Influence of Growth Temperature on the Thermal Resistance of Some Aerobic, Spore-forming Bacteria From Evaporated Milk[1]

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THE HEAT treatment employed in manufacturing evaporated milk ordinarily is satisfactory for the destruction of the microorganisms present. Occasionally, spoilage occurs even though there has been no intentional or discernible modification of the heat treatment used with the milk. The spoilage outbreaks are frequently spasmodic but during their brief existence may cause considerable financial loss to the manufacturer.

In connection with various outbreaks of spoilage in evaporated milk, it has been observed at the Iowa Agricultural Experiment Station, and elsewhere, that the outbreaks often occurred during warm weather. It is entirely possible that the outbreaks were caused by an extensive contamination of the milk, with the causal organisms on the farm or in the plant since the warm weather may have been more favorable for the growth and development of the organisms. During warm weather there is also a greater opportunity for the contamination of milk because of the ease with which dry particles of soil, dirt, etc., containing organisms or spores, can be carried in the air. An extensive contamination increases the difficulty of sterilization because of the large numbers of organisms present. Another explanation may be that, if the causal organisms are thermophilic, they would not grow and develop at ordinary temperatures, even if present, but would grow at summer heat, thereby causing spoilage primarily at that season. From the suggestions advanced the importance of growth temperatures on the organisms responsible for spoilage in evaporated milk is apparent.

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With the importance of growth temperature in mind, the hypothesis has been advanced that certain strains of bacteria causing spoilage in evaporated milk may be capable of withstanding more severe heat treatment when grown at relatively high temperatures. This possibility, together with the frequency with which outbreaks of spoilage have occurred during hot weather, makes important the evaluation of the influence of increased growth temperature on the thermal resistance of the bacteria most frequently present in evaporated milk. The microorganisms commonly found responsible for spoilage in evaporated milk are aerobic, spore-forming bacteria as reported by Hammer (5, 6), Hammer and Hussong (7), Hussong and Hammer (8), Kelly (9), Morrison and Rettger (11), Spitzer and Epple (12) and others.

The primary interest of the problem is centered around the question: What influence does growth temperature have on the heat-resisting ability of aerobic, spore-forming bacteria found in evaporated milk? Data on this question may furnish at least a partial explanation for the heat-resisting ability of bacteria which frequently cause serious spoilage losses in evaporated milk.

METHODS

SOURCES OF ORGANISMS

The organisms used in the study were secured from three sources:

A. Isolated directly from spoiled evaporated milk.

B. Isolated directly from nonspoiled evaporated milk.

C. Isolated by a commercial laboratory from nonspoiled evaporated milk.

Most of the preliminary trials and all the major portion of the study were conducted in a DeKhotinsky constant temperature bath equipped with a high speed turbine agitator. Light mineral oil was used as the heating medium area; temperatures were maintained within the limits of $\pm$ 0.2° C.

Except for a series of comparative trials with evaporated milk, sterile skim milk was always used as the spore suspension medium.

Sterile agglutination tubes were used as the containers for the spore suspensions in all thermal resistance trials. The tubes were approximately 10 mm. in outside diameter by 75 mm. long, with about 1 mm. thickness of wall.

In order to reduce the factor of heat penetration to a minimum, small samples were used in all heating trials. The sterilized agglutination tubes were partially filled with 2.0 cc. samples of the spore suspension used and then sealed in a blast lamp flame.

Various culture media were used with the different cultures in order to secure the best possible growth and spore production. Nutrient agar proved very satisfactory for growth and development of spores with cul-

tures 1, 2, 3, 4, 7, 8, 9, 13, 15 and 16. It was necessary to resort to beef-infusion agar for satisfactory growth and development of spores with cultures 5, 10, 11, 12 and 17. Litmus milk was used as the medium to determine sterility with cultures 1 and 5, while with all other cultures dextrose broth containing bromocresol purple was employed.

Using suitable culture media for the various cultures, as given above, an effort was made to secure growth of each organism at 10°, 21°, 37°, 45° and 55° C. No growth was secured at 10° C., and none of the organisms had a growth range from 21° to 55° C.

