THE BACTERIAL FLORA OF ICE CREAM MIXES WHEN PASTEURIZED AT DIFFERENT TEMPERATURES

by

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INTRODUCTION

In the ice cream industry today there is very little uniformity in the time and temperature employed for pasteurization. In the pasteurization of market milk temperatures above 145°F. even for a short time seriously impair the cream line and flavor of the product. Ice cream mixes on the other hand, may be heated considerably above this temperature without imparting any noticeable cooked flavor or producing any other changes which may be detrimental to the quality of the finished ice cream.

The pasteurization temperature and time (142°F. -145°F. for 30 minutes) which is very widely used in the market milk industry has been carried over into the ice cream industry because it has proved efficient in destroying organisms in milk. In recent years it has been demonstrated experimentally as well as under practical conditions, that mixes were not seriously injured by the use of higher temperatures for shorter holding periods. As a result there are in use today, pasteurization temperatures ranging from 145°F. to 180°F. for time intervals varying from 1 to 60 minutes.

There are but few data available showing the relative efficiencies on ice cream of various temperatures and time

intervals for the destruction of bacteria in ice cream mix. It is a common concept, however, that as the temperature is increased the length of time necessary to attain a given efficiency is decreased. The extent to which the time of heating may be decreased when various temperatures are employed has not been definitely established. The advent of continuous pasteurization and flash pasteurization has rendered this a question of vital importance.

It is reported that some city ordinances require temperatures of 145°F., 150°F., and even as high as 160°F., for 30 minutes. The question immediately arises whether the high temperatures are justifiable when perhaps the same efficiency may be obtained at a lower temperature at less cost.

Some state laws require the pasteurization of the mix at 150°F. for 30 minutes in order to allow an ample margin of safety for the protective action rendered by the mix ingredients. Other states require 145°F. for 30 minutes obviously on the assumption that this allows an ample margin of safety.

The purpose of this investigation has been to determine the bacterial destruction at various temperatures using different holding periods and also to study the types of organisms which survive the different exposures employed.

REVIEW OF LITERATURE

In a discussion of the pasteurization temperatures for ice cream mixes there are three points of major importance to consider. First, the bacterial destruction at the temperature employed; second, the effect of such heating on flavor, whipping ability, freezing time, and overrun of the mix; and third, the cost and time consumed at the temperature used.

Dahle and Barnhart (6) studied the effect of different pasteurizing and homogenizing temperatures on certain properties of the mix. They found that high pasteurizing and homogenizing temperatures (170°F. - 180°F.) decreased the degree of fat clumping, reduced the viscosity and freezing time, and increased the protein stability of the mixes. They concluded that the greatest benefits were derived from the use of high pasteurizing and homogenizing temperatures. They also found that the differences obtained between 170°F. for 10 minutes and 180°F. for 1 minute were not sufficient to warrant using higher pasteurization temperatures. No work was reported on bacterial destruction.

Using pasteurization temperatures of 150°F. for 30 minutes, 160°F. for 20 minutes, and 170°F. for 10 minutes, Dahle, Keith and McCullough (7) found that the mixes pas-

teurized at the higher temperatures had lower viscosities and whipped faster than those pasteurized at the lower temperatures.

Martin, Swope and Knapp (14) using the temperatures 145°F., 150°F., and 160°F. for holding periods ranging from 15 minutes to 2 hours, found that the overrun obtained and the length of time required for freezing was practically the same for all temperatures and holding periods and that there was no definite superiority of one over the other. The higher temperatures and longer holding periods gave the greatest bacterial destruction, although 145°F. for 20 minutes brought about a marked reduction. The highest temperatures were most effective in destroying bacteria of the alkali forming type but the weak acid group survived in large numbers.

These results are in agreement with those of Olson (18) and Hening (13) who confirm the opinion that higher temperatures are as desirable if not more so from a manufacturing standpoint.

The bacteriological aspect of the question of pasteurization is approached from quite a different angle. One must consider the destruction of pathogenic bacteria, as well as the destruction of organisms which may bring about undesirable changes in the mix, such as souring and off flavors.

The thermal death points of various pathogenic organisms in cream and ice cream have been determined by Oldenbusch, Frabischer and Shrader (16). They used one culture of B. diphtheriae, two strains of B. typhosus, two of beta type hemolytic streptococci, and a culture of tubercle bacillus of the bovine type. In their experiments B. typhosus and the streptococci were killed in three minutes at both 145°F. and 150°F., B. diphtheriae was destroyed in less than one minute at these temperatures, while M. tuberculosis was killed in five minutes at 145°F., and in two minutes at 150°F. They conclude that the pasteurization temperature recommended by the Committee on Dairy Products and Eggs, American Public Health Association (143.5°F. for 30 minutes) is sufficiently high to allow an ample margin of safety.

The data given above would tend to show that pathogenic bacteria are as readily destroyed in ice cream mixes as they are in milk, contrary to the concept that the mix ingredients render a protective action to the organisms.

In a study of the thermal death points of pathogenic bacteria in milk, Park (19) stated that all pathogenic organisms commonly found in milk except the tubercle bacilli and the streptococci are destroyed by exposures of 140°F. for 10 minutes. His work showed that 140°F. for 20 minutes uniformly destroyed the tubercle bacilli and that no strep-

tococci were able to survive 138°F. for 30 minutes. This work confirms the results obtained by Sternberg, Newman, Rosenau, and Davis who worked with the common pathogenic bacteria found in milk (cited by Park (19)).

Meanwell (15), however, concludes from his work on milk naturally infected with M. tuberculosis, that 145°F. for 30 minutes does not always kill this organism, although in most cases this exposure is effective. He is of the opinion that the proteins in milk do not afford the same degree of protection to organisms artificially added.

Ayers and Johnson (1) carried on an extensive study of the types of organisms found in retail ice cream in Washington, D. C. They used the "milk tube" method of differentiating the organisms and secured the following results:

TABLE I

TYPES OF BACTERIA IN SUMMER AND WINTER ICE CREAM *

Types of Bacteria	Percentage Distribution	
	71 Summer Samples	28 Winter Samples
1. Acid coagulating	49.82	30.84
2. Acid forming	20.72	38.03
3. Inert	13.98	4.81
4. Alkali	1.86	5.42
5. Peptonizing	13.62	20.90

* (Adapted from Ayers and Johnson (1)).

In summer the acid group comprised 70.54 per cent of the total number of bacteria in ice cream, while in winter this group comprised 68.87 per cent of the total flora.

These same investigators (2) made an extensive study of the bacterial flora of three grades of market milk before and after pasteurization at 145°F. for 30 minutes. A brief summary of part of their work is given in Table II.

TABLE II

DISTRIBUTION OF VARIOUS TYPES OF BACTERIA IN DIFFERENT GRADES OF MILK BEFORE AND AFTER PASTEURIZATION

Type of Organism	Grade A (poor)		Grade B (fair)		Grade C (good)	
	Before P.	After P.	Before P.	After P.	Before P.	After P.
Acid forming	10.71	61.87	9.74	34.87	6.85	61.25
Acid coagulating	36.17	17.91	12.98	31.89	33.85	11.85
Inert	29.31	9.06	43.57	24.00	43.13	22.06
Alkali forming	6.47	9.77	19.66	5.63	3.33	0.36
Peptonizing	17.31	1.39	14.10	3.59	12.81	4.47

Adapted from Ayers and Johnson (2).

These figures indicate that in all grades of milk the total acid group comprised by far the greatest percentage of all the organisms surviving pasteurization. This was contrary to the concept prevalent at that time. Prior to 1913 it was rather generally believed that only the spore forming, peptonizing types of organisms could withstand pasteurization, and that milk subjected to this process would not sour but would undergo proteolytic decomposition unless

lactic acid organisms gained entrance after pasteurization. The thermal death points of lactic acid organisms had been stated to be below 145°F. for 30 minutes, but this work showed conclusively for the first time, that a large number of lactic acid bacteria could survive pasteurization.

Another point of interest is the very marked reduction of the peptonizing group, whereas it was formerly believed that a larger percentage of this type survived pasteurization than any other.

