed and confirmed by Spallanzani in 1765. These early investigators boiled such materials as meat and vegetable extracts for varying periods of time in hermetically sealed flasks. By so doing they observed that no decomposition of the material took place thereafter. In 1782, Scheele advised the exposing of vinegar to the temperature of boiling water for the purpose of preserving it. In 1804, Appert of Paris discovered the process of canning, and in 1811, published a treatise on "The Art of Preserving Animal and Vegetable Substances". In 1810, Durand in England patented a process for preserving certain kinds of foods in tins and glass jars. In 1819, the pioneer canners of America, Daggett, Kensett, Underwood, and Mitchell began on a small scale what is now one of the greatest industries in the world. Up to this time, however, the preservation of foods by heat involved many bacteriological principles which were not clearly and definitely understood, and it was not until

the results of the researches of Louis Pasteur carried on from 1860 to 1870 on the abnormal fermentations of wines were brought to light that these principles became intelligible. He found that by heating wine to a temperature of 62° C. undesirable fermentations could be prevented. This process ultimately became known as "Pasteurization" in honor of the discoverer. The process of pasteurizati on then may be simply defined as a heating process in which practically all of the disease-producing non-spore-forming bacteria as well as most other vegetative bacterial cells are killed. "The temperatures used, however, are not sufficiently high to destroy or otherwise alter the food value of any of the milk constituents.) By such treatment the substance treated is not only rendered safe for human consumption but bacterial decomposition is checked or delayed as well.

The application of the principle of pasteurization to milk dates back to the time of Soxhlet in 1886, who, according to Roseneau, was the first to propose the application of heat to milk for the purpose of infant feeding. In 1889, Jacobi, a well known pediatrician, strongly recommended the use of heated or pasteurized milk. The development of commercial pasteurization was rather slow due to the great public prejudice which had to be gradually overcome. Progress in the art of efficient pasteurization of milk was also slow, but in spite of the many difficulties encountered, three standard methods of milk pasteurization have been perfected and

are in commercial use at the present time. The three generally accepted commercial methods of pasteurization are as follows: 1. the flash, instantaneous, or continuous; 2. the holder, holding, or held; 3. in the bottle or final package method. Besides the above named methods, various modifications of each have from time to time been devised, patented and adapted.

Flash pasteurization consists essentially of rapidly heating the milk to a temperature of about 71° C. for the brief period of a few seconds or more followed by an immediate cooling to 10° C. or lower. In this process the heating is usually effected with live steam.

Holding method - This method consists essentially of heating the milk to 62° C. and holding at that temperature for a definite length of time, usually for 30 minutes, then cooling to 10° C. or lower. In this process the heat is supplied either by hot water, live steam, or exhaust steam.

In the bottle pasteurization - This method consists, as the name implies, of heating the milk after it has been bottled by subjecting the bottles to fine sprays of hot water until the milk reaches the desired pasteurization temperature.

In recent years, however, a fourth method - if it may properly be called a method - has been devised and is steadily being developed and perfected, namely, the electropure process of milk "purification". The theory and mechanics of the electropure process have been thoroughly described in

the literature and will, therefore, not be discussed in detail in this paper. Briefly, the process consists of passing a column or rather a continuous stream of milk through a narrow gage rectangular chamber between two carbon electrodes. The resistance offered by the various electrolytes in solution in the milk immediately causes sufficient heat to affect a reduction of approximately 99% in the bacterial count of the milk without in any way altering the quality of the milk. The apparently valuable and interesting feature of this process is that every particle of the milk undergoing treatment is practically instantaneously heated to the desired temperature for a brief period of time and cooled immediately thereafter. The electropure process is very often referred to as flash pasteurization, but the above mentioned feature of the electropure process serves beyond the shadow of a doubt to differentiate it from the "Flash" method. The electropure process of treating milk, because of the fact that comparatively little work has been done to prove its efficiency (economically and bacteriologically) thus far, and also because of the fact that the process fails to conform to the definitions of "Pasteurization" as set forth by the various Federal, state, and municipal laws, has not as yet been generally accepted.

II. HISTORICAL

The literature bearing on the subject of the electropure treatment of milk is surprisingly scarce. A few articles, mainly of scientific interest, and directly or indirectly pertaining to the subject, will be briefly reviewed here.

Stone (1) in 1909, studied the influence of electricity on microorganisms. His studies were primarily to determine the possibility of obtaining pure water by means of electricity. His experiments were later applied to the purification of milk. In brief, his results show that currents of low voltage had a decided stimulating effect upon bacterial growth and reproduction, whereas on the other hand strong currents produced decided decreases in the numbers of microorganisms present.

Similar results were obtained with milk, and, where static electricity was used, a positive charge was found to favor the development of bacteria to a very considerable extent. Again, where strong electric charges were used, the number of organisms decreased greatly in numbers, but feeble electric currents and small static charges had stimulating effects upon bacteria in milk, increasing their numbers perceptibly.

Beattie (2) in his investigations on the electric treatment of milk gives the following interesting report:

"Thus it will be seen that milk was only in rare instances absolutely sterile, but that it was free from dangerous or disease producing bacteria and from the milk

souring bacteria and that there was an enormous reduction in numbers of all other forms of bacterial life. The actual reduction was over 99 %. The chemical composition is unaltered (so far as chemical analysis can ascertain); chemists' reports also give evidence of non-increase of acidity (which of course is due to the destruction of acid-producing bacteria) and increased keeping quality of milk. The lactalbumin which is coagulated in ordinary sterilized milk is not coagulated in the electrically treated milk. In addition the enzymes are not destroyed."

