THE INFLUENCE OF MILK FAT ON THE THERMAL DESTRUCTION OF *PSEUDOMONAS FRAGI*

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THE INFLUENCE OF MILK FAT ON THE THERMAL DESTRUCTION OF *PSEUDOMONAS FRAGI*

by

Lloyd O. Luedecke

AN ABSTRACT OF A THESIS

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ABSTRACT

THE INFLUENCE OF MILK FAT ON THE THERMAL DESTRUCTION OF PSEUDOMONAS FRAGI

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The thermal destruction rates of Pseudomonas fragi were investigated in skimmilk, milk containing 10 per cent milk fat, half-and-half and cream containing 20 per cent milk fat. P. fragi was also heated in reconstituted skimmilk which contained 0.1 per cent by weight of one of the following fatty acids: butyric, lauric or oleic.

All of the survivor curves prepared from the data obtained in this investigation showed a lag during the initial intervals of the heating trials. The slope of the straight line portion of the survivor curves was determined by linear regression and the D values were determined from the calculated slopes.

A z of 10 was obtained with the cells grown in half-and-half at 25° C. for 20 hours and heated in half-and-half at 48°, 50° and 52° C. A z of 13 was obtained when the cells were grown in half-and-half at 7° C. for 7 days and heated in half-and-half at 48°, 50° and 52° C. The cells grown at 7° C. required longer heating times to reduce the initial

cell population by 99.9999 per cent than did the cells grown at 25° C.

When cells were heated in skimmilk, milk containing 10 per cent milk fat and in cream containing 20 per cent milk fat, the z values were 14, 11 and 22, respectively. At 48° and 50° C. the cells grown and heated in skimmilk required longer heating times to destroy 99.99 per cent of the original cell population than did the cells grown and heated in cream containing 20 per cent milk fat. At 52° C. the times were nearly the same.

The above z values were obtained by heating the cells in a medium which contained the same concentration of milk fat as the medium in which they were grown. Heating trials were also conducted in which the cells were heated in a medium in which the concentration of milk fat differed from the concentration in which they were grown. The data from these trials indicated that the medium in which the cells were heated influenced the z values, but the medium in which the cells were grown did not seem to influence the z values. The largest z values were obtained when the cells were heated in cream containing 20 per cent milk fat and the z values were approximately of the same magnitude

when the cells were heated in skimmilk and in milk containing 10 per cent milk fat. The growth medium seemed to influence the times required to reduce the original cell population by 99.99 per cent, but the times did not seem to be affected by the medium in which the cells were heated. The longest times were required with the cells grown in skimmilk and the shortest times were required with the cells grown in cream containing 20 per cent milk fat.

The z values ranged from 11 to 14 when P. fragi was heated in the skimmilk-fatty acid mixtures. The similarity of the z values would seem to indicate that they were not influenced by the carbon chain length or the characteristics of saturation and unsaturation of the fatty acids used. Heating trials were conducted with and without adjustment of the pH to 6.7. When the pH of the skimmilk-butyric acid mixture was at 5.85 the times required to reduce the original cell population by 99.99 per cent were longer than those required when the pH was at 6.7. With lauric and oleic acid the heating times were approximately the same when the pH of the skimmilk-fatty acid mixtures were 6.7 and less than 6.7.

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INTRODUCTION

The spoilage of dairy products by psychrophilic bacteria has become an important problem to the dairy industry during recent years. This problem has resulted primarily from the present methods of handling and manufacturing dairy products. These methods often involve holding the dairy products at refrigeration temperatures for several days. At these temperatures the psychrophilic organisms are able to increase in numbers and will eventually cause spoilage by producing off-flavors and odors.

The literature contains numerous reports that indicate psychrophiles are destroyed in milk and skimmilk by pasteurization. Most of the work reported has been limited to the results obtained by laboratory pasteurization of samples of whole milk which contained inoculated or naturally occurring psychrophilic organisms. After pasteurization these milk samples were examined to determine if survivors were present. These investigations did not determine the rates at which psychrophiles are destroyed during heating.

The work reported herein was performed to investigate the thermal resistance of a psychrophile, Pseudomonas fragi, in dairy products which contain more milk fat than occurs in

whole milk. Literature reports indicate that in some instances bacterial cells and spores receive a limited amount of thermal protection from fat or oil.

The influence of the following variables on the thermal destruction rates of P. fragi were investigated; (a) the concentration of milk fat in the environment in which the cells were heated, (b) the concentration of milk fat in the environment in which the cells were grown and (c) the presence of butyric, lauric and oleic acids in the medium in which the cells were heated.

LITERATURE REVIEW

Thermal Destruction of Psychrophiles in Dairy Products

The destruction of psychrophilic bacteria in milk at pasteurization temperatures has been reported by many investigators. The psychrophilic species found most commonly in dairy products belong to the genera: Pseudomonas, Achromobacter, Flavobacterium, Alcaligenes, Escherichia and Aerobacter. Most of the species found in dairy products belong to the genus Pseudomonas.