In regard to the number of acid forming groups before and after pasteurization, the authors said; "The percentage of total acid group was greater in raw milk after two days incubation than in pasteurized. After five days, however, the total acid group was higher in percentage in pasteurized milk. After 14 days the total acid group in pasteurized milk was much larger. This shows that the bacteria of the acid group in pasteurized milk develop slower or at least produce acid slower, than those in raw milk".

While this work did not deal with ice cream, it throws some light on the results which may be expected in pasteurizing mixes since milk and milk products are the chief constituents of the mix.

Black, Prouty, and Graham (4), in studying the effect of pasteurization on the bacterial flora of low count milk, obtained results quite different from those of Ayers and

Johnson. They found that the acid group was markedly reduced and that the alkali forming and inert group constituted by far the largest percentage of the flora after pasteurization. They attributed the difference to the use of milk with a lower bacterial count in their experiments.

Investigations as to the percentage destruction of organisms at different pasteurizing temperatures show quite different results, although the amount of work published seems to be rather limited.

Hammer and Sanders (12), who were among the first investigators to determine the percentage destruction of bacteria in ice cream mixes, found that heating the mix to 145°F. destroyed from 91.5 to 99.5 per cent of the organisms, whereas if held 20 minutes the counts were decreased to less than 10,000 bacteria per c.c. in all trials except one.

Fabian and Cromley (8), in studying the influence of manufacturing operations on the bacterial content of ice cream, found that heating the mix to 150°F. for 30 minutes reduced the count of the raw mix to below 50,000 in all cases. The percentage destruction was from 96.5 to 99.9 per cent.

In discussing pasteurization temperatures for ice cream mixes, Fabian (9) stated that, "while 145°F. for 30 minutes is usually satisfactory, plants desiring to consistent-

ly produce ice cream with a low bacterial content should use 150°F. for 30 minutes".

Fay and Olson (11) studying the factors affecting the bacterial count of ice cream found that 150°F. for 30 minutes resulted in greater efficiency than lower temperatures. They found that out of 28 trials there were nine in which the percentage efficiency was less than 99 per cent. In six of these nine instances the pasteurization temperature was below 145°F. for 30 minutes and in two instances the results seemed to be erroneous. It seemed evident that 145°F. for 30 minutes or above had a distinct advantage. They also found that pasteurization destroyed 99 per cent of the acid producers, 99.986 per cent of the acid and gas producers, and 86 per cent of the gelatine liquefiers.

Later work by the same authors (17), working with six commercial plants representative of many small plants in Kansas, led them to the following conclusions on pasteurization. "Pasteurization at 145°F. for 30 minutes and homogenizing at pasteurization temperature results in counts of less than 100,000 bacteria per gram in the finished ice cream, provided equipment contamination is reduced to a minimum.

"If the bacterial flora of the mix is constituted of types easily destroyed by heat, high efficiency of pasteurization may be obtained in less time.

"Extremely high pasteurization temperatures are unnecessary in producing ice cream of a low bacterial count".

In the experiments mentioned the different pasteurization temperatures were not tried on the same mix to determine the relative efficiencies of each temperature. This would be the logical procedure in deciding whether or not one temperature and time combination was superior to another, because of the variability in the bacterial flora of different mixes.

PLAN OF EXPERIMENT

With the introduction of the various pasteurization temperatures and holding periods now in use there has arisen a demand for more definite information in regard to the efficiency of the various temperatures used in bacterial destruction. In the literature no work was found in which the same mix has been subjected to different temperatures and holding periods to measure the pasteurization efficiency. Obviously the effect of a given pasteurization exposure on the bacterial flora of different ice cream mixes is variable.

In this experiment a standard ice cream mix was subjected to various temperatures and holding periods. Total and differential counts were made before and after pasteurization in an attempt to determine which types were destroyed. The characteristic of the organisms surviving pasteur-

ization were also studied.

In other experiments sterile ice cream mixes inoculated with pure cultures were subdivided and subjected to various exposures.

Preparation of Mixes. A standard ice cream mix containing 12 per cent fat, 10 per cent serum solids, 15 per cent sugar, and 0.4 per cent gelatine was prepared from either pasteurized or raw ingredients as indicated for each mix. Small mixes of five pounds each were made for the first laboratory studies, and later, larger mixes of 100 pounds each were prepared under plant conditions. Sweetened condensed milk, skim milk, sweet cream, cane sugar and gelatine were used in making all the mixes. The gelatine was added to the skim milk and heated to 110°F. and then combined with the other ingredients. The mixes were held in the cooler at 40°F. More than one complete pasteurization trial was run on a single mix.

Pasteurization of the Mixes. The pasteurization exposures used in the trials were as follows:

145°F. for 20, 25, and 30 minutes.
150°F. for 20, 25, and 30 minutes.
160°F. for 10, 15, and 20 minutes.
170°F. for 2, 5, and 10 minutes.
180°F. flash.

Unless otherwise indicated, all the above temperatures

and time intervals were run on a mix at the same time, which will be referred to as a "complete trial".

The raw mix was first thoroughly agitated and plate counts were made using the dilutions 1-1,000, 1-10,000, 1-100,000, and 1-1,000,000. By means of a sterile pipette, 10 c.c. portions of the mix were transferred to a series of large test tubes. Four of these tubes were prepared for each temperature, one for each time interval and one for the thermometer. By means of a small water bath, which could be quickly heated, the four tubes were brought to the desired temperature, as indicated by the thermometer in the check tube. When the mix had reached the desired temperature all four tubes were transferred to a constant temperature bath regulated to the desired temperature, ±1C. At the end of each holding period a tube was removed from the bath and plates were made immediately.

The same procedure was carried out for all the temperatures and time intervals except 180°F. In this case the mix was heated by immersing small tubes of mix in boiling water until 180°F. was reached. The sample was removed and dilutions prepared immediately.

Method of Plating. The methods recommended by the American Dairy Science Association (5) were closely followed throughout these trials. Bacto Nutritive Caseinate Agar was used for the differential counts. This medium is a

modification of the milk powder agar described by Ayers and Mudge (3) and is claimed to give results closely comparable to the original agar. Brom-cresol purple indicator (0.8 per cent) was added to the medium at the time of sterilization.

Duplicate plates were poured with each medium. Extract agar plates were prepared, incubated, and counted as described by the American Dairy Science Association (5).

Caseinate agar plates were first counted after 48 hours incubation to record the "rapid acid producers" and the peptonizers. At the end of five to seven days the slow acid formers and the alkali formers and inert group were counted on the next higher dilution plate. Usually the first count was made on the 1-100 dilution plate and the final count on the 1-1,000 dilution plate.

RESULTS OF EXPERIMENTS

It was found by preliminary experiments that 80°F. was the most suitable temperature for the differentiation of types with the medium employed. At this temperature the acid groups showed up much more clearly. The trouble encountered when the plates were incubated at 98°F. was that the spreaders, which were numerous in the pasteurized mixes, produced sufficient alkali to render the entire plate alkaline, thereby making it impossible to differentiate between the acid and inert groups.

Various buffers added to the media did not seem to remedy this trouble. The retardation of growth of alkali formers at 80°F. lessened the tendency for the diffusion of alkali throughout the medium, and hence facilitated the identification of acid formers. Incubation at this temperature reduced the size of colonies formed by proteolytic types although they were readily distinguishable.

The data in Table III are typical of the results obtained when the plates were counted after 48 hours incubation at 98°F.

Results of duplicate plates such as those obtained when the mix was pasteurized at 150°F. for 25 minutes, and 145°F. for 30 minutes show that when 98°F. was employed for incubation a reliable differential count could not be obtained. This fact was also very evident on examination of the plates.

In an effort to obtain a more accurate differential count an experiment was carried out in which the plates were incubated at a lower temperature. A mix was pasteurized at 160°F. for 10, 15, and 20 minutes, and at 170°F. for 2, 5, and 10 minutes. Two sets of duplicate plates were poured, one set of which was incubated at 80°F. and the other at 98°F. The plates were examined at 24 hour intervals and the results as in Table IV were recorded.