Anderson and Finkelstein (3) conducted a series of experiments on the electropure process of treating milk with the following points in view:

1. To determine the efficiency of the electropure process in destroying bacteria together with the keeping quality of the milk so treated.

2. To determine whether the bactericidal action of the process is a result of the current alone, the heat produced by the current, or by a combination of these two factors.

3. To study the chemical changes, if any, which are produced by the treatment.

4. To study the effect of the process on some of the enzymes in milk, the time required for coagulation with rennin, the cream line, acidity, etc.

5. To determine if there is any change in nutritive value, such as the destruction of the so-called "vitamine".

These investigators found that the electropure process brought about a reduction of 98.7% of the total count of milk which had a count of 161,500 for an average of sixteen samples. A small percentage of lactose-fermenting organisms were found, however, to escape destruction in the process for some reason or other.

As regards the effect of the electric current on bacteria, the results of these workers show that the heat factor was mainly responsible for the destruction of bacteria. In respect to the keeping qualities of the electro treated milk as well as the effects on the cream line of the milk and the effect on its food value, the conclusions reached by them agree closely with the conclusions reached by others in this field.

As regards the effect of the electric current on bacteria Christian (4) states that the electric current - both continuous and alternating - is said to have a slight retarding effect on bacterial development, but not sufficient to kill bacteria when the auxiliary influences, heat, electrolysis, and the production of ozone are disregarded.

Brannon (5) in his studies on the effect of pasteurization on the individual organisms found in milk states that only two out of forty-seven non-spore-forming organisms survived pasteurization. Ayers and Johnson (6) found that of twenty-two typical streptococci, none survived a temperature of 62° C. for thirty minutes. Tanner and Dubois (7) found

that, when members of the colon-typhoid group were present in milk in numbers equal to which they would occur naturally, they were destroyed by heating for thirty minutes at 60° C. C. M. Carpenter (8) reports complete destruction of tubercle bacilli in milk at 62.5° C., the heat being obtained by passing the milk between electrodes using a 220 volt 60 cycle alternating current. Guinea pigs injected with the milk treated at 55° C. developed typical lesions of the disease. Similar results had previously been reported by such investigators as Carpenter, Curren, Huddleson (17), and others, not only in connection with the tubercle bacillus but various other pathogenic organisms as well.

Resistance of Certain Types of Microorganisms to the Pasteurization Process

Since one phase of the present work will deal to some extent with thermophilic and thermoduric microorganisms, a brief review of some of the work of the leading investigators in this field might prove of interest. It must be said, however, that the work here reviewed has been done mainly on the holding methods of pasteurization. Practically no work of this nature has thus far been done in connection with the electropure process.

Robertson, Yale, and Breed (9), 1926, found that of 140 cultures of spore-forming non-thermophilic bacteria isolated from either freshly pasteurized milk or pasteurizing equipment, the greater majority proved to be such organisms as

Bacillus mesentericus, Bacillus subtilis, Bacillus vulgatus, etc. These workers found the above species to abound in the dried material cooked onto the pasteurizing equipment. Hucker (10), 1928, in his work on cocci which resist pasteurization temperatures, found that the holding temperatures previous to pasteurization did not appear to effect the numbers of organisms appearing on plates incubated at 45° C. made from pasteurized milk. He found, however, that Streptococcus thermophilis appeared to be more prevalent in samples of milk which were held at 30° C. or higher for four hours or more previous to pasteurization. Fay (11), 1927, states that when milk is judged on a basis of its bacterial count, the improvement in its sanitary qualities through pasteurization may not be recognized, if various thermoduric organisms (pin-point colonies) constitute a major part of the flora. The omnipresence of thermo-resistant and thermophilic bacteria in pasteurized milk is usually considered to be of little significance, since they seldom find conditions favorable for their rapid multiplication. Fay (11) further states that the most resistant cultures isolated during his investigations were killed by heating in the steamer for one minute, or by treating for an equal time with a commercial hypochlorite disinfectant. If the organisms are resistant to pasteurization, then subsequent destruction in milk, particularly by the holding processes of pasteurization, is hopeless, and the solution of the so-called pin-point

colony problem must be through preventive measures. North (12) in his investigations of the resistance of Mycobacterium tuberculosis strains found that there is no material difference in the resistance to heat between bovine and human strains of tubercle bacillus and between different strains of either species, or between the tubercle bacilli of naturally infected milk, tissue, lesions, and pure cultures.

Eckford (13), 1927, states that thermo-tolerant organisms may cause annoyance to pasteurizing plants by multiplying during the process and giving the pasteurized milk a high bacterial count. They may cause pin-point colonies at 37° C. Only two groups of these organisms isolated by this investigator ferment lactose. He found thermophiles to survive not only pasteurization but 100° to 120° C. for fifteen minutes in the De Khotinsky water bath. Breed (14), 1928, in his studies on bacteria resisting pasteurizing temperatures concludes that all the procedures that develop true thermophilic bacteria in pasteurizing and milk powder plants seem to be properly classed as faulty or undesirable rather than as unsanitary plant practices. The original presence of thermophilic bacteria in raw milk, on the other hand, seems to be due to the inoculation of the milk with dirt, dust, etc. Breed (14) further showed that the real development of the truly thermophilic organisms, when they occur in pasteurized milk, takes place during the process of pasteurization. Such faulty procedures as allowing foam to remain

in the vats, holding of milk for pasteurization, dead ends, rough surfaces, etc., all offer places for thermophilic bacteria to grow and should be eliminated as far as possible.