Hussong et al. (19) stated that Pseudomonas fragi, which is a common psychrophilic contaminant in dairy products, was killed by "relatively low pasteurization exposures." According to Seleen and Stark (42) no Pseudomonas survived heating in milk at 60° C. for 30 minutes. Thomas and Sekhar (44) and Chandrasekhar (7) reported that laboratory pasteurization was sufficient to destroy those organisms which develop in milk at 3° to 5° C. Thirty minutes at 62.5° C. was sufficient to prevent a significant bacterial increase in milk during storage for 15 days at 4.4° to 7.3° C. (3). A total of 305 psychrophilic cultures were isolated from milk and none of the cultures survived laboratory pasteurization

(44, 47, 48). Thomas et al. (45) and Rogick and Burgwald (41) were unable to isolate psychrophilic organisms when they examined samples of milk that had been removed aseptically from vat and high-temperature-short-time (HTST) pasteurizing systems. Parker et al. (36) heated 10 psychrophilic cultures that had been incubated 64 hours at 25° C. and found that all of the cells were destroyed when heated in milk at 61.7° C. for 30 minutes. Each culture contained approximately 1,000,000 to 5,000,000 cells per milliliter. Olson et al. (35) reported that psychrophiles were destroyed when heated at pasteurization temperatures in thermal death time tubes. Only 6 of 41 cryophilic (psychrophilic) cultures isolated from butter by Jezeski and Macy (21) survived laboratory pasteurization at 150° F. (65.6° C.) for 30 minutes. Davis and Babel (11) observed that bacteria capable of forming slime on cottage cheese were destroyed by 2.5 minutes of exposure at 62.5° C. Thomas et al. (46) stated that all types of psychrophilic bacteria commonly found in milk, especially those able to grow and multiply at temperatures between 0° and 7° C., are destroyed by pasteurization.

In contrast to the above data, Kennedy and Weiser (25) reported that all but one of 15 psychrophilic cultures isolated from milk survived when heated in milk at 145° F. (62.8° C.) for 30 minutes.

Powell (37) reported on the destruction of organisms that caused a bitter flavor in cream stored at 35° F. (1.7° C.). He found that when cream containing the organisms was heated to 165°, 175° and 185° F. (73.9°, 79.5° and 85° C.) for 30 to 45 seconds the bitter flavor did not develop during 10 days of storage at 35° F. (1.7° C.). Erdman and Thornton (16) found that bacteria capable of growth at 10.5° C. did not survive commercial pasteurization in homogenized milk containing 10 per cent milk fat. These workers were not concerned with specific psychrophiles, therefore, they used milk that was known to contain a large number of psychrophiles representing a natural mixed flora.

El Sedek and Richards (15) reported on the destruction of the lipolytic flora found in raw cream. They found that one day of storage in "cool conditions" permitted the Pseudomonas and Achromobacter genera to increase in number, but this increase could be nullified by pasteurization.

None of the investigations mentioned above included a study of the thermal destruction rate of the psychrophiles. The studies were limited to experimental work in which the organisms were heated at a specific temperature for a specific length of time in various dairy products. The

products were examined after heating to determine whether survivors were present.

Kaufmann and Andrews (24) were the first to report on the thermal destruction rate of psychrophiles in dairy products. They used Pseudomonas viscosa and an organism tentatively identified as Pseudomonas mephitica. The authors found z values of 14.3 and 6.7 with P. viscosa and P. mephitica, respectively. They compared the two thermal death time curves to the milk pasteurization curve and noted that neither of the death curves crossed the pasteurization curve, indicating the lethality of the pasteurization process was sufficient to inactivate these two organisms, although the margin of safety was much less with P. viscosa. These workers suggested that on the basis of their results, some of the psychrophiles might survive HTST pasteurization in whole milk. Normally the margin of safety is less with the HTST process. Also, bacteria that are trapped in the fat or oil of a product may be protected from thermal destruction.

Other workers (5,8) have studied the effect of the cottage cheese cooking operation on common psychrophilic contaminants. Bonner and Harmon (5) found that none of the 17 cultures isolated from spoiled cottage cheese survived when heated to 48.9° C. for 15 minutes in whey at pH 4.55. Chaudhary et al. (8) reported

that three species of Pseudomonas failed to survive cooking at 120° F. (48.9° C.) for 30 minutes. These workers found z values of 12.4, 8.8 and 8.0 when P. fragi, P. viscosa and P. fluorescens, respectively, were heated in skimmilk. They concluded that none of these organisms would survive pasteurization in skimmilk or whole milk.

Influence of Fat on the Thermal Destruction of Microorganisms

Many workers have studied various factors influencing the thermal resistance of both spore-forming and non-spore-forming organisms. Some of the factors affecting the heat resistance of cells or spores are: (a) inherent resistance of the organism, (b) the conditions under which the cells or spores were formed and (c) the nature of the medium in which the cells or spores are heated.

Inherent resistance is illustrated by the fact that different strains of the same species will have different resistances even when propagated under the same conditions. Incubation temperature and composition of the growth medium are environmental influences that are active during the growth of the cells or formation of the spores of an organism.

The nature of the heating medium will affect the thermal resistance of cells or spores. The presence of fat in the heating medium is one factor which may influence the thermal destruction of microorganisms. There is a lack of agreement among workers concerning the amount of protection that cells or spores receive from the presence of fat in the heating medium and also, if the organisms are protected, there is lack of agreement concerning the mechanism of protection. For example, Lang (27) suggested that the poor conductivity of oil media resulted in increased heat resistance.