TABLE III

DIFFERENTIAL COUNT OBTAINED WHEN PLATES WERE INCUBATED AT 98°F. FOR 48 HOURS

Pasteurization Temperature °F.	Time Min.	CASEINATE AGAR Total	Acid	Peptonizer	Alkali and Inert
Raw		167,000 107,000	90,000 70,000	1,000 1,000	67,000 23,000
165	20	8,500 8,200	4,000	300-300	
165	25	5,700 4,900	3,300 900	600-900	
165	30	5,500 7,900	200 2,800	500-500	
145	20	11,900 14,900	9,600 11,300	200-100	
145	25	13,900 14,800	2,700	400-700	
145	30	13,700 13,800	4,200 10,400		
160	10	6,400	4,800	500-400	
160	15	5,500	3,600	700-500	
160	20	5,500	4,300	600-1900	
170	2	3,000 3,500	1,600	600	
170	5	1,300 900	100	400	
170	10	1,000 1,500	100	400	
180		1,500		300	

TABLE IV

A COMPARISON OF COUNTS OBTAINED WHEN PLATES ARE INCUBATED AT 98°F. AND 80°F.

Temperature °F.	Time Min.	98°F. Count	98°F. Acid	98°F. Peptonizers	80°F. Count	80°F. Acid	80°F. Peptonizers
Raw					180,000 130,000	140,000 90,000	
160	10	2,500 2,600	-	* *	2,000 1,400	-	* *
160	15	2,700 2,300	-	* *	1,700	-	* *
160	20	2,300 1,800	-		2,500 2,200	-	
170	2	1,500 2,300	-	*	2,600 2,200	-	*
170	5	2,500 1,600	-	*	2,700 1,800	-	*
170	10	2,600 2,600	-	*	2,900 1,900	-	*
48 Hours							
170	2	2,300 1,800	-	1,600 1,000	27,600 19,800	1,300 -	1,300 1,700
170	5	2,100 1,600	-	1,300 1,400	13,500 10,700	- -	700 400
170	10	3,400 2,700	-	2,900 2,200	14,000 10,900	-	2,100 1,200
160	10	7,300 5,400	-	1,900 2,000	23,700 30,600	-	1,300 1,700
160	15	2,900 2,300	-	1,600 1,800	18,300 22,200	-	1,900 2,000
160	20	2,100 2,700	-	1,200 1,800	6,000	-	500
Raw		220,000 150,000	50,000 30,000	-	430,000 390,000	140,000 160,000	-

* Proteolytic counts not determined.

The spreaders were very large on the plates incubated at 98°F. and the medium was distinctly alkaline over the entire area of the plate. The medium on the plates incubated at 80°F. showed no change in reaction.

In addition this table brings out two points of interest. First, that apparently no acid formers survived pasteurization and second, that much higher counts were obtained when the plates were incubated at 80°F. Although the counts at the end of 24 hours were practically the same at each incubation temperature, after an additional 24 hours the plates at 80°F. showed a very large increase whereas at 98°F. the counts remained about the same. The organism responsible for this increase produced a rather small elliptical or round colony, showing no change in reaction at the end of 48 hours, but distinct acidity after 96 hours or longer.

This type of colony was frequently encountered in these trials, and was especially numerous on plates made from mixes composed of pasteurized ingredients. Since these types of organisms were found in practically all the mixes and constituted the largest percentage of the bacteria surviving pasteurization, a few of their characteristics will be noted at this time. A rather detailed study of the organisms was made which will be presented later.

The fact that growth was better at 80°F. than at 98°F. partially accounts for the lower counts obtained when the higher temperatures were employed. It was found that when these organisms were isolated in pure culture they grew slightly better on caseinate agar than on extract agar, although the growth was quite abundant on the latter. Since they produced acid very slowly, they were designated as "slow acid" formers to distinguish them from the "rapid acid formers" which showed distinct acidity to brom-cresol purple after 48 hours incubation.

The fact that this particular group of organisms grew slowly necessitated incubation of the plates five days before a final count was made.

The data in Tables V and VI show the difference in counts after 48 and 120 hours incubation. The count after 48 hours was made on the 1-100 dilution, whereas the count after 96 hours was made on the 1-1,000 dilution plates.

The data in Table V represent the average of three trials in which the plates were counted after 48 hours and again after five days. The results in Table VI are an average of four complete trials made on a single mix on different days.

At the end of 48 hours it appears as if no acid formers survived pasteurization, whereas if the plates were incubated 96 to 120 hours most of the colonies which at first ap-

TABLE V

TOTAL AND DIFFERENTIAL COUNTS AFTER 48
AND 120 HOURS INCUBATION
(Average of Three Trials)

Pasteurization Temperature °F.	Time Min.	48 HOURS					5 DAYS				
		Total Count	Acid Formers		Peptonizer	Alkali and Inert	Total Count	Acid Formers		Peptonizer	Alkali and Inert
			Rapid	Slow				Rapid	Slow		
Raw		61,666	15,000	-	3,160	-	81,666	-	43,333	-	-
145	20	55,000	-	-	1,440	53,560	62,000	-	51,000	-	-
145	25	53,142	-	-	1,466	51,676	78,000	-	68,666	-	-
145	30	39,328	-	-	1,550	37,778	77,000	-	65,550	-	-
150	20	35,211	-	-	1,416	33,795	58,000	-	32,000	-	-
150	25	35,730	-	-	1,760	33,970	61,333	-	39,333	-	-
150	30	34,657	-	-	1,883	32,774	72,666	-	64,606	-	-
160	10	47,442	-	-	1,433	46,009	60,000	-	53,000	-	-
160	15	46,416	-	-	1,616	54,800	74,333	-	64,666	-	-
160	20	36,642	-	-	1,516	35,126	69,000	-	47,750	-	-
170	2	39,266	-	-	1,333	37,933	65,500	-	52,750	-	-
170	5	40,080	-	-	1,366	48,742	71,750	-	58,250	-	-
170	10	28,650	-	-	1,366	27,284	62,500	-	52,000	-	-
180		19,533	-	-	1,666	17,867	45,800	-	35,400	-	-

TABLE VI

TOTAL AND DIFFERENTIAL COUNTS AFTER 48
AND 120 HOURS INCUBATION
(Average of Four Trials)

Pasteurization Temperature °F.	Time Min.	48 HOURS Total Count	48 HOURS Acid Formers Rapid	48 HOURS Acid Formers Slow	48 HOURS Peptonizer	48 HOURS Alkali and Inert	5 DAYS Total Count	5 DAYS Acid Formers Rapid	5 DAYS Acid Formers Slow	5 DAYS Peptonizer
Raw		1,647,000	33,000	-	108,000		5			
145	20	41,644	-	-	1,216		51,250	-	36,500	
145	25	36,287	-	-	810		45,250	-	31,625	
145	30	38,157	-	-	714		49,500	-	36,625	
150	20	33,800	-	-	900		47,666	-	31,000	
150	25	36,487	-	-	680		37,000	-	25,666	
150	30	32,712	-	-	700		51,000	-	35,600	
160	10	28,000	-	-	880		40,333	-	29,000	
160	15	28,988	-	-	842		35,873	-	25,166	
160	20	22,971	-	-	783		26,000	-	15,400	
170	2	22,600	-	-	500		30,750	-	29,333	
170	5	22,828	-	-	757		31,166	-	24,000	
170	10	19,100	-	-	437		29,857	-	24,000	
180		30,242	-	-	480		33,666	-	23,333	

peared to be inert showed definite acid formation. To check the accuracy of the indicator, typical organisms of this type were transferred to litmus milk and it was found they produced sufficient acid to coagulate litmus milk in from 7 to 14 days. Although acid production was slow, sufficient acid was produced to coagulate milk.

Results of Pasteurizing the Mixes Made From Ingredients Which Had Been Previously Pasteurized

The data in Tables VII to XII, inclusive, show the results obtained by pasteurizing different mixes at the various temperatures and time intervals. Each table is the average of two or more complete trials on a single mix as indicated at the heading of the table.