Breed and Brickett (15), 1929, conclude that it does not seem probable that especially favorable conditions for growth of milk thermophiles occur normally before the milk reaches the pasteurizing plant. The significance of the presence of occasional large numbers of these organisms in milk is largely dependant upon their origin, upon the conditions under which they grow in the milk, and upon the effect of their activity in the milk. Prolonged holding at high temperatures favors the development and growth of thermophiles. Most thermophiles found in pasteurized milk are non-pathogenic, but many of these types are liquefiers and alkali producers and are objectionable for these reasons.

IV. EXPERIMENTAL

A.

To attempt to demonstrate the efficiency of the electropure treatment of market milk with special reference to the occurrence of members of the colon-aerogenes group in the treated product.

A series of preliminary tests were conducted with a laboratory model of the electropure machine on various lots of good, average, and poor market milk in order to determine the percentage reduction in bacterial numbers at various temperatures, and also to ascertain, if possible, the o occurrence, if any, of Escherichia coli and Aerobacter aerogenes, in the milk after treatment.

The samples for these tests were obtained from the receiving vat of the college creamery. The milk of various patrons was selected at random, and at least two liters were collected from each lot in a flask of suitable size. The samples were immediately taken to the laboratory and a standard plate count was made before subjecting them to the electropure treatment. The samples were run through the machine at such a rate that a constant temperature of 71° C. was maintained, cooled immediately to 25° C. (tap water temperature), and again plated. Dunham fermentation tubes containing lactose broth were inoculated with the milk (0.1 cc., 0.5 cc., and 1 cc. respectively) both before and after treatment, and smears were made on plates containing Salle's medium from those tubes showing gas.

All plate counts were made on standard nutrient agar adjusted to pH 6.8. Dilutions of 1-100, 1-1000, 1-10,000, and 1-100,000 were made on the raw milk, and 1-10, 1-100, and 1-1000 were made on the treated milk. In general the methods as set forth by the American Public Health Association were used for all plate counts. The medium (Salle's) used for demonstrating the presence of and differentiating the colon-aerogenes group was made up as follows:

Peptone	5 gms.
K_2HPO_4	5 gms.
KH_2PO_4	1 gm.
Powdered agar	20 gms.
Distilled water	1000 cc.

When made up, the agar was sterilized in 100 cc. portions, and immediately before using, the following ingredients were added to each 100 cc.:

Erythrosin (2% aqueous)	2 cc.
Methylene blue (1% aqueous)	1 cc.
Bromcresol purple (1% aqueous)	2 cc.
Lactose	1.5 gm.

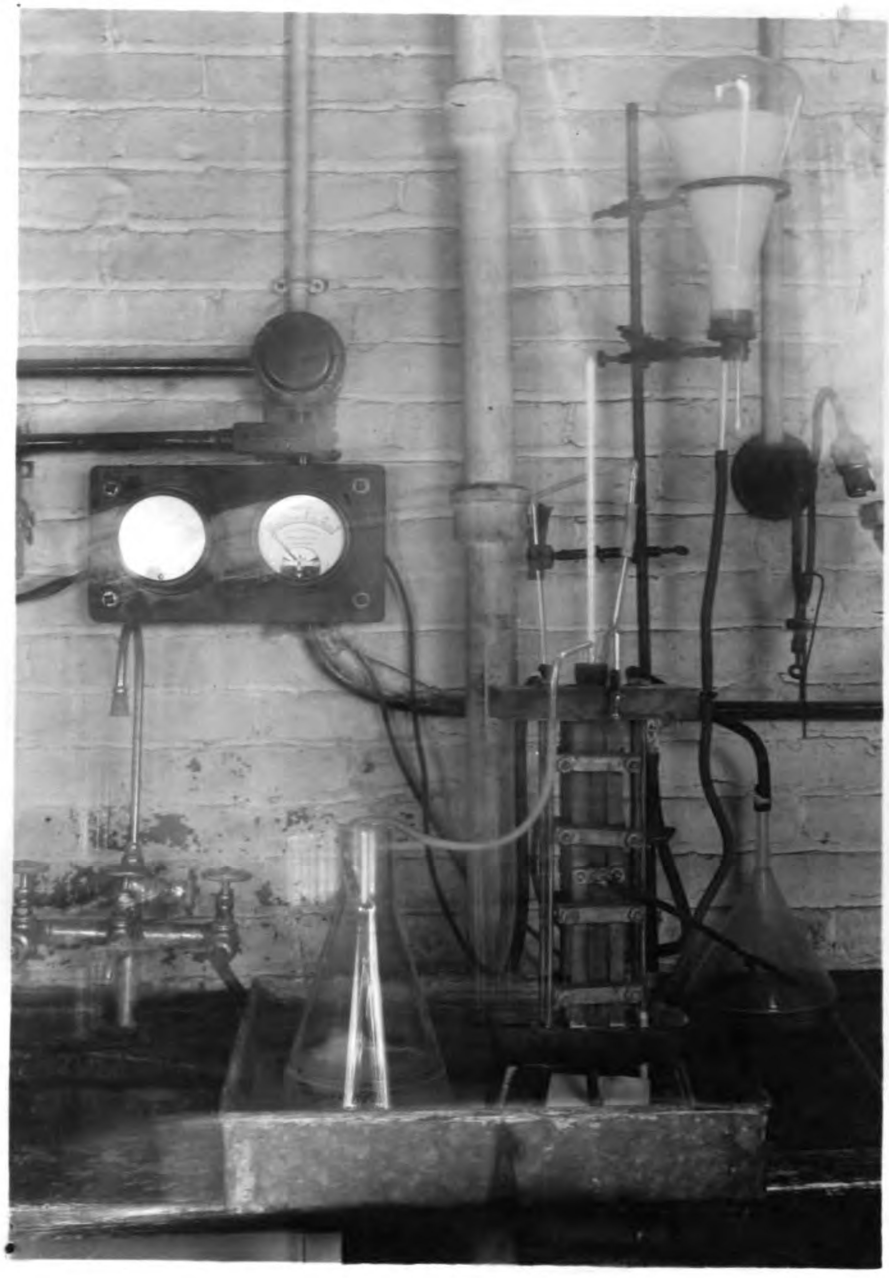
This medium gave excellent colon-aerogenes differentiation in all cases tried. The inoculated plates were incubated at 37° C. for twenty-four hours.