In the preparation of spore suspensions nutrient or beef-infusion agar slants were inoculated with a pure culture of the organism to be tested and grown at one of the temperatures which preliminary work indicated would permit growth and development of spores. The periods of incubation at the different growth temperatures were varied in order to permit as large a production of spores as possible and yet secure comparisons of spores of approximately the same age. The production of spores was slower at low-growth temperatures than at higher temperatures, which necessitated a longer period of incubation at the low temperatures in order to secure approximately the same spore development as at the higher temperatures. The maximum periods of incubation at the higher temperatures were in turn limited by the injurious effect of the higher temperatures on the spores. Long periods of incubation at high temperatures decreased the number of viable spores on the agar slants. A portion of each growth was transferred to 60 cc. of sterile skim milk and the skim milk then agitated vigorously for several minutes. The spore content per cubic centimeter of skim milk in comparative thermal resistance trials was relatively constant due to the care with which definite quantities of growth were transferred. Thus, the growth temperature was the only variable factor. Each of eight sterilized agglutination tubes was then partially filled with 2.0 cc. of the spore suspension and sealed in a blast lamp flame. The tubes were immediately heated in the oil bath, and the time between removal of the spores from the incubator and immersion in the oil bath never exceeded 20 minutes. Although eight tubes were prepared for each trial, only seven exposures were used; and the eighth tube was needed only when a tube was accidentally broken. The skim milk remaining after the agglutination tubes were filled was heated at 80° C. for 10 minutes, and plated for spore content on the assumption that the spores but not the vegetative cells would survive this exposure.

In each trial on the comparative heat resistance of spores of different ages, every effort was made to secure spore suspensions containing approximately the same number of spores per cubic centimeter. In some instances it was necessary to make several runs before satisfactory spore counts were secured in comparative trials. By this means the only variable factor influencing heat resistance was the growth temperature.

The spores were exposed to heat by immersing the sealed tubes containing the spore suspension in the oil bath. Before the tubes were subjected to the desired temperature, they were exposed to the same temperature for 15 seconds in a preliminary oil bath. The exposure temperatures used, except for slight variations with specific cultures, were: 104° C., 108° C., 112° C., 116° C. and 120° C. The series of eight agglutination tubes, each containing 2.0 cc. of the spore suspension, was exposed to the desired temperature for definite periods of time.

With each culture the exposure periods used in the preliminary trial were 3, 5, 7, 10, 15, 20 and 25 minutes. After the first trial, the exposure periods were usually varied in order to have about the same number of periods above and below the period last showing growth. For example, if the preliminary trial showed the last survival at 7 minutes, the exposure periods were then changed to 3, 4, 5, 7, 9, 12 and 15 minutes. In practically all trials other than the preliminary ones the differences between exposures were 1 minute from 0 to 5 minutes, 2 minutes from 5 to 9 minutes, 3 minutes from 9 to 15 minutes and 5 minutes from 15 to 40 minutes. At the end of each exposure period a tube was removed, immediately placed in a bath of cold water and tested for sterility within 10 minutes.

Sterility of a spore suspension after exposure to heat was determined by inoculating a tube of litmus milk or dextrose broth containing bromocresol purple with a 1 cc. portion of the contents of the heated tube and incubating for 7 days at the optimum growth temperature for the culture. As a check, several loops of the heated spore suspension were streaked on a standard nutrient or beef-infusion agar slant and the slant incubated at the optimum growth temperature for 7 days.

All hydrogen ion determinations were made electrometrically, using quinhydrone, and calculated to the nearest 0.1.

RESULTS

COMPARISON OF HEAT RESISTANCE OF SPORES SUSPENDED IN SKIM MILK AND IN EVAPORATED MILK

Various investigators, notably Ayers and Johnson (1), Barthel and Stenström (2), and Brown and Peiser (3), have demonstrated that milk, when used as the suspension medium for the vegetative cells or spores of bacteria, aids them in resisting destruction by heat. Therefore, in determining the thermal resistance of any organism in milk, due consideration should be given to the influence of the protective action of the kind of milk used.

In studying the influence of growth temperature on the thermal resistance of some aerobic, spore-forming bacteria from evaporated milk, it was planned to use either sterilized skim milk or evaporated milk as the suspension medium instead of sterile water or broth; thus the conditions of heating would be more nearly comparable to those existing during the

TABLE 1. *Comparison of heat resistance of spores suspended in skim milk and in evaporated milk*

(Temperature of exposure 116° C.)