Jensen (20) reported the results of an experiment with streptococci which indicated large differences in the heat resistance of the cells in fatty and aqueous media. The streptococci were destroyed in "moist melted butter" after 15 minutes at 100° C., whereas in "dry butter," 50 minutes at 115° C. were required and at 120° C., 20 minutes were required. In milk the organisms were destroyed in 30 minutes at 61.7° C. Bartlett and Kinne (4) reported that Bacillus subtilis, Bacterium anthracis (Bacillus anthracis) and Bacillus vitalis were significantly more heat resistant in glycerine, olive oil, cottonseed oil and paraffin than in water. Rogacheva (40) suggested that the heat resistance of non-spore-forming bacteria was not increased by heating in fats and oils and

that the heat resistance of spores from the (Bacillus) subtilis-mesentericus group would increase only if the fats and oils were dehydrated.

Nichols (33) reported that when milk or cream was used as a heating medium, an increase in the fat content failed to increase the resistance of Bacillus subtilis spores and that non-sporulating bacteria may have been more subject to thermal destruction as the milk fat content was increased. Daoust et al. (13) heated Corynebacterium diphtheriae in whole milk, 40 per cent cream and ice cream mix and found that the cells were the least resistant in 40 per cent cream. However, Brown and Peiser (6) observed that Bacterium lactis acidi (Streptococcus lactis) and Bacterium coli (Escherichia coli) were protected by milk fat from thermal destruction.

Mudd and Mudd (31) developed a method for determining the behavior of various cells, including bacteria, at an oil-water interface. These workers found that cells with hydrophobic surfaces readily pass from the aqueous phase into the oil phase, whereas cells with hydrophilic surfaces usually remain at the interface. The behavior of bacteria at an oil-water interface can be influenced by various materials, such as fatty acids and soaps which enhance the passage of

bacteria into oils. Jensen (20) reported that an aqueous solution containing 0.05 per cent sodium oleate allowed all of the microorganisms tested to pass into the oil. In discussing the preparation of canned meats, he suggests that fatty acids and soaps should be eliminated as far as possible because the long chain fatty acids enhance the passage of bacteria into fat.

Mellon (30) postulated that calcium ions bring the lipoidal phase to the external surface of the bacterial cell wall, as can be shown by the fact that organisms will pass from an aqueous suspension to lipoidal solvents. This does not occur in the presence of the sodium ion. Later Jones and Lorenz (22) showed that in oil-water mixtures containing bacteria, calcium ions facilitate the passage of bacteria into the oil layer. The layer of oil around the cell may protect the organisms from destruction, but there is also evidence that this same layer of oil may trap the surviving cells. Consequently, cell development may not occur due to the lack of an aqueous environment. Thus, the food product may not spoil even though surviving cells are present. The above theory is based on the assumption that the cells receive thermal protection while they are coated with a layer of fat or oil.

Influence of Fatty Acids on the Growth of Microorganisms

Nieman (34) reviewed the influence of trace amounts of fatty acids on the growth of microorganisms. He reported that fatty acids in very low concentrations may inhibit or stimulate the growth of bacteria. The amount of inhibition or stimulation is dependent on the concentration and nature of the fatty acids and on the bacterial species involved. Generally only Gram positive organisms are susceptible to the influence of minute amounts of fatty acids, although influences on Gram negative organisms have been observed. Both saturated and unsaturated fatty acids may influence the growth of microorganisms. The antibacterial activity of unsaturated fatty acids increases with the number of double bonds in the molecule and the natural cis-forms are generally more inhibitory than the trans-isomers. Antibacterial activity of saturated fatty acids is optimal when the chain length is approximately 12 carbon atoms.

Nieman (34) concluded his review by pointing out that a completely satisfactory theory had not yet been advanced explaining growth inhibition by fatty acids, but the most logical explanation is attributed to the changes in cell

permeability caused by fatty acids adsorbed at the cell membrane.

Costilow and Speck (10) tested the effect of all the fatty acids common to milk and found that the growth of Streptococcus lactis was inhibited by caprylic, capric and lauric acid.

Nashif and Nelson (32) studied the effect of fatty acids on the lipase production of P. fragi. They found that the addition of small amounts of tricaprylin, caprylic and capric acid to vitamin-free casamino acids or peptone media caused a pronounced increase in lipase production. However, caprylic acid was the only one of the three that increased both cell population and lipase production. Lauric acid was found to be inhibitory to the strain of P. fragi used by these workers.

EXPERIMENTAL PROCEDURES

This investigation was conducted to determine the influence of milk fat and selected fatty acids on the thermal resistance of Pseudomonas fragi, a common psychrophilic contaminant of dairy products which grows well at refrigeration temperatures, but as is the case with most psychrophilic bacteria, the optimum growth temperature is 20° to 25° C.

The studies on thermal destruction were divided into four different sections which involved heating the cells in:

I commercial half-and-half

II (a) fresh fluid skimmilk

(b) cream containing 20 per cent milk fat which was prepared by blending fresh fluid skimmilk and pasteurized whipping cream

III (a) reconstituted skimmilk

(b) reconstituted milk containing 10 per cent milk fat which was prepared by blending reconstituted skimmilk and cream containing 42 per cent milk fat

(c) reconstituted cream containing 20 per cent milk fat which was prepared by blending reconstituted skimmilk and cream containing 42 per cent milk fat

IV reconstituted skimmilk containing 0.1 per cent by weight of a selected fatty acid

The heat resistance of cells grown at 7° C. was compared to the heat resistance of cells grown at 25° C. when commercial half-and-half was used as the heating medium.