In order to obtain a fair representative sample of milk, and also in order to secure proper adjustment of temperature and milk flow in the experimental machine it was found necessary to use at least two liters of milk for each

trial.

The results of the trials at the various temperatures, the occurrence of the colon-aerogenes group before and after treatment, and the percentage reduction in total numbers are given in the tabulations Nos. I and II. The accompanying photograph shows the complete apparatus used in this work.

The Electropure Machine with which all tests presented herein were conducted



Laboratory model in operation showing a sample of milk inoculated with a pure culture being passed through.

No. 1. "Electro-purification" of Milk

Raw

Sample No.	Plate count	Lactose fermented			Salle's medium	
		0.1 cc.	0.5 cc.	1.0 cc.	E. coli	A. aerogenes
1	110,000	+	+	+	+++	++
2	185,000	-	+	+	+++	-
3	125,000	+	+	+	+++	+
4	350,000	+	+	+	+++	+
5	65,000	+	+	+	+++	+
6	60,000	+	-	+	+++	+
7	168,000	-	+	+	+++	+
8	295,000	+	+	+	+++	+
9	210,000	-	-	+	++	+
10	16,000	-	-	-	-	-
11	145,000	+	+	+	+++	+
12	130,000	+	+	+	+++	+
13	135,000	-	+	-	++	+
14	233,000	+	+	+	+++	++
15	175,000	-	+	+	+++	+

\+ = Few colonies
++ = Medium growth
+++ = Heavy growth
\- = No growth

No. 2. "Electro-purification" of Milk

Treated

Sample No.	Temperature Degrees C.	Plate count	Reduction %	Lactose fermented			Salle's medium	
				0.1 cc.	0.5 cc.	1.0 cc.	E. coli	A. aerogenes
1	65	22,000	80.0	+	+	+	+	+
2	68	22,580	87.8	+	-	+	+	-
3	70	12,000	90.5	-	-	-	-	-
4	70	35,000	90.0	-	-	+	-	+
5	71	4,000	92.3	-	-	-	-	-
6	72	1,200	98.0	-	-	+	-	+
7	73	3,024	98.2	-	-	-	-	-
8	74	4,000	98.6	-	-	-	-	-
9	74	3,360	98.4	-	-	-	-	-
10	74	350	97.8	-	-	-	-	-
11	75	610	99.6	-	-	-	-	-
12	75	750	99.4	-	-	-	-	-
13	75	640	99.6	-	-	-	-	-
14	75	1,170	99.5	-	-	-	-	-
15	76	453	99.7	-	-	-	-	-

+ = Growth

- = No growth

B.

To test the resistance of a series of the various milk borne disease producing bacteria, and to find, if possible the safety range for these organisms in the electropure process of milk "purification".

The following tests were conducted in order to point out the efficiency of the electropure process in destroying various milk borne disease producing bacteria, and to determine, if possible, the extent of the safety range for each individual organism.

A two liter sample of fresh raw whole milk was rendered sterile by autoclaving for twenty minutes at eight to ten pounds pressure. A plating was made to check its sterility. A twenty-four hour agar culture (in the case of those organisms capable of growing on agar medium) was suspended in a physiological saline solution and thoroughly shaken and filtered through cotton to remove all bacterial clumps. The sterile milk was then heavily inoculated with this suspension, from 0.5 to 1 cc. of inoculum being used in each case. This inoculation usually gave a preliminary count of from 100,000 to 1,000,000 or more organisms per cc. of milk. After thoroughly shaking the inoculated sample in order to insure even distribution of the organisms, a plate count was made to determine the extent of the initial inoculation. The milk was then subjected to the electropure process at temperatures ranging from 65° C. to 75° C. Small samples (about 50 cc. per sample) were collected aseptically at the various tem-

peratures in small sterile flasks and cooled immediately to tap water temperature. The samples were plated as soon as possible after the last one was collected. The above process was repeated for each organism used in these tests.

The milk was admitted to the machine in a specially improvised flask arrangement which permitted of easy cleaning and thorough sterilization. The temperatures were easily attained and held constant by adjusting the rate of flow of the milk into the machine. The media used in these tests varied with the type of organism being tested. Whenever possible "Difco" dehydrated media were used. In the case of the haemolytic streptococci the blood agar used was prepared by adding 5 cc. of sterile rabbit blood obtained by heart punctures to each 100 cc. of dextrose agar containing 2.5 per cent agar.

The presence of the haemolytic streptococci in the milk before and after treatment was demonstrated by making smears on blood agar plates of the sediment obtained by thoroughly centrifuging 10 to 12 cc. lots of the milk.

The organisms used in these tests are listed in table III, and the results of the various tests are shown in table IV.