The figures given in Table VII are an average of three trials run on a mix of standard composition, which had been made from pasteurized skim milk and cream, sweetened condensed skim milk, sugar, and gelatine. In this particular trial the extract agar had become contaminated, consequently there is no comparison to make with the caseinate agar.

The extreme left hand column of the table shows the pasteurization temperatures and times. In the second and third columns are the counts and percentage destruction as shown by the standard plate method. In column four is given the total count obtained on caseinate agar and in successive

TABLE VII

EFFECT OF VARIOUS PASTEURIZATION EXPOSURES ON THE TOTAL AND DIFFERENTIAL COUNT OF AN ICE CREAM MIX
(Average of Three Trials)

Pasteurization Temperature °F.	Time Min.	EXTRACT AGAR Count	EXTRACT AGAR Per cent destruction	CASEINATE AGAR Total Count	CASEINATE AGAR Acid Formers Rapid	CASEINATE AGAR Acid Formers Slow	CASEINATE AGAR Peptonizer	CASEINATE AGAR Alkali and Inert	CASEINATE AGAR Per cent destruction	Per Cent of Types Rapid	Per Cent of Types Slow	Per Cent of Types Peptonizer	Per Cent of Types Alkali and Inert
Raw				81,666	15,000	43,333	3,160	20,173	-	18.36	53.06	3.86	-
145	20	*		62,000	-	51,000	1,440	9,560	24.08	-	82.25	2.32	15.43
145	25	*		78,000	-	68,666	1,466	7,868	4.48	-	88.03	1.87	10.10
145	30	*		77,000	-	65,550	1,550	9,900	5.71	-	85.06	2.01	12.93
150	20	3,400		58,000	-	32,000	1,416	24,584	28.97	-	40.00	2.41	57.56
150	25	5,400		61,333	-	39,333	1,760	20,243	24.89	-	64.12	2.86	33.02
150	30	3,800		72,666	-	64,666	1,883	6,117	11.02	-	88.99	2.59	8.42
160	10	10,700		60,000	-	53,000	1,433	5,567	26.53	-	88.33	2.38	9.29
160	15	4,200		74,333	-	64,666	1,616	8,051	8.97	-	86.99	2.17	15.18
160	20			69,000	-	47,750	1,516	19,734	15.50	-	69.20	2.19	28.61
170	2	*		65,500	-	52,750	1,333	11,417	19.79	-	80.45	2.03	17.52
170	5	*		72,750	-	58,250	1,366	12,134	12.14	-	81.18	1.90	16.92
170	10	*		62,500	-	52,000	1,366	9,134	23.46	-	83.20	2.18	14.62
180		4,900		56,133	-	38,900	1,750	15,483	31.26	-	69.29	3.11	27.60

* Contaminated

columns are recorded the numbers of rapid acid producers, slow acid producers, peptonizers, and alkali forming and inert groups. The percentage destruction as shown by the caseinate agar plates and the percentages of each group present in the mix before and after pasteurization are shown in the columns on the right of the table.

It will be seen by observing the counts in Table VII that in this mix the percentage destruction is very low, ranging from 4.5 to 31.0 per cent. This is to be expected since the mix was made from pasteurized ingredients and the organisms present had survived the previous pasteurization.

Further, it will be observed in column four, that none of the temperatures nor time intervals employed yielded results sufficiently different to be measurable by the plate count. That is to say, all the counts after pasteurization are well within the limits of error inherent to the plate count.

As to the types surviving pasteurization it is seen that in no instance did any of the rapid acid producers survive, whereas there was no reduction of the slow acid producing group. The same is true in Tables VIII, IX and X, based on mixes made from pasteurized ingredients and in which the rapid acid producers constituted but a minor portion of the total flora of the raw mix.

TABLE VIII

EFFECT OF VARIOUS PASTEURIZATION EXPOSURES ON THE TOTAL AND DIFFERENTIAL COUNT OF AN ICE CREAM MIX
(Average of Three Trials)

Pasteurization Temperature °F.	Time Min.	EXTRACT AGAR Count	Per cent de-struction	CASEINATE AGAR Total Count	Acid Formers Rapid	Acid Formers Slow	Peptonizer	Alkali and Inert	Per cent de-struction	Per Cent of Types Rapid	Per Cent of Types Slow	Per Cent of Types Peptonizer	Per Cent of Types Alkali Inert
Raw		23,000	-	208,250*					-				
145	20	3,970	82.73	53,500	-	29,500	1,600	22,400	74.30	-	55.14	2.99	41.87
145	25	3,650	84.13	45,000	-	15,000	1,550	28,450	78.39	-	33.33	3.44	63.23
145	30	2,737	88.10	38,000	-		1,750		81.75	-	-	4.60	
150	20	3,050	86.73	31,000	-	17,000	2,700	11,300	85.11	-	54.83	8.70	36.47
150	25	2,750	88.04	29,000	-	16,000	1,700	11,300	86.07	-	55.17	5.86	38.97
150	30	2,970	87.08	31,000	-	18,000	1,810	11,150	85.11	-	58.06	5.96	35.98
160	10	3,728	83.79	39,000	-	27,333	1,650	10,017	81.27	-	70.08	4.23	25.69
160	15	2,416	89.49	33,750	-	21,666	1,800	10,314	83.79	-	64.19	5.34	30.47
160	20	2,320	89.91	24,000	-	14,500	1,150	8,350	88.47	-	60.41	4.79	34.80
170	2	2,650	88.47	35,000	-	11,500	1,800	21,700	83.19	-	32.85	5.14	62.01
170	5	2,333	89.85	34,250	-	15,000	1,350	17,900	83.55	-	43.79	3.94	52.27
170	10	2,333	89.85	30,000	-	16,333	1,350	12,317	85.59	-	54.44	4.50	40.16
180		3,166	86.23										

* Alkali formers on the plate made it impossible to make a differential count.

TABLE IX

EFFECT OF VARIOUS PASTEURIZATION EXPOSURES ON THE TOTAL AND DIFFERENTIAL COUNT OF AN ICE CREAM MIX
(Average of Four Trials)

Pasteurization Temperature °F.	Time Min.	EXTRACT AGAR Count	Per cent destruction	CASEINATE AGAR Total Count	Acid Formers Rapid	Acid Formers Slow	Peptonizer	Alkali and Inert	Per cent destruction	Per Cent of Types Rapid	Per Cent of Types Slow	Per Cent of Types Peptonizer	Per Cent of Types Alkali and Inert
Raw		1,452,500	-	1,647,000	33,000	*	108,800	*		200		6.60	
145	20	48,871	96.63	51,250	-	36,500	1,216	13,534	96.88	-	71.21	2.37	26.42
145	25	19,812	98.63	45,250	-	31,625	810	12,815	97,25	-	69.88	1.79	28,33
145	30	27,412	98.11	49,500	-	36,625	714	12,161	96.99	-	73.98	1.44	25.38
150	20	19,783	98.63	47,666	-	31,000	900	15,766	97.10	-	65.03	1.88	33.09
150	25	22,337	98.46	37,000	-	25,666	680	9,654	97.75	-	69.36	1.83	28.81
150	30	26,685	98.16	57,000	-	35,600	700	14,700	96.90	-	69.80	1.37	28.83
160	10	26,200	98.16	40,333	-	29,000	880	10,453	97.55	-	71.90	2.18	25.92
160	15	17,357	98.80	35,833	-	25,166	842	9,825	97.82	-	70.23	2.34	27.43
160	20	18,087	98.79	26,000	-	15,400	783	9,817	98.42	-	59.23	3.01	46.76
170	2	21,842	98.49	30,750	-	29,333	500	917	98.13	-	95.39	1.62	2.99
170	5	17,912	98.79	31,166	-	24,000	757	6,409	98.08	-	77.00	2.42	20.58
170	10	15,325	98.94	29,859	-	24,000	437	5,420	98.21	-	80.38	1.46	18.16
180		24,562	98.30	33,666	-	23,333	480		97.95	-	69.30	1.42	29.28