The influence of varying concentrations of milk fat on the heat resistance of P. fragi was investigated using the two media listed in II above. The data obtained by heating the cells in these two media were compared with the data obtained from the cells which were grown in half-and-half at 25° C. After analyzing the data obtained from using these three media, it seemed that a second study should be made using media which were prepared from the same milk fat and serum solids. The three media listed in III above were prepared from non-fat-dry-milk-solids and cream containing 42 per cent milk fat. These three media were used to compare the influence of the heating medium on the thermal resistance of P. fragi cells and also to determine the influence of the growth medium on the heat resistance of P. fragi. This was accomplished by growing the cells in a medium with a specific concentration of milk fat and heating the cells in media which contained different concentrations of milk fat. For example, cells grown in reconstituted skimmilk were heated in (a) reconstituted skimmilk, (b) reconstituted milk containing 10 per cent milk fat and (c) reconstituted cream

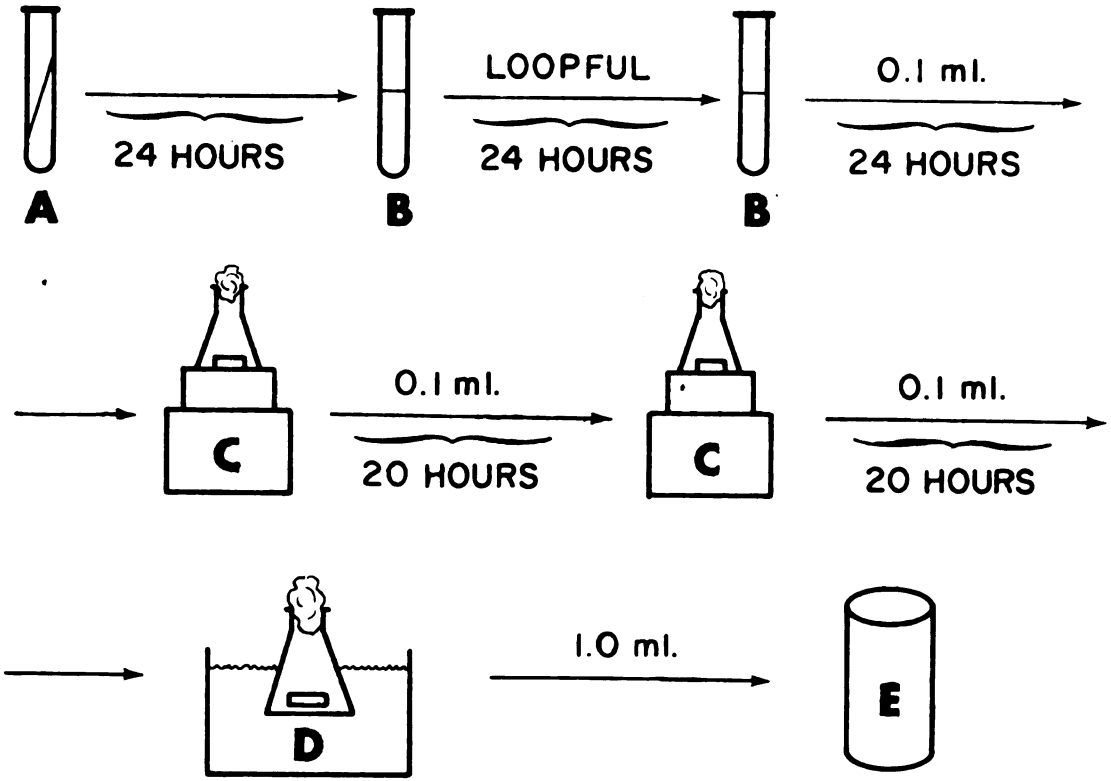
containing 20 per cent milk fat. Utilizing these three media, tests were performed on cells subjected to all of the nine possible combinations of growing and heating the cells.

The thermal destruction studies involving selected fatty acids were performed by growing the cells in reconstituted skimmilk and then heating the cells in reconstituted skimmilk containing 0.1 per cent by weight of each fatty acid. The fatty acids were butyric, lauric and oleic.

Preparation of Cells

The procedure used to culture *P. fragi* was carefully standardized to obtain cells with the same heat resistance and also to obtain approximately the same number of cells per milliliter each time they were propagated.