No. III. Cultures Used in Experiment

No.	Organism	Source
1	Eberthella typhi	(Rawlings) State Lab.
2	Salmonella paratyphi	M. S. C. Lab.
3	Salmonella schottmüller i	(Schotmuller) M.S.C. Lab.
4	Staphlococcus aureus	Squibb and Son
5	Streptococcus scarlatinae	Stock # 1171 (State Lab.)
6	Escherichia coli	Yale S. of M.
7	Aerobacter aerogenes	Yale S. of M.
8	Eberthella dysenteriae	(Flexner) State Lab.
9	Eberthella dysenteriae	(Shiga State Lab.
10	Alcaligines melitensis	M. S. C. Lab.
11	Alcaligenes abortus	Bovine (M. S. C. Lab.)

No. IV

Culture No.	Medium used	Initial inoculation	Temperature Range C. Degrees					
			65°	67°	69°	71°	73°	75°
1	Standard agar	17,000,000	+	+	+	-	-	-
2	Standard agar	1,250,000	+	+	+	-	-	-
3	Standard agar	8,300,000	+	+	+	-	-	-
4	Standard agar	1,780,000	+	+	+	-	-	-
5	Blood agar	heavy	+	+	+	-	-	-
6	Standard agar	21,000,000	+	+	-	-	-	
7	Standard agar	14,800,000	+	+	+	-	-	-
8	Standard agar	22,600,000	+	+	-	-	-	
9	Standard agar	19,100,000	+	+	-	-	-	
10	Liver Infu. agar	455,000	+	+	+	-	-	-
11	Liver Infu. agar	6,500,000	+	+	-	-	-	

+ = Growth

- = No growth

C.

To study to a limited extent the effect of the electropure process upon thermoduric organisms, and to compare their resistance to this process with that of the holding method.

The cultures used in this experiment were isolated from several samples of milk pasteurized by the holding method at the Michigan State College creamery. Practically all of the cultures appeared as pin-point colonies on standard agar plates after forty-eight hour incubation period at 37° C.

The holding method was simulated by placing 10 cc. lots of skimmed milk in test tubes and autoclaving these at ten pounds pressure for twenty minutes. Platings were made to check the sterility of the milk. Skimmed milk was used to avoid any interference from the fat layer in the test tubes. Each tube was then inoculated with a 0.1 cc. broth culture (24 hour culture) of the particular organism to be tested. Dilutions of 1-100, 1-1000, and 1-10,000 were made, and 1 cc. from each of these was plated on plain nutrient agar cleared with egg albumin and adjusted to pH 6.9. In this manner initial counts were taken for each organism before pasteurization. The tubes were then placed in an automatically controlled water bath at 62.7° C. and held at that temperature for thirty minutes (five minutes being allowed for the milk to reach the desired temperature). At the end of thirty minutes the tubes were immediately transferred to an ice water bath and cooled to 10° C. Each tube was then

plated, (dilutions of 1-10, 1-100, 1-1000, and 1-10,000 being used), and the percentage reduction before and after treatment, if any, was determined. The above process was repeated in order to check the results of the first trials, and these final results were found to check with the first within reasonable experimental error.

Each culture used in the above tests was individually subjected to the electropure process according to the following procedure. A two liter sample of skimmed milk previously rendered sterile by autoclaving at ten pounds pressure for twenty minutes was heavily inoculated with a saline suspension of a pure culture of the organism to be tested. The suspension was obtained by washing a twenty-four hour agar slant culture into a tube of sterile salt solution and thoroughly shaking and filtering to remove any bacterial clumps. All milk samples used were carefully checked for sterility before making inoculations, and all results for that particular trial were immediately discarded when control plates showed any signs of growth. The flasks of milk were inoculated with such quantities of suspension that 1 cc. of the milk gave a plate count of 50,000 or over in dilutions of 1-1000 to 1-100,000 before treatment. After thoroughly shaking to insure even distribution of organisms, the inoculated milk was plated according to standard methods of milk analysis in order to obtain accurate initial counts. The milk was then subjected to the electropure treatment at

a temperature of 71° C. A sample was drawn in a sterile flask (about 75 to 100 cc.) after the rate of flow and temperature had been properly adjusted and was remaining constant. Immediately upon drawing the sample it was cooled to the temperature of tap water and plated as above. The organisms used in these tests are listed in table V and partly described in table VI. The results of the tests are shown in tables VII and VIII.

No. V Organisms Used in Experiments

Culture	Source	
1P	Pasteurized milk	
2P	"	"
3P	"	"
4P	"	"
5P	"	"
6P	"	"
7P	"	"
8P	"	"
9P	"	"
10P	"	"
11P	"	"
12P	"	"
13P	"	"
14P	"	"
15P	"	"