* (Could not be determined)

TABLE X

EFFECT OF VARIOUS PASTEURIZATION EXPOSURES ON THE TOTAL AND DIFFERENTIAL COUNT OF AN ICE CREAM MIX
(Average of Four Trials)

Pasteurization Temperature °F.	Time Min.	EXTRACT AGAR: Count	EXTRACT AGAR: Per cent destruction	CASEINATE AGAR: Total Count	CASEINATE AGAR: Acid Formers: Rapid	CASEINATE AGAR: Acid Formers: Slow	CASEINATE AGAR: Peptonizer	CASEINATE AGAR: Alkali and Inert	CASEINATE AGAR: Per cent destruction	Per Cent of Types: Rapid	Per Cent of Types: Slow	Per Cent of Types: Peptonizer	Per Cent of Types: Alkali and Inert
Raw		516,400		13,099,500	1,052,788	*	*	*	-	7.89			
145	20	48,616	90.58	57,500	-	41,833	560	17,107	99.56	-	72.75	.97	26.28
145	25	56,916	88.97	62,400	-	48,800	450	13,150	99.52	-	78.20	.72	21.08
145	30	47,550	90.75	56,666	-	42,500	433	13,733	99.56	-	75.00	.76	24.24
150	20	43,400	91.59	62,833	-	44,500	625	17,708	99.52	-	70.82	.99	28.19
150	25	68,000	86.83	57,500	-	38,250	775	18,475	99.56	-	66.52	1.34	32.14
150	30	45,400	91.20	53,800	-				99.58	-	-	-	-
160	10	42,060	92.04	50,833	-	37,500	425	12,908	99.61	-	73.77	.83	25.40
160	15	54,933	89.36	72,500	-	52,166	500	19,834	99.44	-	71.95	.68	27.37
160	20	43,616	91.55	38,833	-	29,500	620	8,713	99.70	-	75.96	1.59	22.45
170	2	54,140	87.57	53,166	-	38,833	400	13,933	99.58	-	73.04	.75	26.21
170	5	36,850	92.86	41,800	-	32,000	520	9,087	99.68	-	77.03	1.24	21.72
170	10	36,010	93.02	41,000	-	25,800	600	14,600	99.68	-	62.91	1.46	35.63
180		34,650	93.29	52,400	2,400	43,880	240	8,180	99.59	4.58	83.74	.64	15.62

* (Could not be determined)

The peptonizing and the alkali forming and inert group showed but slight reduction in numbers after pasteurization, the percentage surviving being very constant at each temperature and time interval employed. It will be observed in Table VII that the slow acid formers constitute the largest per cent of the organisms surviving pasteurization. In only one instance did this group constitute less than 50 per cent and in many instances more than 80 per cent of the flora after pasteurization.

The peptonizing group constitutes but a minor part of the flora whereas the alkali forming and inert group make up from 10 per cent to 30 per cent of the flora.

Table VIII presents much the same picture as Table VII, as far as the differential counts are concerned. The count on the raw mix is somewhat higher, and the counts after pasteurization slightly lower than for the mix represented in Table VII, although it will be observed that the rapid acid producers were completely destroyed. From 32.8 per cent to 70.0 per cent of the flora after pasteurization consists of the slow acid formers.

A comparison of the extract and caseinate agar counts shows that the latter are approximately ten times as high as the former.

The mix represented in Table IX had a considerably higher count than either of those represented in Tables VII

and VIII and also showed a much greater percentage destruction after pasteurization. However, the counts and the flora after pasteurization are approximately the same as those in the two preceding tables.

In these trials, the extract agar plates were incubated at 80°F. and counted after five days incubation. It will be seen that the counts are approximately the same on both media.

The data in Table X show that this mix had a very high initial count. Of the 13 million bacteria in the raw mix, only one million were of the rapid acid producing type, none of which survived pasteurization. The counts after pasteurization were much the same as those reported in the three previous tables, and the predominating type after pasteurization was the slow acid formers. The percentage destruction was very high in all cases, ranging from 99.5 to 99.7 per cent.

In these trials the extract agar plates as well as the caseinate agar plates were incubated at 80°F. It will be seen that there is very little difference in counts obtained with the two media.

Results of Pasteurizing Mixes Made From Raw Dairy Products

The results shown in Table XI are an average of two

TABLE XI

EFFECT OF VARIOUS PASTEURIZATION EXPOSURES ON THE TOTAL AND DIFFERENTIAL COUNT OF AN ICE CREAM MIX
(Average of Two Trials)

Pasteurization Temperature °F.	Time Min.	EXTRACT AGAR Count	Per cent destruction	CASEINATE AGAR Total Count	Acid Formers Rapid	Acid Formers Slow	Peptonizer	Alkali and Inert	Per cent destruction	Per Cent of Types Rapid	Slow	Peptonizer	Alkali and Inert
Raw		192,000,000		370,000,000	356,000,000					94.68		3.72	
145	20	10,650	99.994	37,750	28,500	-	1,225	8,025	99.986	75.49	-	3.24	21.27
145	25	9,375	99.995	9,100	2,725	-	1,150	5,225	99.998	30.27	-	12.63	57.10
145	30	7,825	99.995	4,125	750	-	1,300	2,075	99.998	18.18	-	32.96	48.86
150	20	4,300	99.9985	3,050	250	-	1,100	1,900	99.998	8.19	-	36.06	55.75
150	25	4,050	99.9987	2,950	50	-	700	2,200	99.9989	1.69	-	23.72	74.59
150	30	3,450	99.998	2,900	250	-	850	1,800	99.9990	8.62	-	29.31	62.07
160	10	3,750	99.998	3,850	-	-	1,150	2,700	99.998	-	-	29.87	70.13
160	15	4,600	99.998	3,750	-	-	900	2,850	99.998	-	-	24.00	76.00
160	20	3,800	99.998	2,880	-	-	1,200	1,650	99.999	-	-	42.10	57.90
170	2	6,850	99.997	3,850	-	175	1,000	2,675	99.998	-	-	25.97	74.03
170	5	3,500	99.998	3,425	-	-	1,375	2,050	99.998	-	-	40.14	59.86
170	10	5,700	99.998	3,425	-	-	1,075	2,350	99.998	-	-	31.38	68.62
180	1	15,000	99.994	10,200	4,500	-	1,200			44.11	-	11.76	44.13
180	Instant			42,200,000					88.64		-		

trials run on a mix made of raw ingredients. It will be seen that the count on the raw mix is extremely high and consists mostly of the rapid acid producing group. This is quite in contrast to the data shown in Tables VII, to X, inclusive.

At all pasteurization temperatures and time intervals employed, it will be noticed that there was a tremendous decrease in counts, although it is evident that noticeably less destruction took place when heated at 145°F. for 20 minutes than at the other temperature. The other counts in column four are not significant since they are within the limit of error inherent to the plate count.

It is interesting to note from Table XI that after pasteurization none of the slow acid formers survived. The number of slow acid formers in the raw mix could not be determined because of the high dilution requisite for plating. Consequently,it is not known whether this group was destroyed by pasteurization or was not present in the original mix.

At 145°F. for 20 and 25 minutes many rapid acid formers survived but at all other temperatures and time intervals employed this group was uniformly reduced to a negligible number. This is to be expected since the rate of destruc- is a function of time, temperature, and the number of organisms present. With such a large number of rapid acid producers present, the lower temperature and shorter holding period did not destroy them as completely as the higher

temperatures.

In all instances the peptonizers were reduced to a relatively constant figure as were also the alkali forming and inert group.

The rapid acid producers constituted a considerable part of the flora that survived 145°F. for 20 minutes. At the other temperatures, the flora was divided between the peptonizing and the alkali forming and inert group, with no slow acid producers present.

The extract agar counts and the caseinate agar counts were approximately the same on this mix.

The data in Table XII which are an average of seven complete trials run on a mix made of raw ingredients, presents much the same picture as did Table XI. That is, a high count on the raw mix, and a flora consisting largely of the rapid acid producing group, and a uniformly high destruction at all temperatures and time intervals. No slow acid producers were found to survive pasteurization. The types after pasteurization consisted of approximately equal numbers of the peptonizing group and the alkali forming and inert groups.