Figure 1 is a schematic diagram of the procedure followed in growing the cells for thermal destruction studies. The procedure as shown was used to grow the cells in skimmilk and in milk containing 10 to 11 per cent milk fat. When the cells were grown in cream containing 20 per cent milk fat the procedure was modified slightly. In growing the cells a tube of trypticase soy broth was inoculated and incubated 24 hours at 25° C. After 24 hours of incubation, a loopful of culture was transferred to a



A. 24 hour culture on a plate count agar slant
B. Trypticase soy broth
C. 50 ml. of the desired growth medium. The Erlenmeyer flask, containing a magnetic stirring bar, was held 2 inches above the surface of the magnetic agitator by a copper wire stand
D. Ice bath
E. Medium at the desired heating temperature

Fig. 1. A schematic diagram of the procedure used in preparing the cells for thermal destruction studies.

second tube of broth and after additional incubation at 25° C. for 24 hours, 0.1 ml. of inoculum was placed into 50 ml. of the desired medium. The inoculated medium was agitated continuously with a magnetic stirrer throughout incubation at 25° C. for 20 hours. After the 20 hours, 0.1 ml. of culture was transferred to a second flask containing the same medium. This inoculated medium was also incubated with agitation for 20 hours at 25° C., after which the cells were used in the heating trials. To minimize conduction of heat from the motor of the agitator into the contents of the flask, a small copper wire stand was used to provide a two inch air space between the flask and the top of the agitator.

The cells propagated in cream containing 20 per cent milk fat were grown as illustrated in Figure 1, except agitation was not used as it resulted in churning of the milk fat; the incubation time was increased to 24 hours without agitation. The cells grown in half-and-half at 7° C. were also grown according to the procedure in Figure 1, except the time between transfers was 7 days. Agitation was not used in growing the cells at 7° C. because of the

reason given above. Preliminary studies showed that the cells would be in the same growth phase after incubation under the following conditions: (a) 20 hours with agitation at 25° C., (b) 24 hours without agitation at 25° C. and (c) 7 days without agitation at 7° C.

After the final incubation period the cultures were placed in an ice bath and held at 0° C. until one milliliter portions were removed for use in the heating trials at 48°, 50° and 52° C. Placing the cells in the ice bath stopped their growth and assured having cells that were in the same growth phase at the time of heating at each of the three temperatures.

Preparation of Half-and-half as a Heating Medium

Commercial half-and-half was used as a heating medium in the preliminary part of this research program. The half-and-half was purchased as needed from the Michigan State University Dairy Store. Two-hundred milliliter quantities of this heating menstrum were placed in stainless steel heating chambers (24) and covered with parchment paper. The heating chambers and contents were sterilized and allowed to cool before the sterile closure was attached to the heating

chamber. Allowing the menstrum to cool before attaching the closure prevented the formation of a vacuum which pulled the rubber gasket into the chamber.

Preparation of Fresh Skimmilk and Cream Containing 20 Per Cent Milk Fat as Heating Media

The need for a standardized heating medium became quite evident when half-and-half was used as the heating medium. Therefore, the skimmilk and 20 per cent cream were prepared in sufficient quantities to adequately provide the needs of this experiment. These media were prepared from fresh, pasteurized skimmilk and whipping cream. The 20 per cent cream was heated to 60° C. and homogenized in a commercial three piston homogenizer at 2500 pounds per square inch (psi). Only one stage of the homogenizer was used, consequently the 20 per cent cream was very viscous. The skimmilk and 20 per cent cream were dispensed in 200 ml. quantities into 8 ounce jars with screw-cap lids, sterilized at 121° C. for 15 minutes, and stored at 4° C. Immediately before the heating trial the desired heating medium was aseptically transferred into a sterilized heating chamber and sealed.

Preparation of Reconstituted Skimmilk, Reconstituted Milk Containing 10 Per Cent Milk Fat and Reconstituted Cream Containing 20 Per Cent Milk Fat as Heating Media

These three heating media were prepared from reconstituted skimmilk containing 8.30 per cent total solids and raw 42 per cent cream. This skimmilk was used as a heating medium and also to standardize the other two media to the desired concentration of milk fat. All three media were prepared in sufficient quantity to provide media for all of the experiments. After standardization each medium was treated as described in the previous section. These media were used in two separate experiments: (a) the growth and heating media contained the same concentration of milk fat and (b) the growth and heating media contained different concentrations of milk fat.

Preparation of Reconstituted Skimmilk Containing Selected Fatty Acids as Heating Media

Reconstituted skimmilk was used as a base to which the fatty acids were added. The lauric and oleic acids were added directly to the skimmilk to give a 0.1 per cent concentration by weight. These skimmilk-fatty acid mixtures were then homogenized at 2500 psi in a single piston pilot

plant homogenizer. Since lauric and oleic acids are essentially insoluble in water, homogenization was necessary to obtain uniform dispersion in the skimmilk. After homogenization the mixtures were dispensed in 200 ml. quantities into 8 ounce jars and sterilized. Prior to sterilization nitrogen was bubbled for one minute through the skimmilk containing oleic acid to minimize oxidation of the fatty acid. The skimmilk-fatty acid mixtures were held at 4^{o} C. until the time of use. The heating menstrum containing butyric acid was prepared by aseptically adding sterile butyric acid to sterile skimmilk immediately before beginning the heating trial. This menstrum was prepared in this manner since a skimmilk-butyric acid mixture would coagulate during sterilization.

When the skimmilk contained butyric, lauric or oleic acid in a 0.1 per cent concentration, the pH was reduced to 5.85, 6.6 and 6.45, respectively. Therefore, in order to eliminate differences due to pH, heating trials were conducted with and without the pH adjusted to 6.7 which is normal for skimmilk. In the trials requiring pH adjustment, a sterile saturated solution of trisodium phosphate (Na_3PO_4) was used. The trisodium phosphate was added aseptically to the menstrum in the heating chambers immediately before beginning the heating trial.