No. VI

Culture No.	Morphology	Gram	Gelatin liquefaction	Nitrates reduced	Litmus milk	Sugar fermentation									
						Dextrose		Lactose		Maltose		Mannite		Sucrose	
						A	G	A	G	A	G	A	G	A	G
1P	Staphyloccus	+	-	-	Acid	+	-	+	-	-	-	-	-	-	-
2P	Staphyloccus	+	-	-	Reduction	+	+	+	-	-	+	-	-	-	-
3P	Short rod	-	-	-	No change	+	+	-	-	-	-	-	-	-	-
4P	Short rod	-	-	-	Coag. Pepto.	-	-	-	+	-	+	-	-	-	-
5P	Slender rod	-	-	+++	No change	-	+	-	-	-	-	-	-	-	-
6P	Streptobacillus	-	-		Acid, coag.	+	+	+	+	+	-	+	-	+	-
7P	Staphylococcus	+	-	+++	No change	+	-	+	-	-	-	-	-	-	-
8P	Short rod	-	-	-	Reduction	+	+	-	-	+	+	+	-	+	-
9P	Micrococcus	+	-	+	Coag. pepto	+	+	+	-	-	-	-	-	+	-
10p	Coccus forms	+	-	-	Acid, Reduc.	+	+	+	+	-	-	-	-	-	-
11P	Large rod	+	+	-	Acid, Coag.	+	+	+	-	+	-	-	-	-	-
12P	Long slender rod	+	-	-	Coag, Pepto.	+	+	-	-	-	-	-	-	+	+
13P	Streptococcus	+	+	-	Coag. Reduct.	+	+	-	+	+	+	-	+	+	-
14P	Long rod	-	-	-	Reduction	+	+	-	+	+	+	-	-	+	-
15P	Short rod	-	-	+	Acid, slime	+	+	-	-	-	-	-	-	+	-

No. VII

	Holding Process (62.7°C. - 30 min.)			Electropure Process (71° C.)		
Culture	Initial Count	Final Count	% Reduction	Initial Count	Final Count	% Reduction
1P	585,000	541,000	7.5	1,210,000	976,000	10.1
2P	938,000	664,000	29.1	1,520,000	850,000	33.7
3P	782,000	698,000	10.7	51,000	36,000	20.8
4P	128,200	77,600	38.0	338,000	22,500	89.0
5P	242,000	20,400	91.0	90,860	5,420	94.0
6P	1,520,000	815,000	44.0	3,700,000	580,000	84.3
7P	2,260,000	2,017,000	11.0	22,270,000	5,580,000	79.0
8P	2,550,000	1,380,000	46.0	9,100,000	930,000	89.0
9P	2,220,000	1,300,000	41.0	6,400,000	538,000	91.5
10P	637,000	635,000	3.7	872,000	868,000	4.8
11P	122,000	54,000	55.7	600,000	270,000	55.0
12P	4,280,000	343,000	92.2	6,390,000	400,000	93.0
13P	2,300,000	1,930,000	16.0	4,800,000	2,300,000	52.0
14P	862,000	425,000	50.8	570,000	193,000	66.0
15P	124,000	67,000	46.0	40,000	11,800	70.0.

No. VIII

	Holding Process (62.7° C. for 30 min.)			Electropure Process (71° C.)		
Culture	Initial Count	Final Count	% Reduction	Initial Count	Final Count	% Reduction
1P	340,000	330,200	2.5	369,000	331,000	10.3
2P	5,801,000	4,580,000	21.9	839,000	597,000	28.7
3P	2,620,000	2,500,000	4.5	555,000	546,000	16.2
4P	50,000	39,400	21.2	210,000	30,000	85.7
5P	2,500	200	92.0	974,000	91,000	90.6
6P	595,000	356,000	40.0	577,000	60,000	89.6
7P	394,000	361,000	8.0	22,500,000	4,470,000	80.0
8P	600,000	350,000	41.6	24,000,000	4,700,000	80.0
9P	80,000	32,200	60.0	62,000,000	125,000	99.7
10P	958,000	1,100,000	0	1,590,000	1,470,000	7.5
11P	543,000	141,000	70.4	11,500,000	3,450,000	70.0
12P	562,000	84,000	85.0	431,000	75,520	82.4
13P	840,000	823,000	20.2	2,520,000	610,000	36.5
14P	1,500,000	493,000	67.0	220,000	100,000	54.5
15P	1,950,000	980,000	49.7	1,050,000	220,000	79.0

D.

To compare the resistance of some milk thermophilic organisms to the holding process of pasteurization with that of the electropure process.

The organisms used in these tests were obtained from the Tanner collection at the University of Illinois. The methods used were similar in most respects to those used in the preceding experiments. Agar slant cultures grown at 45° C. were suspended in physiological saline solutions, and thoroughly shaken and filtered to remove clumps. Flasks containing two liters each of sterile skimmed milk were heavily inoculated with each culture respectively. Each flask of inoculated milk was then subjected to the holding process of pasteurization at 62.7° C. for thirty minutes, and similarly inoculated flasks were then subjected to electropure process at 71° C. In the case of the holding operation, 10 cc. portions of sterile skimmed milk in test tubes were inoculated with about 0.1 cc. of the suspensions of the respective organisms, thoroughly shaken to insure even distribution of the organisms, exposed to a temperature of 62.7° C. for thirty minutes in an automatic water bath, and cooled immediately thereafter to 25° C. In the case of the electropure process a 50 to 75 cc. sample was drawn directly from the delivery spout of the machine a minute or so after the temperature had been adjusted to the desired point. The sample was immediately cooled to 25° C. and plated within a few minutes after drawing. The time of exposure of

the milk to the electric current in the machine was approximately 14 seconds. Standard plate counts on "Difco" nutrient agar were made of each sample immediately before and after each treatment using in each case dilutions of 1-100, 1-1000, 1-10,000, and 1-100,000. Duplicate plates were poured of each dilution, and all counts were made with a counting plate when necessary and a counting lens. All plates were incubated at 45° C. and counted at twenty-four and forty-eight hours. In order to insure consistent results it was found very necessary to use cultures of exactly the same age for each trial, as the older the culture the more heat resistant it was found to be. Therefore, in order to standardize conditions as much as possible, cultures which were exactly twenty-four hours old were used throughout. A series of seventy-two hour old cultures, however, were also used for comparison. The results of these tests (Plate counts and percent reduction in each case) are shown in tables X and XI. The cultures used in these tests are listed in table IX.