A very striking difference was noted in the results obtained from pasteurizing mixes made of raw ingredients and mixes made of dairy products which had been previously pasteurized. A comparison of the data in Tables V to XII pre-

TABLE XII

EFFECT OF VARIOUS PASTEURIZATION EXPOSURES ON THE TOTAL AND DIFFERENTIAL COUNT OF AN ICE CREAM MIX
(Average of Seven Trials)

Pasteurization Temperature °F.	Time Min.	EXTRACT AGAR: Count	Per cent destruction	CASEINATE AGAR: Total Count	Acid Formers: Rapid	Slow	Peptonizer	Alkali and Inert	Per cent destruction	Per Cent of Types: Rapid	Slow	Peptonizer	Alkali and Inert
Raw		999,142	-	3,516,428	1,019,285	-	41,107	2,456,036		28.98	-	1.16	69.86
145	20	4,314	99.56	5,607	-	-	1,323	4,284	99.84	-	-	23.59	76.41
145	25	3,985	99.60	4,463	-	-	1,600	2,863	99.87	-	-	35.85	64.15
145	30	3,957	99.60	4,171	-	-	1,571	2,600	99.88	-	-	37.66	62.34
150	20	4,478	99.55	4,242	-	-	1,478	2,764	99.87	-	-	34.84	65.16
150	25	3,564	99.64	3,992	-	-	1,615	2,377	99.88	-	-	40.45	59.55
150	30	4,114	99.58	3,758	-	-	1,500	2,258	99.89	-	-	39.91	60.09
160	10	3,675	99.63	2,916	-	-	1,200	1,716	99.91	-	-	41.15	58.85
160	15	3,458	99.65	3,318	-	-	1,490	1,828	99.90	-	-	44.90	55.10
160	20	3,325	99.66	3,440	-	-	1,734	1,706	99.90	-	-	50.40	49.60
170	2	3,750	99.62	3,225	-	-	1,533	1,692	99.90	-	-	47.53	52.47
170	5	3,150	99.68	2,810	-	-	1,380	1,430	99.91	-	-	49.11	50.89
170	10	3,175	99.68	2,585	-	-	1,321	1,264	99.92	-	-	51.10	48.90
180		28,487	97.51	53,960	43,333	-	1,744	8,883	98.46	80.30	-	3.23	16.46
180	1	5,142	99.48	4,950	-	-	1,475	3,475	99.85	-	-	29.79	70.21

sents the following generalizations.

1. Mixes made of raw ingredients showed a very high initial count, consisting mostly of rapid acid producers. After pasteurization these were uniformly reduced to sufficiently low numbers to permit plating in low dilutions. In these low dilution plates none of the slow acid producing types was observed. This suggests that they were either not present in the mix before pasteurization or had been destroyed by the heat.

2. In the mixes made from dairy products which had been previously pasteurized, lower initial counts were obtained as well as a lower efficiency of pasteurization. It was of special interest to note, however, that the flora after pasteurization consisted mostly of slow acid types. This suggests that these organisms were more resistant to heat when not grossly outnumbered by the rapid acid producing organisms. It is further suggestive that the acidity produced may affect the thermal resistance of the cells.

Another possibility is that the slow acid formers may be present in very few numbers in the raw products and constitute such a small percentage of the flora that they are immeasurable by the plate method. During pasteurization these products may become contaminated from the pasteurizing vat and other equipment. This possibility seems quite plausible in view of the fact that these organisms usually

survived pasteurization and would be expected to remain on the equipment unless complete sterilization was practiced. This contamination together with the elimination of competitive types of organisms by pasteurization would easily account for their presence in large numbers in the mixes made of pasteurized ingredients.

In Table XIII is given the average per cent of types of organisms in the mixes before and after pasteurization for all the trials run in this experiment. In the raw mix the slow acid producers could not be determined because of a predominance of alkali forming colonies that rendered the entire plate alkaline, thereby obliterating the slow acid formers. The slow acid producing group, the alkali producing group, and the alkali forming and inert combined, constituted approximately 88 per cent of the flora of the raw mixes.

After pasteurization these two groups constituted, roughly, about 97 per cent of the flora, of which 75 per cent were slow acid formers. The observation that the flora after pasteurization consisted mostly of the slow acid formers is in keeping with the results of Ayers and Johnson (15) whose findings are reported in table II.

TABLE XIII

SUMMARY OF THE PER CENT OF TYPES OF ORGANISMS IN THE MIX BEFORE AND AFTER PASTEURIZATION

Pasteurization Temperature °F.	Time: Min.	PER CENT OF TYPES: Acid Formers: Rapid	Acid Formers: Slow	Pepto-nizers	Inert
Raw		11.55		1.11	
145	20	10.77	73.33	2.57	13.33
145	25	1.91	78.02	2.27	17.40
145	30	.25	77.19	2.77	20.29
150	20	.09	62.23	2.55	35.93
150	25	.02	64.60	3.02	32.36
150	30	.09	59.01	3.18	37.72
160	10	-	77.55	2.55	19.90
160	15	-	76.35	2.39	21.26
160	20	-	67.63	3.38	28.99
170	2	.09	79.12	2.46	18.33
170	5	-	77.57	2.72	19.71
170	10	-	74.88	2.73	22.39
180		-	70.89	2.76	26.35

With one exception, the rapid acid producers were uniformly reduced to a negligible figure. It may be well to relate that in only one mix did any of this group survive pasteurization and that was a mix very heavily contaminated with these organisms.

The peptonizers in all cases made up but a minor portion of the flora both before and after pasteurization. A

large portion of these organisms were destroyed by pasteurization although some survived all temperatures and time intervals in this experiment.

Comparison of Counts on Extract Agar at 98°F. and Caseinate Agar at 80°F.

The "total counts" in Tables VII, VIII, XI, and XII show the results obtained by pouring duplicate sets of plates from the same mix. Extract agar was poured into one set of plates and incubated at 98°F. Caseinate agar was poured into the other set and incubated at 80°F. It will be seen in Tables VII, VIII, XI, and XII that when the flora consisted of a high per cent of slow acid forming types the count obtained at 80°F. was frequently ten times that at 98°F. On the other hand, in those mixes in which the predominating organism was a rapid acid producer, very little difference was noted in the counts obtained at either incubation temperature.

That the higher count on caseinate agar is due to the appearance of the slow acid formers is further proved in Tables XIV and XV, in which the growth of pure cultures of the slow acid formers was compared at 98°F. and 80°F.

It is evident from the data in these tables that the slow acid forming organisms do not grow, or grow but slowly at 98°F., whereas they grow rather rapidly at 80°F.

TABLE XIV

COMPARISON OF GROWTH OF PURE CULTURES OF SLOW ACID FORMING ORGANISMS AT 98°F. AND 80°F. IN LITMUS MILK

Culture	Incubation Temperature											
	98°F.						80°F.					
	Reaction (days)						Reaction (days)					
	2	4	6	8	10	20	2	4	6	8	10	20
1 A	-	-	-	-	-	±	-	-	-	-	+	+
3 A	-	-	-	-	-	±	-	-	+	+	++	++
4 A	-	-	-	-	-	±	-	-	+	+	++	++
5 A	-	-	-	-	-	±	-	-	+	+	++	++
15	-	-	-	-	±	±	-	-	+	+	++	++
16	-	-	-	-	-	±	-	-	+	++	++	++
Strong Acid	++	++	++	++	++	++	+	++				

\- = No change
\+ = Acid
++ = Acid and curd
± = Slightly acid

TABLE XV

COMPARISON OF GROWTH OF PURE CULTURES OF SLOW ACID FORMING ORGANISMS ON CASEINATE AGAR

Culture	98°F. Incubation Temperature 80°F.								
	Growth after (days)					Growth after (days)			
	2	4	6	8	10	2	4	6	8	10
1 A	-	-	-	-	-	-	+	+++		
3 A	-	-	-	-	-	-	+	+++		
4 A	-	-	-	-	-	-	+	+++		
5 A	-	-	-	-	-	-	+	+++		

\+ = Slight growth - = No growth
+++ = Abundant growth

The question may arise as to whether the higher count was due to the agar or the incubation temperature. In order to determine this, duplicate sets of plates were poured with extract and caseinate agars and both sets incubated at 80°F. The results of these counts are shown in Tables IV, IX, and X. It is obvious that when incubated at 80°F. the extract agar, gave counts almost as high as the caseinate agar. This suggests that the higher counts on the caseinate agar were due primarily to incubation temperature rather than the medium. Also, the fact that in those cases where the extract agar counts were about one-tenth those of the caseinate agar there was always a predominance of slow acid producers which do not grow at 98°F. further suggests that the incubation temperature was the major factor.