Heating Procedure

The thermal destruction studies were made using equipment and methods similar to those described by Kaufmann and Andrews (24). This method permits tempering of the heating menstrum to the desired temperature before adding the cells.

A copper-constantan thermocouple and recording potentiometer were used to determine when the heating menstrum was at the desired temperature. When the menstrum was at the desired temperature a sterile 2 ml. glass hypodermic syringe fitted with a sterile 2-inch, 16-gauge needle was used to inject 1.0 ml. of the prepared culture into the heating chamber. At intervals throughout the heating period samples were taken with sterile 5 ml. glass hypodermic syringes fitted with 6-inch, 16-gauge needles. Approximately eight seconds were required to remove a sample and place it in a sterile chilled test tube. The tube was shaken in an ice bath for an additional 20 seconds to further aid in cooling. At the end of 20 seconds the sample temperature was near 0° C.

The samples were held in an ice bath until plated; this holding time was normally 30 to 45 minutes. Triplicate plates were prepared using tryptone glucose extract agar

containing 0.2 per cent yeast extract (TGEY). The plates were incubated for 48 to 72 hours at 25° C. The samples that were heated at 48° and 50° C. were incubated 48 hours, while those heated at 52° C. were incubated 72 hours. The organisms heated at 52° C. were much slower in recovering and required the additional time to form visible colonies.

Determination of Thermal Destruction Rates

The thermal resistance of *P. fragi* was determined at 48°, 50° and 52° C. in nineteen different sets of experiments which involved variations in pre-incubation of the cells and variations in the media in which the cells were grown and heated. In each of these nineteen different studies, at least two to four heating trials were conducted at each of the heating temperatures. The data obtained from the heating trials at each of the three temperatures were pooled and the statistical method of linear regression was used to prepare a single destruction curve which represented the pooled data. The organisms were not destroyed at a logarithmic rate during the initial interval of the heating period; therefore, the data obtained during the initial intervals were not included in the pool. The approximate shape of the curve during the initial interval

of heating is shown as a "broken line." The linear regression calculations determined the slope of each individual destruction curve. The "D" value of each curve was obtained by determining the reciprocal of the calculated slope. The definition of "D" is the number of minutes required to reduce the cell population by 90 per cent during the logarithmic death rate. Confidence limits at the 95 per cent level were also applied to the D values.

The slope of the thermal resistance curve for each of the different experiments was determined by applying the statistical method of least squares to the D values. The "z" value of the thermal resistance curve was obtained by determining the reciprocal of the calculated slope. The definition of "z" is the number of degrees Fahrenheit necessary to cause a ten-fold change in the time required to destroy 90 per cent of the cells.

The thermal resistance curve is a phantom thermal death time curve in that it has shape but not position. In order to prepare thermal death time curves that could be compared to the milk pasteurization curve, the time required to decrease the initial cell population by 99.9999 per cent at 48^{o}, 50^{o} and 52^{o} C. was determined. These times were

determined from the data obtained with the three media listed under I and II on page 13. The 99.9999 per cent destruction times were obtained by extrapolation from the survivor curves when they were extended to 99.9999 per cent destruction. The times required to reduce the initial cell populations by 99.9999 per cent are longer than six D values because the survivor curves always contained a lag period during the initial interval of the heating trials. Thermal death time curves were not prepared from the data obtained when P. fragi was heated in the media listed under III and IV on page 13. When P. fragi was heated in these media the times required to reduce the initial cell population by 99.99 per cent were determined. This amount of destruction could normally be determined from the survivor curves without extending the lines.

RESULTS

Thermal Destruction of P. fragi in Half-and-half

Grown in half-and-half at 25° C. for 20 hours and heated in half-and-half. A number of trials were made in which the thermal resistance of P. fragi was measured when commercial half-and-half was used as a growth medium and as a heating medium. The data in Table 1 show the average number of P. fragi per milliliter of sample at various time intervals during heat treatment in half-and-half at 48°, 50° and 52° C. The average was determined from triplicate plates. The "straight line" portion of each survivor curve shown in Figure 2 was prepared by the linear regression method. During the initial interval of heating a lag in cell destruction occurred, consequently the survivor curves show an initial deviation from linearity. This portion of the curve was not considered in the analyses; a broken line has been used to indicate the approximate shape of the curves. The D values calculated from the straight line portion of the survivor curves in Figure 2 were 5.4, 3.0 and 1.0 at 48°, 50° and 52° C., respectively. The slope of the thermal resistance curve shown in Figure 3 was determined

TABLE 1. Number of P. fragi per milliliter surviving heat treatment in half-and-half after growth in half-and-half at 25° C. for 20 hours*