Culture used to secure spores			Milk used for suspension	Spores per cc. of milk heated	Period of heating (minutes)	Survival (minutes)
No.	Growth temperature °C.	Age (days)				
1	37	5	skim	17,400	2, 3, 4, 5, 7, 9, 11	7.0
			evap.	18,000	2, 3, 4, 5, 7, 9, 11	7.0
2	37	5	skim	3,500	16, 18, 20, 22, 24, 26, 28	20.0
			evap.	4,500	16, 18, 20, 22, 24, 26, 28	18.0
4	37	5	skim	250,000	16, 18, 20, 22, 24, 26, 28	20.0
			evap.	290,000	16, 18, 20, 22, 24, 26, 28	18.0
5	55	3	skim	450	26, 28, 30, 32, 34, 36, 38	30.0
			evap.	500	26, 28, 30, 32, 34, 36, 38	30.0
9	45	3	skim	40,000	1, 2, 3, 4, 5, 7, 9	2.0
			evap.	45,000	1, 2, 3, 4, 5, 7, 9	2.0

process of sterilizing evaporated milk. In order to determine the difference, if any, between the protective action of sterilized skim milk and evaporated milk, a series of comparative trials was run with five representative cultures.

Every effort was made to have the conditions as nearly identical as possible, except for the type of suspension medium. In each comparison the spore content per cubic centimeter of skim milk and evaporated milk varied only a little, and since the spores were from one source the growth temperature and age were the same. The evaporated milk used was not resterilized but was transferred aseptically from commercial cans of evaporated milk. It had a pH of 6.5, while the pH of the skim milk was 6.4.

Results of the trials are presented in table 1. In 3 of the 5 comparisons there was no difference in the protective action of the two suspension media. With cultures 2 and 4, however, the skim milk apparently had a slightly greater protective action than did the evaporated milk. In each instance the difference was only two minutes at the temperature used and does not appear to be significant, especially since the results of the three other comparisons showed no difference between the two media.

From the data presented it appears that results of thermal resistance trials using sterilized skim milk as the spore suspension medium are comparable to the results secured with evaporated milk as the suspension medium.

When it was found that there was little difference between the protective action of skim milk and of evaporated milk, it was decided to use

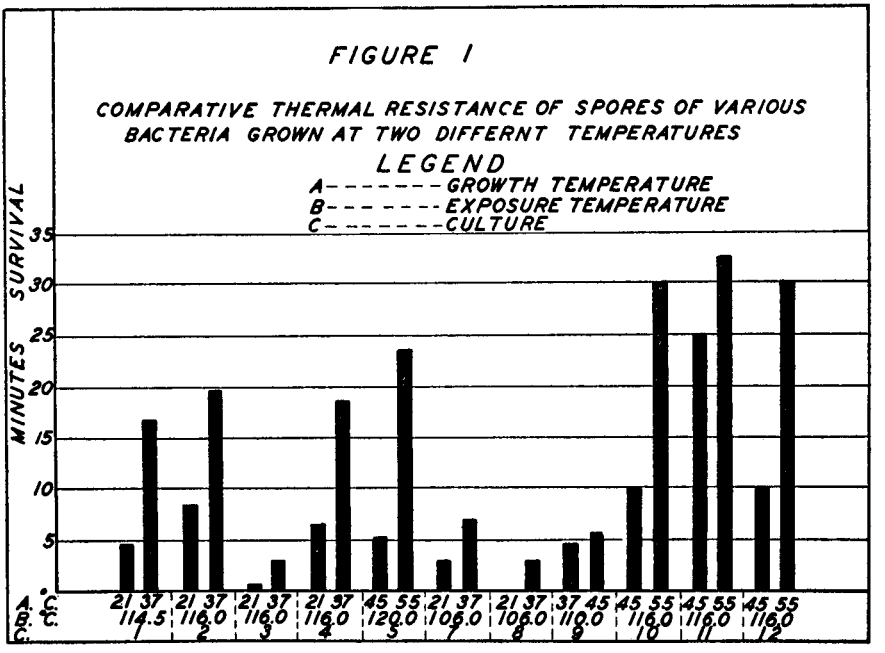
skim milk as the suspension medium for the spores in the thermal resistance trials. The decision was also based upon two other reasons: First, evaporated milk is difficult to handle in large quantities without contamination; and, second, it was considered inadvisable to resterilize evaporated milk since there is a possibility of changing its composition and consequently modifying its protective action on the suspended spores.