In all the trials in which the extract agar plates were incubated at 98°F. for 48 hours, the counts after pasteurization were under 10,000, even when pasteurized at 145°F. for 20 minutes. These results are much the same as those reported by Hammer and Saunders (7) in which with one exception, 145°F. for 20 minutes reduced the count to less than 10,000. They differ, however, from the results obtained by Fay and Olson (12) in which they report 150°F. for 30 minutes resulted in greater efficiency than lower temperatures for that holding period.

It is evident, however, that the extract agar counts

obtained when incubated at 98°F. are not a true index of the actual number of organisms present since the caseinate agar plates incubated at 80°F. gave very much higher counts.

Effect of Pasteurization Under Plant Conditions on the Bacterial Flora of Mixes

In order to check the results of the laboratory method of pasteurizing in test tubes against the results obtained under more practical conditions, three complete trials were run under plant conditions. For each trial a 100 pound batch of mix was prepared and then divided into four 25 pound lots. Only four pasteurization temperatures were used because no satisfactory method of flash pasteurizing was available. All the mixes were made of pasteurized cream and skim milk, sweetened condensed skim milk, sugar, and gelatine. Some of the mixes were heated in a small steam kettle and were constantly agitated, while others were heated in a pasteurizing vat.

At the end of each holding period a sample was taken by means of a sterile pipette and immediately iced until plated.

The results in Table XIX show the results obtained by three complete trials run as described. In mixes made from pasteurized ingredients very little difference in results were noted under laboratory and plant conditions. The rapid

acid producers were always destroyed, and the slow acid formers predominated the flora after pasteurization. The peptonizers were uniformly reduced to a very low figure.

TABLE XIX

EFFECT OF PASTEURIZATION UNDER PLANT CONDITIONS
ON THE TOTAL AND DIFFERENTIAL COUNT
OF AN ICE CREAM MIX
(Average of Three Trials)

Pasteurization Temperature °F.	Time: Min.	Total Count	Acid Formers: Rapid	Acid Formers: Slow	Peptonizer	Alkali and Inert
Raw		3,658,000	68,000		32,000	
145	20	50,000	-	46,000	525	3,475
145	25	39,500	-	36,000	250	3,250
145	30	42,000	-	45,000	400	3,600
150	20	78,500	-	69,000	525	8,975
150	25	-	-	-	-	-
150	30	42,500	-	31,000	350	11,150
160	10	-	-	-	-	-
160	15	44,500	-	37,500	400	6,600
160	20	47,000	-	43,000	450	3,550
170	2	29,500	-	22,500	500	6,500
170	5	44,500	-	27,500	300	16,700
170	10	28,000	-	18,000	300	9,700

Study of Slow Acid Forming Organisms

Since the slow acid forming group comprised the majority of the organisms surviving pasteurization, it was of interest to obtain some results with cultures of these types of bacteria.

Careful examination of the caseinate agar plates revealed that there were five different types of colonies prevalent. A representative of each of these types was transferred to litmus milk and agar slants, and numbered 1 A, 2A*, 3 A, 4 A, and 5 A. Detailed studies of these cultures revealed that they could be classified into three distinct groups as indicated in Chart I.

CHART I

	Nitrate	Raffinose	Xylose	Rhamnose	Arabinose	Inulin	Dulcitol
Group I	-	+	+	+	+	+	+
Group II	-	-	-	-	-	-	-
Group III	+	-	+	-	-	-	-

In addition to these five cultures 23 slow acid types were picked at random from the plates and transferred to litmus milk. All but two of these 23 cultures (15 and 16, Chart I) were classified into one of the three groups. Chart II shows the morphological and biochemical characteristics of the three groups of organisms as well as for the two atypical cultures 15 and 16.

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* Lost in first transfer.

CHART II

CULTURAL CHARACTERISTICS OF SLOW ACID FORMERS

Culture No.	Morphology	Gram Stain	Litmus Milk	Nitrate reduction	Gelatine liquefaction	Carbohydrates												Motility	H_2S
						Mannose	Raffinose	Xylose	Sucrose	Rhamnose	Arabinose	Maltose	Dulcitol	Inulin	Dextrose	Lactose			
1 A	Short, slender rod occurring singly	G+	Sufficient for acid curdling	-	-	+	+	+	+	+	+	+	+	+	+	+		-	-
3 A	Short, thick rod occurring singly	+	Sufficient for acid curdling	-	-	+	-	-	+	-	-	+	-	-	+	+		-	-
4 A	Short, thick rod occurring singly	+	Sufficient for acid curdling	+	-	+	-	+	+	-	-	+	-	-	+	+		-	-
5 A	Short, thick rod occurring singly	+	Sufficient for acid curdling	-	-	+	+	+	+	+	+	+	+	+	+	+		-	-
15 A	Very small rods occurring singly	+	Sufficient for acid curdling	-	-	+	+	+	+	+	+	+	+	+	+	+		-	-
16	Short, thick rods	+	Sufficient for acid curdling	-	-	+	+	+	+	+	+	+	+	+	+	+		-	-

The litmus milk reactions at indicated intervals are given in Table XVI. It will be seen that although the organisms produce acid rather slowly, litmus milk is eventually coagulated. The curd in all cases was very firm and gave no evidence of gas formation. In each case the dye was reduced except for a pink band at the top.

TABLE XVI

LITMUS MILK REACTIONS

Culture	Reaction in: (days) 1	2	3	4	5	6	7	8	9	10	14
1 A	-	-	-	-	+		+	+		++	
3 A	-	-	+	+	+		++	++		++	
4 A	-	-	+	+	+		++	++		++	
5 A	-	-	+	+	++		++	++		++	
15	-	-	-	(+)	(+)		++	++		++	
16	-	-	-	-	-		+	++		++	

\- = No reaction
\+ = Acid
++ = Acid and curd
(+) = Slightly acid

That these organisms are resistant to heat is demonstrated in Table XVII. Pure cultures of the slow acid forming organisms were inoculated into flasks of sterile mix and incubated 24 hours. A portion of each culture was then transferred to sterile test tubes and pasteurized at 170°F.

for 10 minutes and 145°F. for 30 minutes. The results show very definitely that these pasteurization temperatures do not materially reduce the counts.

TABLE XVII

EFFECT OF PASTEURIZATION ON
PURE CULTURES OF ORGANISM

Culture	COUNTS		
	Raw	76.6°C. - 10 Min.	62.8°C. - 30 Min.
1 A	116,000,000 123,000,000	91,200,000	110,000,000
3 A	31,000,000 41,000,000	34,200,000	34,000,000
4 A	52,000,000 41,000,000	32,500,000	34,800,000
5 A	51,000,000 38,000,000	43,300,000	43,700,000
16	76,000,000 59,000,000	69,200,000	72,900,000

Pure cultures of these organisms were also inoculated into flasks of sterile mix and held at 80°F. The change in pH was observed at stated intervals and is shown in Figure I. It will be seen that the slow acid formers produce acid more slowly than S. lactis, although culture 5 A produced sufficient acid to coagulate the mix in six days. It is doubtful if these organisms are of any practical significance in the mix unless it were held for an extended period.