Temperature (° C.)					
48		50		52	
Time (Min.)	Survivors	Time (Min.)	Survivors	Time (Min.)	Survivors
0	**	0	**	0	**
4	2,100,000	2.5	410,000	3	200,000
8	350,000	6	220,000	4	72,000
12	140,000	8	50,000	5	6,900
16	**	10	5,700	6	490
20	2,400	12	1,000	7	60
24	500	14	110	8	30
0	6,300,000	0	6,300,000	0	6,300,000
4	6,500,000	3	6,100,000	3	1,300,000
8	4,100,000	6	1,400,000	4	300,000
12	1,600,000	8	630,000	5	43,000
16	100,000	10	150,000	6	120
20	**	12	21,000	7	20
24	4,600	14	3,800		
0	2,900,000	0	2,900,000	0	2,900,000
4	3,800,000	3	4,100,000	3	1,300,000
8	3,500,000	5	2,400,000	4	430,000
12	1,200,000	8	520,000	5	73,000
20	130,000	12	74,000	7	6,200
24	7,600	14	11,000	8	360

* Counts represent the average of triplicate plates
** Laboratory accident

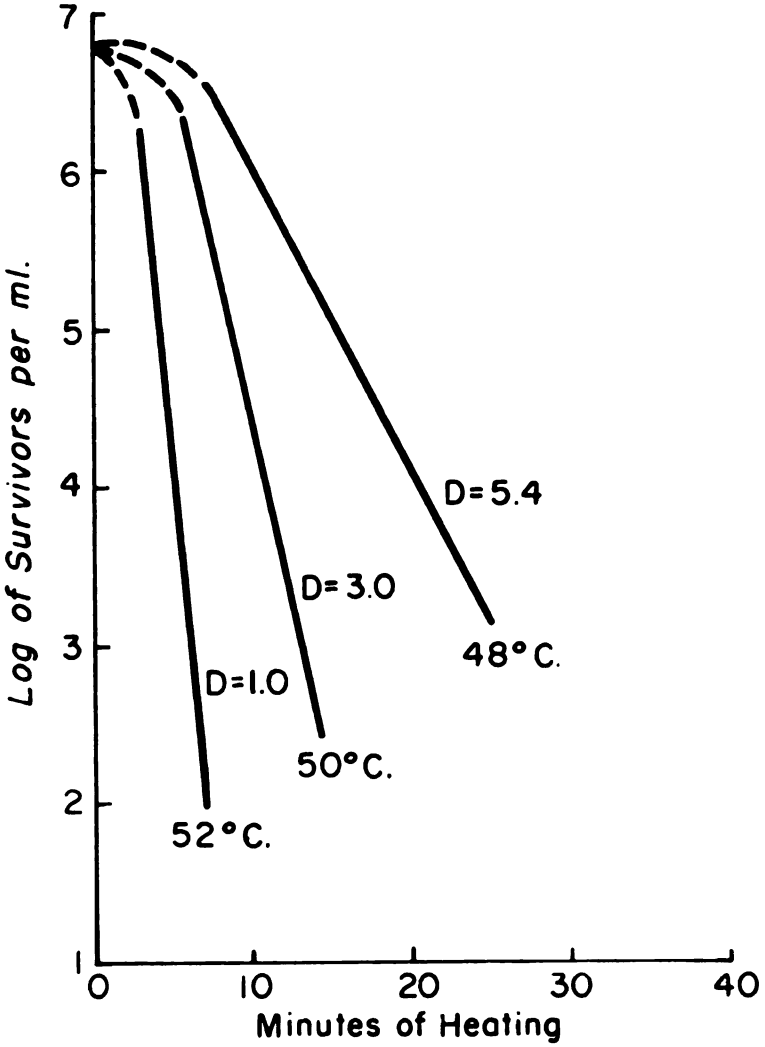


Fig. 2. Rate of destruction of P. fragi in half-and-half after growth in half-and-half at 25° C. for 20 hours.

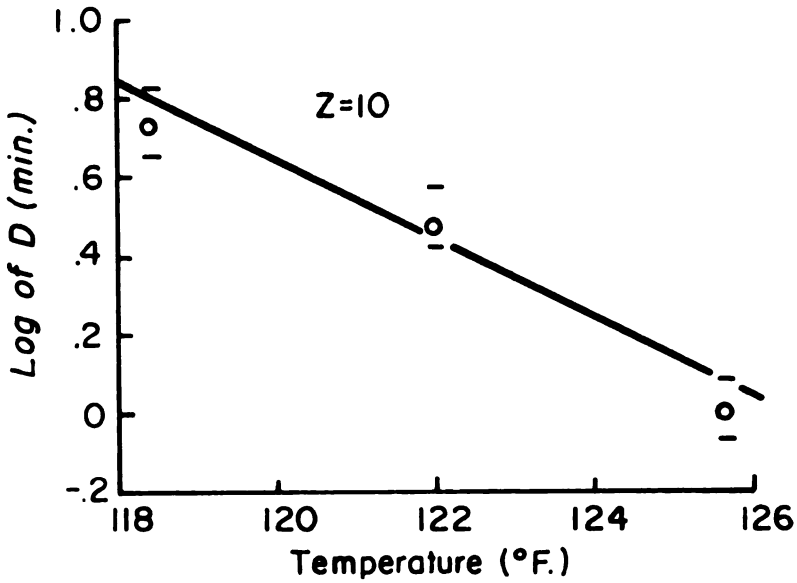


Fig. 3. Thermal resistance curve of P. fragi in half-and-half after growth in half-and-half at 25° C. for 20 hours.

from the D values using the method of least squares. The z value obtained from this thermal resistance curve was 10. The 95 per cent confidence limits of the D values are shown in Figure 3 and in Table 8. The times required to reduce the initial cell population by 99.9999 per cent were 38, 18 and 6 minutes at 48°, 50° and 52° C., respectively.