COMPARATIVE THERMAL RESISTANCE OF SPORES OF VARIOUS BACTERIA GROWN AT TWO DIFFERENT TEMPERATURES

In an effort to secure information on the influence of growth temperature on the thermal resistance of bacterial spores, all of the cultures examined which showed any appreciable resistance to heat were grown at two different temperatures and the spores tested for heat resistance. The higher of the two growth temperatures was, in all instances, the optimum or at least the temperatures which gave the most luxuriant growth of the culture being studied. The optimum growth temperatures employed were 37°, 45° and 55° C., while the lower growth temperatures used in comparison with these temperatures were 21°, 37° and 45° C., respectively. Before being used in comparative trials, each culture was carried through at least three transfers at the respective growth temperatures.

The results of the comparative trials with the various cultures are presented in fig. 1, which clearly shows the differences in average thermal resistance of the spores when grown at two temperatures. The figure shows that every culture possessed a lower average thermal resistance when grown below the optimum. The greater thermal resistance exhibited at the optimum growth temperature was, except for culture 9, very significant. With culture 9 the greater thermal resistance caused by growth at its optimum growth temperature was comparatively small, being only one minute. It is well to note that there was no instance of spores from a culture grown at a temperature below the optimum exhibiting a thermal resistance greater than that of spores from a culture grown at the optimum temperature. The spore content, per cubic centimeter of skim milk, varied somewhat in the comparative trials; but no significant difference in thermal resistance could be attached to any definite difference in the spore content. Within the limits of the spore contents used in the individual trials, there did not appear to be any correlation between the number of spores present per cubic centimeter of skim milk and the time of survival of the spores. The data definitely demonstrate that, with the cultures studied, growth at a temperature below the optimum decreased the thermal resistance.

The increases in thermal resistance of the cultures when grown at the optimum growth temperatures were particularly striking with cultures 1, 2, 4, 5, 10, 11 and 12, being 12.3, 11.4, 12.5, 17.5, 20.0, 7.5 and 20.0 minutes, respectively. These cultures were exposed to temperatures approximating the sterilization temperature used for evaporated milk. Cul-



ture 1 was exposed to 114.5° C., cultures 2, 4, 10, 11 and 12 to 116.0° C., and culture 5 to 120.0° C. At these temperatures the spores, when grown at the optimum growth temperature, survived longer than the normal holding period used in sterilizing evaporated milk, which is from 15 to 17 minutes. The average survival was 16.9, 19.9, 18.8, 23.6, 30.0, 32.5 and 30.0 minutes for cultures 1, 2, 4, 5, 10, 11 and 12, respectively. Outstanding in resistance to heat was culture 5, which, when grown at 55° C., survived a temperature of 120° C. for 23.6 minutes. This thermal resistance is sufficient to enable the organism to survive the ordinary autoclaving procedure used in laboratory work which demands a temperature of 120° C. for 20 minutes.

These observations indicate that spores of some of the organisms found in evaporated milk can survive, when grown at their optimum temperature and present in large numbers, the sterilization process normally used in the manufacture of evaporated milk.

COMPARATIVE THERMAL RESISTANCE OF SPORES OF VARIOUS BACTERIA GROWN AT THREE DIFFERENT TEMPERATURES

Having established the fact that a growth temperature below the optimum lowered the thermal resistance of the spores of the cultures studied, it was thought advisable to try other growth temperatures. Cultures 2, 4, 7, 8 and 9 were the only cultures which, with the temperatures