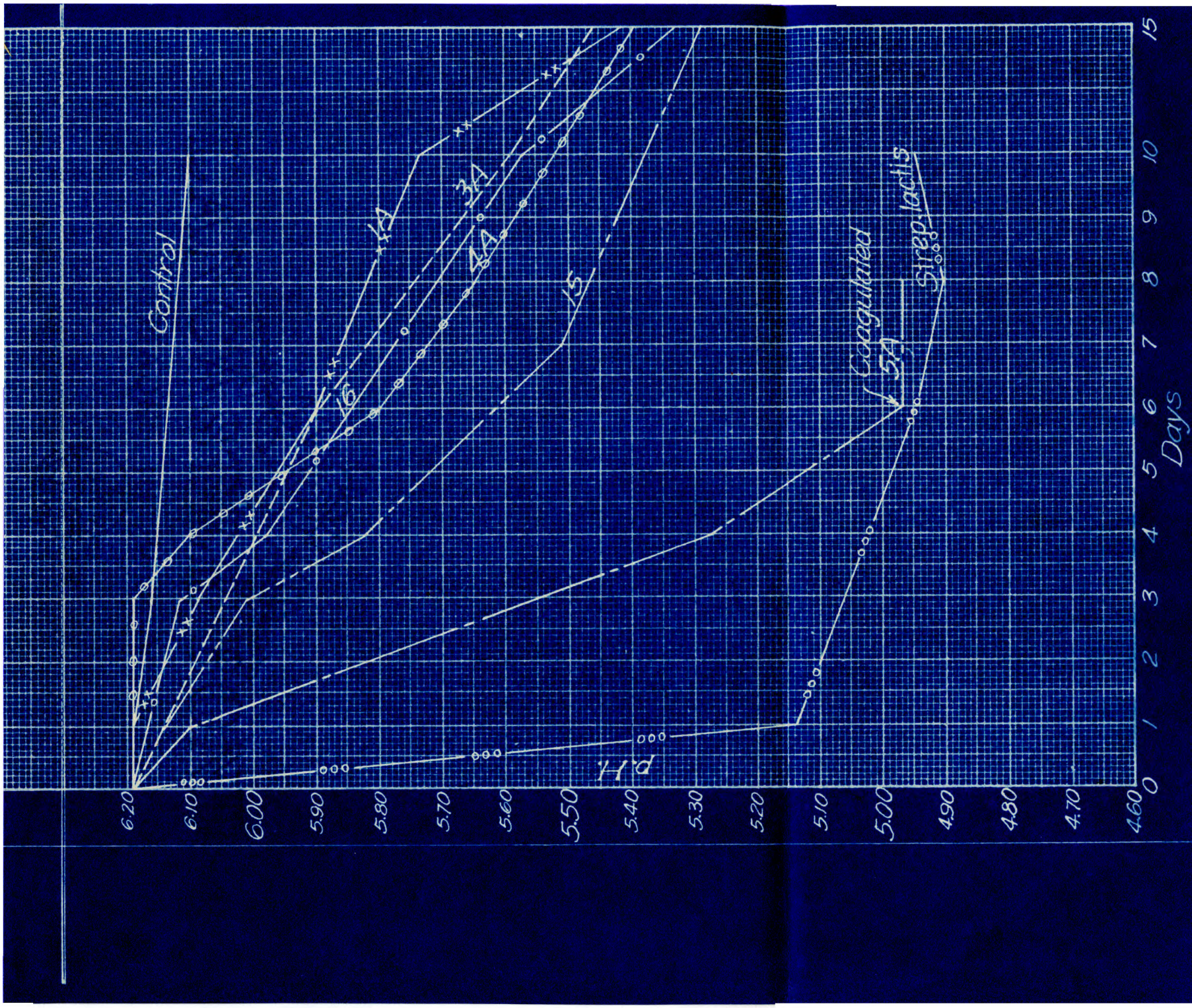

Fig. 1. pH Changes of Slow Acid Forming Organisms in Ice Cream Mixes

Effect of Various Pasteurization Temperatures on Cultures of *E. coli*

In an attempt to measure the relative efficiencies of the pasteurization temperatures used in this experiment, six strains of *E. coli* were pasteurized at each temperature. Flasks of sterile mix were heavily and uniformly inoculated with *E. coli*. After thorough agitation a portion of the mix from the flask was removed and pasteurized at one of the temperatures indicated in Table XVIII.

It will be seen that only one culture (No. 57) pasteurized at 145°F. for 30 minutes survived pasteurization. Upon repeating the experiment this culture was destroyed at this temperature. Since each temperature was sufficiently high to completely destroy all the cultures, no statement can be made as to the superiority of one exposure over another. It was thought that the relative efficiencies of the various exposures employed could be measured by this method because the thermal death points of different strains of *E. coli* approximate these exposures. Various strains were used because the thermal resistance of strains is variable. The variability of the results obtained with one culture out of only six suggests caution in the utilization of the colon test as an index to the efficiency of pasteurization.

TABLE XVIII

EFFECT OF PASTEURIZATION ON
COLI CULTURES

Pasteurization Temperature °F.	Time Min.	Culture No.	Reaction in 18 Hrs.	24 Hrs.	36 Hrs.	48 Hrs.	72 Hrs.
Raw		25	+	+++	+++		
		26	+	+++	+++		
		27	+	+++	+++		
		52	+	+++	+++		
		56	+	+++	+++		
		57	+	+++	+++		
170	10	25	-	-	-	-	
		26	-	-	-	-	
		27	-	-	-	-	
		52	-	-	-	-	
		56	-	-	-	-	
		57	-	-	-	-	
160	20	25	-	-	-	-	
		26	-	-	-	-	
		27	-	-	-	-	
		52	-	-	-	-	
		56	-	-	-	-	
		57	-	-	-	-	
150	30	25	-	-	-	-	
		26	-	-	-	-	
		27	-	-	-	-	
		52	-	-	-	-	
		56	-	-	-	-	
		57	-	-	-	-	
145	30	25	-	-	-	-	
		26	-	-	-	-	
		27	-	-	-	-	
		52	-	-	-	-	
		56	-	-	-	-	
		57	+	+++	+++	+++	
180		25	-	-	-	-	
		26	-	-	-	-	
		27	-	-	-	-	
		52	-	-	-	-	
		56	-	-	-	-	
		57	-	-	-	-	

SUMMARY

Standard ice cream mixes were made from raw dairy products and from ingredients which had been previously pasteurized. These mixes were pasteurized at various exposures and total and differential bacterial counts were made.

In the first trials pasteurization was carried out in test tubes, but later, larger mixes were made and pasteurized under plant conditions to afford a comparison of the two methods.

It was found that none of the exposures employed for pasteurization yielded results sufficiently different to be measurable by the plate method. The results obtained by pasteurizing mixes under plant conditions were essentially the same as those obtained when pasteurization was done in test tubes.

It was further found that in mixes made from raw dairy products the flora after pasteurization was about evenly divided between the peptonizing and alkali forming and inert groups, whereas in mixes made from pasteurized ingredients the flora after pasteurization consisted largely of slow acid formers. The predominating types of these organisms were isolated and their characteristics determined.

CONCLUSIONS

The results of this experiment show that:

1. The differences in efficiency between the pasteurizing temperatures and time intervals used in this experiment are not sufficiently great to be measurable by the plate method.

2. When mixes composed of pasteurized dairy products were subsequently pasteurized, about 75 per cent of the flora which survived consisted of the slow acid forming types of bacteria. On the other hand, when mixes made of raw dairy products were pasteurized the flora consisted chiefly of the alkali forming and inert types. In either case, pasteurization uniformly destroyed the rapid acid producing types and reduced the number of peptonizing bacteria to a low figure.

3. If the slow acid formers constituted a large per cent of the flora, higher counts were obtained when the plates were incubated at 80°F. for five days than when a temperature of 98°F. for 48 hours was employed.

4. The slow acid forming organisms isolated were small Gram positive rods, which grew well at 80°F. but not at 98°F. Since they produce acid only slowly in ice cream mix, it is doubtful if they are of any significance in the souring of ice cream mix.

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