No. IX Organisms Used in Experiments

Culture	Source
27	U. of Ill. (Milk)
31	U. of Ill. (Milk)
41	U. of Ill. (Milk)
49	U. of Ill. (Milk)
50	U. of Ill. (Milk)
56	U. of Ill. (Milk)
66	U. of Ill. (Milk)
75	U. of Ill. (Milk)
135	U. of Ill. (Feces)
142	U. of Ill. (Feces)

No. X 24 Hour Cultures

	Holding Process (62.7° C. - 30 min.)			Electropure Process (71° C.)		
No.	Initial Count	Final Count	% Reduction	Initial Count	Final Count	% Reduction
27	44,000	0	100	85,210	0	100
31	511,000	5,000	99	386,000	31,000	92
41	393,000	20,210	94.8	980,000	13,000	98.6
50	333,000	0	100	782,000	0	100
49	318,000	0	100	380,000	1,200	99.6
56	360,000	0	100	1,250,000	1,520	99.9
66	356,000	0	100	176,000	0	100
75	696,000	4,380	99	568,000	1,232	99.8
135	236,000	130,300	44.7	350,000	27,000	90
142	520,000	32,500	93.8	7,200,000	18,200	99.9

No. XI 72 Hour Cultures

	Holding Process (62.7° C. - 30 min.)			Electropure Process (71° C.)		
No.	Initial Count	Final Count	% Reduction	Initial Count	Final Count	% Reduction
27	510,000	25,700	94.9	96,000	39,000	59.0
31	513,000	109,000	78.0	250,000	50,250	79.0
41	982,000	326,000	66.0	2,360,000	584,000	75.0
50	2,040,000	1,600,000	21.5	730,000	110,000	84.0
49	1,060,000	200,000	81.0	76,000	14,000	81.0
56	5,390,000	4,240,000	21.0	410,000	290,000	29.0
66	64,400	2,800	95.0	30,000	5,300	82.0
75	972,000	750,000	22.0	280,000	220,000	21.0
135	461,000	240,000	48.0	365,000	115,000	69.0
142	60,000	27,000	71.0	214,000	27,000	87.0

E.

To determine the effect of the electropure process upon some spore-forming organisms commonly or occasionally found in milk.

The organisms used in these tests were all obtained from the stock cultures of the Michigan State College Bacteriological laboratories. The general technique and methods employed were essentially the same as those outlined in the previous experiments. The cultures were grown for four to six weeks on agar slants at room temperature. Before using each culture was examined microscopically to determine the approximate extent of sporulation. All cultures showed from 80-90 % spores when used. The cultures which were too dry to be put in suspension by the usual method were ground in a sterile mortar with sterile sand, then washed with salt solution and filtered through sterile filter paper. Flasks of sterile skimmed milk were heavily inoculated with these suspensions. The remainder of the tests were carried as previously described.

The spore-formers used in the above tests comprise culture members 15S, 16S, 17S, 18S, and 19S and are described in table XII.

The results of these tests, including plate counts and percent reduction before and after each method of pasteurization, are shown in detail in table XIII.

No. XII Spore-formers Used in Experiments

Culture	Source
15S	Bacillus anthracis
16S	Bacillus megatherium (M.S.C.)
17S	Bacillus subtilis (M.S.C.)
18S	Bacillus mycoides (M.S.C.)
19S	Bacillus mesentericus (M.S.C.)

No. XIII

First trial

	Holding Process (62.7° C. - 30 min.)			Electropure Process (71° C.)		
No.	Initial Count	Final Count	% Reduction	Initial Count	Final Count	% Reduction
15S	2,170,000	2,110,000	2.7	2,400,000	4,200	99.5
16S	4,810,000	4,812,000	0	2,600,000	740,000	71.5
16S	490,000	550,000	0	2,100,000	360,000	82.0
18S	678,000	682,000	0	3,000,000	5,000	99.9
19S	1,162,000	1,140,000	1.7	7,800,000	150,000	98.0

Second trial

	Holding Process (62.7° C. - 30 min.)			Electropure Process (71° C.)		
No.	Initial Count	Final Count	% Reduction	Initial Count	Final Count	% Reduction
15S	947,000	944,000	0.3	1,850,000	4,700	99.7
16S	3,720,000	3,780,000	0	14,500,000	570,000	90.6
17S	1,960,000	1,960,000	0.5	1,140,000	13,000	98.0
18S	1,400,000	1,400,000	13.0	4,200,000	2,000	99.9
19S	2,160,000	2,160,000	0.9	5,200,000	310,000	94.0

V. SUMMARY AND CONCLUSIONS

1. A series of samples of raw market milk taken at random from various patrons of the Michigan State College creamery were subjected to the electropure treatment at temperatures ranging from 65° C. to 76° C. The results of plate counts made before and after treatment, and the occurrence of members of the colon-aerogenes group were tabulated. The condition of the milk after being subjected to the various temperatures as to flavor and cream line were noted. From the results obtained in these tests it would seem that temperatures ranging from 71° - 73° C. are the most efficient. The percent reduction in total count at these temperatures ranged from 92.3 to 98.2. Though the percent reduction is greatly increased at 74° C. and 75° C., a marked effect on flavor and cream line becomes apparent at temperatures above 73° C. Some of the colon-aerogenes group, apparently, occasionally escaped destruction for some reason or other at temperatures up to 72° C. The extent of the reduction will naturally depend upon the kinds of organisms present and very probably upon the age of the organisms themselves. That the age of non-spore-forming organisms in milk affects their heat resistance has been clearly demonstrated by Robertson (16) and to a limited extent by the author in some of the work presented in this thesis.