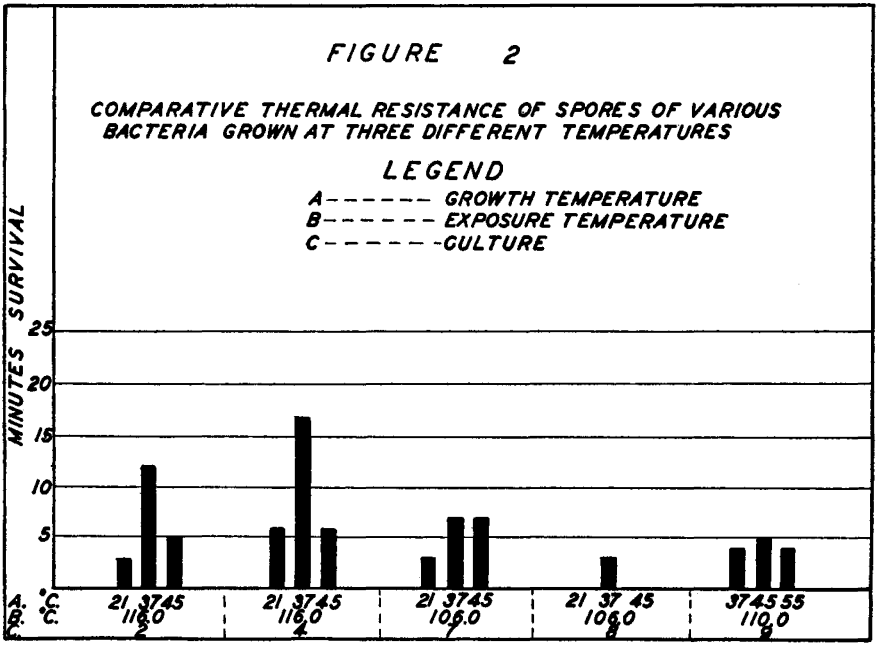
used, showed growth at a temperature above the optimum and also at a temperature below the optimum. These cultures were carried through at least three transfers at the respective growth temperatures before being used in comparative thermal resistance trials. The pH of the skim milk was 6.3 in all cases.

Figure 2 clearly shows that the average thermal resistance of the spores of the cultures employed was usually lowered when growth temperatures either above or below the optimum were used. In other words, unfavorable growth temperatures decreased the thermal resistance. Individual comparative trials likewise showed the same general tendency. These results substantiate those secured when two growth temperatures were studied.

If the cultures investigated are representative of those found in evaporated milk, it appears that a low resistance to heat can be effectively secured by maintaining low growth temperatures. From the data secured, temperatures of 21° C. or below would be considered as low temperatures. With all the cultures studied a temperature of 21° C. or below resulted either in no growth or in a low thermal resistance of the spores formed.

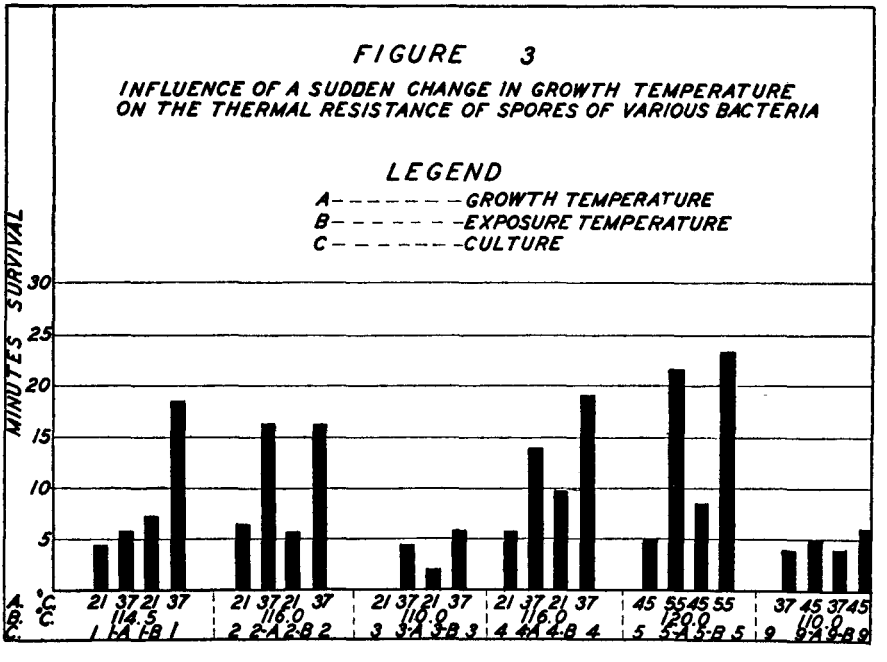
INFLUENCE OF A SUDDEN CHANGE IN GROWTH TEMPERATURE ON THE THERMAL RESISTANCE OF SPORES OF VARIOUS BACTERIA

In all the trials thus far reported each culture was carried through a series of at least three transfers or generations at a certain temperature



before it was used in the thermal resistance trials. Speculation as to the influence of a sudden change in the growth temperature naturally arose, and a series of trials was planned in an effort to evaluate this factor.

After cultures 1, 2, 3, 4, 5 and 9 had been growing for some time at two different temperatures, of which the higher temperature was the optimum temperature, the growth temperatures of the cultures were suddenly changed. The same growth temperatures were used for each culture as formerly, but the transfers of the cultures were held at a temperature different from the one used with the cultures from which the transfers came. For example, if a transfer was made from a culture growing at 37° C. (the optimum temperature for the culture), the transfer was grown at 21° C.; while if the transfer was made from a culture of the same organism growing at 21° C., it was grown at 37° C. Figure 3 shows that with all cultures studied a sudden change in growth temperature from below the optimum to the optimum caused an increase in the average thermal resistance of the spores. In contrast, a sudden change of growth temperature from the optimum to a lower temperature resulted in a decrease in the average thermal resistance of the spores of each culture. Individual comparative trials showed the same differences in resistance of the spores. Figure 3 shows also that, with the exception of culture 2, all cultures were more resistant to heat when grown for several generations at their optimum temperatures than when grown for only one generation. When evaluating this observation, it should be pointed out that all cultures with the letters



A or B after the culture number (e. g., 1-A, 2-A, 1-B or 2-B) represent only one generation at the specific growth temperature. Culture 1, when grown for several generations at 37° C., produced spores with an average thermal resistance of 18.5 minutes; but, when grown for only one generation at the same temperature (culture 1-A), the average thermal resistance of the spores was only 5.7 minutes. This same tendency was shown by cultures 3, 4, 5 and 9, but to a lesser degree. With culture 2, however, spores produced after only one generation at 37° C. exhibited the same resistance to heat as did the spores from cultures grown several generations at 37° C. In contrast there appeared to be some tendency for the cultures grown for only one generation at a temperature below the optimum to produce spores with a greater thermal resistance than those grown for several generations at the same temperatures. This tendency appeared with cultures 1, 3, 4 and 5. Culture 2, however, when grown for one generation at a temperature below the optimum, produced spores with a lower thermal resistance than the spores from cultures grown for several generations at the same temperature. Spores of culture 9 showed no difference in a comparison of this kind.

It is well to note that the average spore contents of the spore suspensions, in comparative trials, were approximately the same and that the only variable factor influencing growth or resistance to heat was the growth temperature.

EFFECT OF CONTINUED GROWTH OF VARIOUS BACTERIA AT A CHANGED GROWTH TEMPERATURE ON THE THERMAL RESISTANCE OF THE SPORES

Observations made when there was a sudden change in growth temperature suggested that spores of cultures grown for some time at the optimum temperature were more resistant to heat than spores of cultures grown for only one generation at that temperature. In order to measure this tendency cultures which had been suddenly changed to a lower or to a higher growth temperature were carried through a series of transfers at that temperature and then tested for heat resistance.

Table 2 shows the thermal resistance of the spores after the cultures had been carried through a series of from 5 to 11 transfers or generations at the changed growth temperature. In addition there is given, for each culture, the average thermal resistance of the spores when the culture was originally tested and also the average thermal resistance of the spores when the culture had grown only one generation after the growth temperature was changed. It should be noted that, when the cultures were originally tested, they had been carried through at least three generations.