2. A series of pathogenic organisms commonly or occasionally found in milk was subjected to the electropure process in

pure culture. Temperatures ranging from 65° C. to 75° C. were used on each organism tried. The object was to test their individual resistance to the process, and also to determine, if possible, the safety range in temperature for these various organisms. The results obtained in these trials tend to indicate that though the safety range is very narrow, it is clear-cut and apparently very constant, that is, most pathogens escaped total destruction at 69° C. but were all destroyed at 71° C.

From this narrow safety range in temperature it naturally follows that the success of the electropure process will depend in the last analysis upon an efficient and absolutely dependable mechanical device which will control the temperature to within at least three degrees.

3. A number of organisms which seemed to persistently resist the holding process of pasteurization were isolated and studied in pure culture. Each of these organisms were subjected to the holding method of pasteurization and also to the electropure process. At least two trials (and in some instances four and five) were made for each culture. The percent reduction in numbers before and after treatment for each method of pasteurization was carefully noted. Although as a whole the organisms studied were very resistant to both the holding and the electropure processes, the difference in percent reduction between the two methods is markedly in favor of the electropure process, there being only two instances out of the thirty trials recorded where

the reduction was greater by the holding process. These results are certainly worthy of note, especially when the brief period of time at which the milk was exposed to the desired temperature (usually less than ten seconds) is taken into consideration, and this would undoubtedly lead one to believe that some factor or factors other than heat are most probably responsible for the destruction of bacterial cells. Whatever these factors may be, however, still remains to be determined. Various suggestions have been offered in explanation for the phenomenon manifested in the chamber of the electropure machine. Some of these, such as the possible production of ozone, electrolysis, production of ultra-violet or other rays, etc., are merely speculative, however, and the field still lies open for experimentation and further investigation.

4. A number of thermophilic organisms were subjected both to the electropure process and holding method of pasteurization, and their individual resistance to each process was compared. The results of these tests do not appear nearly as favorable with respect to the electropure process as some of the results previously obtained when other types of organisms were used. Though the cultures here employed grew well at 45° C. to 50° C., they proved to be not very resistant to pasteurizing temperatures. In this series of cultures No. 135, an organism of fecal origin, was the only one which showed any great degree of heat resistance. This organism was equally resistant to both processes.

From the data collected in these tests it would seem reasonable to state that the electropure process, although as fully efficient as the holding method in reducing the numbers of microbes present, shows no particular advantage over the holding method in this respect. In view of the present conception, however, that the role played by thermophiles in milk is of no great significance, the electropure process could not very well be severely criticized from this point of view.

Here again we have evidence of an increase in heat tolerance of an organism with an increase in age of the culture. Table X shows that the cultures were markedly more heat resistant at 72 hours than they had been at 24 hours. The percent reduction, however, in the 24 hour and 72 hour cultures was, in general, proportionately the same for each method of pasteurization.

5. A series of tests were conducted with some common spore-forming organisms in order to compare their relative resistance to the electropure and holding methods of pasteurization. The cultures were from six to eight weeks old when used, and microscopic examinations showed them to contain from 80 to 90 percent spores. The results obtained in these tests, as shown in table XI, are markedly in favor of the electropure process. Particular attention is drawn to those results obtained with Bacillus anthracis (No. 15S) which is known to be an extremely resistant organism. The percent

reduction in the case of this organism ranged from 0.3 to 2.7 in the holding method, and from 99.5 to 99.7 in the electropure process. The results of the tests with Bacillus subtilis, Bacillus mycoides, Bacillus mesentericus, and Bacillus megatherium are equally striking.

From the figures shown in table XI, the electropure process is undoubtedly effective in the destruction of spores. Indications of this fact were found in the early part of this work when raw milk samples were being subjected to the treatment. At this time the organisms escaping destruction were isolated and examined microscopically. No spore-formers were found during the course of these examinations.

The results of this experiment indicate beyond further doubt that there is something else besides the heat factor which causes the destruction of bacterial cells in the electropure process. There seems to be a strong possibility, however, that, in the case of spores, the destruction might be brought about indirectly by the effect of the electric current. It is a known fact that the more concentrated an electrolytic solution becomes, the less resistance it offers to an alternating current, and the greater the amount of heat produced in consequence. The cytoplasm in the sporulated cells becomes more concentrated due to loss of water, and consequently the electrolytic substances in solution within the cells offer less resistance to the electric current than does the surrounding medium (milk). As a

result, an instantaneous and marked increase in temperature within the cells themselves is affected. The heat thus created is probably sufficiently intense to cause the destruction of the spores.

The idea brought out in the above statement has been demonstrated in the laboratory by immersing an artificial cell (consisting of a parchment sack filled with a concentrated NaCl solution) between two carbon electrodes in a vessel containing a salt solution of lesser concentration. A sensitive thermometer was suspended in each solution respectively, and, when the current was applied (110 v.AC), the rise in temperature in the parchment sack was shown to be much more rapid than the rise in temperature of the surrounding medium. The medium in the parchment sack contained the greater amount of free ions and, therefore, offered the least resistance to the current, and as a result more current flowed through the cell, and consequently more heat was generated.

Since the results of this experiment show so definitely that the "purification" of milk by the electropure process is brought about by the aid of another factor besides heat, then, the process, by definition, cannot properly be called a pasteurization process. Whatever the factor or factors might be, whether their effect is direct or indirect, the process is superior to pasteurization and should not be classed as, regarded as, or confused with "Pasteurization".

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