Results presented in table 2 indicate that the average thermal resistance of the spores of the various cultures, when grown for a period at a changed growth temperature, tended to approximate the average thermal resistance of the spores of the culture when originally tested after growth at the same temperature. The spores of culture 1-A, which was culture 1

TABLE 2. *Effect of continued growth of various bacteria at a changed growth temperature on the thermal resistance of the spores*

No. of transfers	Growth temperature °C.	Spores per cc. milk heated	Periods of heating (minutes)	Survival (minutes)	Survival original culture	
					Several transfers (ave.)[1]	One transfer (ave.)[2]
Culture 1-A, 5 days old, heated at 114.5° C. pH of milk 6.3						
7	37	65,000	3, 5, 7, 10, 15, 20, 25	15.0	16.9	5.7
Culture 1-B, 5 days old, heated at 114.5° C. pH of milk 6.3						
7	21	71,000	2, 3, 4, 5, 7, 9, 12	3.0	4.6	7.2
Culture 2-A, 5 days old, heated at 116.0° C. pH of milk 6.3						
5	37	100,000	7, 9, 12, 15, 20, 25, 30	20.0	19.9	16.2
Culture 2-B, 5 days old, heated at 116.0° C. pH of milk 6.3						
5	21	120,000	3, 4, 5, 7, 9, 12, 15	7.0	8.5	5.7
Culture 3-A, 5 days old, heated at 110.0° C. pH of milk 6.4						
7	37	2,000,000	1, 2, 3, 4, 5, 7, 9	5.0	3.0	4.3
Culture 3-B, 5 days old, heated at 110.0° C. pH of milk 6.4						
7	21	1,500,000	1, 2, 3, 4, 5, 7, 9	3.0	0.6	2.0
Culture 4-A, 5 days old, heated at 116.0° C. pH of milk 6.3						
11	37	4,000	9, 12, 15, 20, 25, 30, 33	15.0	18.8	14.0
Culture 4-B, 5 days old, heated at 116.0° C. pH of milk 6.3						
11	21	20,000	3, 4, 5, 7, 9, 12, 15	3.0	6.3	9.8
Culture 5-A, 3 days old, heated at 120.0° C. pH of milk 6.4						
5	55	100	9, 12, 15, 20, 25, 30, 35	25.0	23.6	21.6
Culture 5-B, 3 days old, heated at 120.0° C. pH of milk 6.4						
5	45	200	3, 4, 5, 7, 9, 12, 15	5.0	6.1	8.3

A = Change from lower to higher growth temperature
B = Change from higher to lower growth temperature

[1] Data taken from fig. 1.
[2] Data taken from fig. 3.

grown at 21° C. and then changed to a temperature of 37° C., had a thermal resistance of 5.7 minutes after one generation; but after 7 generations the thermal resistance had increased to 15.0 minutes, or almost the same thermal resistance as that exhibited by the spores of the culture when originally tested after growth at 37° C. Culture 1-B, which was culture 1 grown at 37° C. and then changed to a temperature of 21° C., produced spores which had an average thermal resistance of 7.2 minutes after one generation; but after 7 generations the thermal resistance had decreased to 3.0 minutes, which was less than that of the spores of the culture when originally tested after growth at 21° C. Culture 1-A and 4-A, after 7 and 11 generations, respectively, at the changed growth temperature, produced spores which had a thermal resistance almost equal to that of the spores of the cultures when originally tested after growth at 37° C. Spores of cultures 3-A and 5-A, after 7 and 3 generations, respectively, exceeded slightly the thermal resistance of the spores of the cultures originally tested after growth at 37° C.; and spores of culture 2-A, after 5 generations, equalled the thermal resistance of the spores of the culture when originally tested after growth at 37° C. In contrast, spores of cultures 1-B, 2-B, 4-B and 5-B, after 7, 5, 11 and 5 generations, respectively, were lower in thermal resistance than the spores of the cultures when originally tested after growth at 21° C. Spores of culture 3-B, however, after 7 generations, were not quite as low in thermal resistance as the spores of the culture when originally tested after growth at 21° C.; in fact, their thermal resistance was slightly increased.

The results given in table 2 indicate that continued growth at a changed growth temperature generally resulted in a thermal resistance approximating that of the culture when originally tested after growth at the same temperature. Apparently the cultures, after a period of time, had become acclimatized and the growth temperature exerted its influence on the heat-resisting ability of the spores produced.

DISCUSSION OF RESULTS

The importance of low temperatures for holding milk which is to be manufactured into evaporated milk is indicated by the data showing the relatively low thermal resistance of aerobic, spore-forming organisms grown at temperatures below the optimum. It appears, therefore, that the maintenance of low temperatures would markedly lower the thermal resistance of bacteria likely to be present at the time of sterilization and thereby reduce, if not prevent, the spoilage of evaporated milk. An exception to the beneficial influence of low temperatures would be in the case of using temperatures low enough to inhibit the growth of spoilage organisms but still having present in the milk spores which had developed at high or optimum temperatures. In this instance the maintenance of low temperatures would perhaps not markedly lower the thermal resistance of the spores. The observation emphasizes the necessity of main-

taining low temperatures, wherever possible, from the time the milk is drawn until the time of sterilization. The data presented give support to the observations of Weil (13) that growth temperatures influence the ability of microorganisms to resist heat.

The results show that the temperature to be avoided in attempting to secure a lower thermal resistance of spores of organisms isolated from evaporated milk is the optimum growth temperature. Although growth temperatures above the optimum tend to reduce the heat resistance of the spores, such holding temperatures are objectionable since some of the organisms found in evaporated milk have a high optimum growth temperature and high temperatures (37° to 55° C.) adversely affect the quality of the milk by stimulating the development of bacteria which may lower the heat stability of the evaporated milk. Realization of the great thermal resistance exhibited by some of the aerobic, spore-forming bacteria gives a better appreciation of the possibility of milk spoilage even when employing a sterilizing procedure that is usually efficacious. In fact, culture 5, at its optimum growth temperature, produced spores that could survive the ordinary autoclaving process used in laboratory work at 120° C. for 20 minutes.

The observations indicating that each growth temperature may give a different plane of heat resistance to the spores of specific organisms are of interest. From the commercial viewpoint they emphasize the necessity of continually maintaining temperatures which are unfavorable for growth. The data presented show that growth of organisms at favorable temperatures for only one generation resulted in a material increase in the heat resistance of the spores. The observations, therefore, form a basis for an explanation of the sudden outbreaks of spoilage in evaporated milk. Briefly: (Assuming the presence of causal organisms on the farm or in the plant) the prevalence of a favorable growth temperature for several days (sufficiently long to produce spores) might increase the heat resistance of the spores enough so that if they should gain entrance into the milk they would survive the usual heat treatment given the evaporated milk.

The relative constancy of the results obtained in the various heat resistance trials with each culture, as long as the growth temperature was the same, is in harmony with the work of Morrison and Rettger (11) but is not in agreement with the observations of Esty and Williams (4) and Magoon (10). The latter investigators consider the resistance of spores to heat as not a fixed property but a variable characteristic, influenced by a host of conditions rather than by one factor such as temperature of growth.

SUMMARY AND CONCLUSIONS

The work reported involved a study of the influence of growth temperature on the thermal resistance of spores of certain aerobic, spore-forming bacteria isolated from normal and spoiled evaporated milk.

Within the limits of the study, as imposed by the number and species of organisms used, the following points were established:

1. The thermal resistance of spores was influenced by the temperature at which the cultures were grown.

2. Growth temperatures below the optimum decreased the thermal resistance of the spores.

3. Growth temperatures above the optimum tended to decrease the thermal resistance of the spores, and the decreases were generally as great as that caused by growth at temperatures below the optimum.

4. Maximum thermal resistance of the spores was obtained by growth at the optimum temperature.

5. Some cultures isolated from evaporated milk, grown at the optimum temperature, produced spores which, when present in large numbers, survived the sterilization exposure normally used in manufacturing evaporated milk. This was true of cultures 1, 2, 4, 5, 10, 11 and 12.

6. Sudden decreases in growth temperature from the optimum always decreased the average thermal resistance of the spores, while sudden increases in growth temperature to the optimum always increased the thermal resistance, thus establishing the important influence of growth temperature on thermal resistance.

7. Continued growth of cultures at changed growth temperatures generally resulted in spores with a thermal resistance approximating that of spores of the cultures when originally grown and tested at the same temperatures.

8. The results of the study suggest that, in order to decrease the thermal resistance of bacteria likely to be found in evaporated milk and thus minimize or prevent spoilage losses, low temperatures should be maintained in the raw milk from the time of production until it reaches the forewarmer or preheater. Likewise, during any storage period previous to the sterilization process, low temperatures should be maintained.

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