Grown in half-and-half at 7° C. for 7 days and heated in half-and-half. The data presented in Table 2 are the number of P. fragi surviving at various time intervals during the heating trials. These data are shown in the form of survivor curves in Figure 4. The D_{48}, D_{50} and D_{52} values obtained from these curves were 10.0, 5.0 and 2.7, respectively. The z value obtained from the thermal resistance curve shown in Figure 5 was 13. The extrapolated survivor curves showed that 99.9999 per cent of the initial cell population was destroyed in 65 minutes at 48° C., 40 minutes at 50° C. and 19 minutes at 52° C.

TABLE 2. Number of P. fragi per milliliter surviving heat treatment in half-and-half after growth in half-and-half at 7°C. for 7 days*

Temperature (° C.)					
48		50		52	
Time (Min.)	Survivors	Time (Min.)	Survivors	Time (Min.)	Survivors
0	4,100,000	0	4,100,000	0	4,100,000
12	730,000	4	2,700,000	3	1,500,000
18	180,000	8	830,000	6	250,000
24	51,000	12	190,000	8	62,000
30	11,000	16	20,000	10	6,100
35	3,000	20	2,700	12	1,000
40	1,200	24	660	14	180
0	11,000,000	0	11,000,000		
12	1,400,000	4	5,300,000		
18	330,000	8	1,400,000		
24	84,000	12	340,000		
30	29,000	16	61,000		
35	7,200	20	9,200		
40	2,700	24	1,700		
0	3,600,000	0	3,600,000	0	3,600,000
12	430,000	4	1,500,000	3	1,200,000
18	68,000	8	380,000	6	210,000
24	9,900	12	64,000	8	31,000
30	2,100	16	4,400	10	3,500
35	580	20	560	12	330
40	240	24	120	14	130
0	4,100,000			0	4,100,000
12	1,200,000			3	1,800,000
18	390,000			6	620,000
24	150,000			8	200,000
30	33,000			10	53,000
35	8,800			12	11,000
40	3,900			14	3,000

* Counts represent the average of triplicate plates

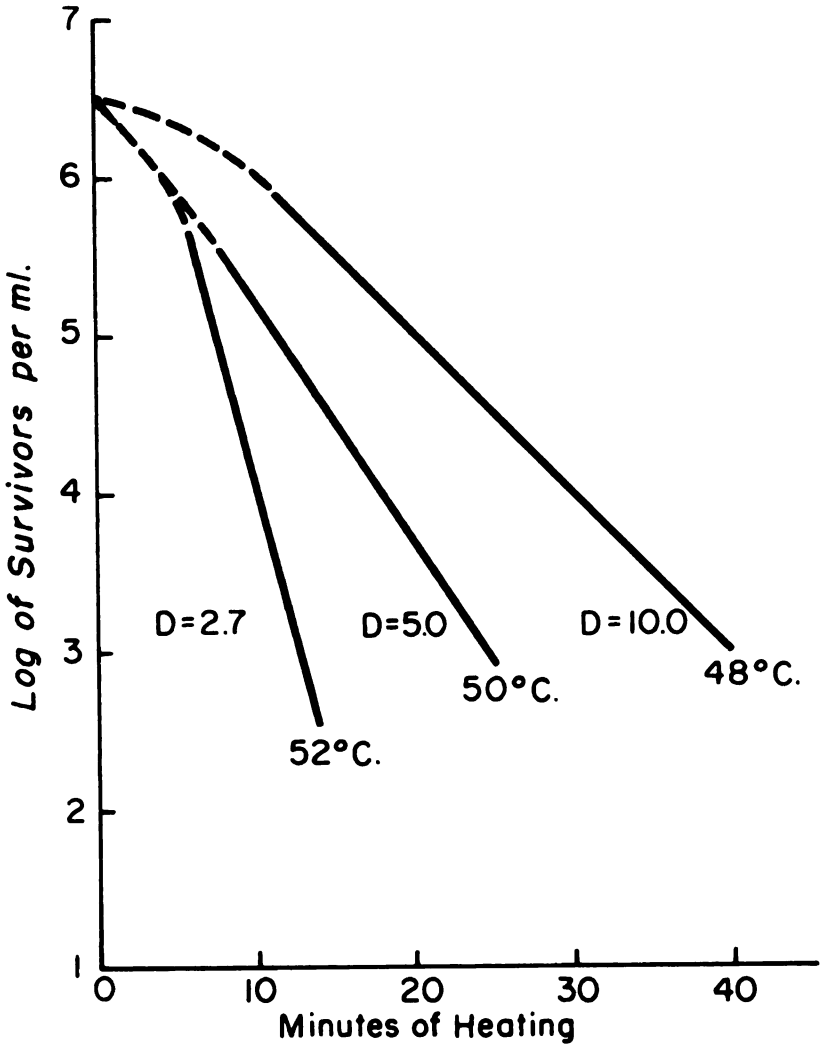


Fig. 4. Rate of destruction of P. fragi in half-and-half after growth in half-and-half at 7° C. for 7 days.

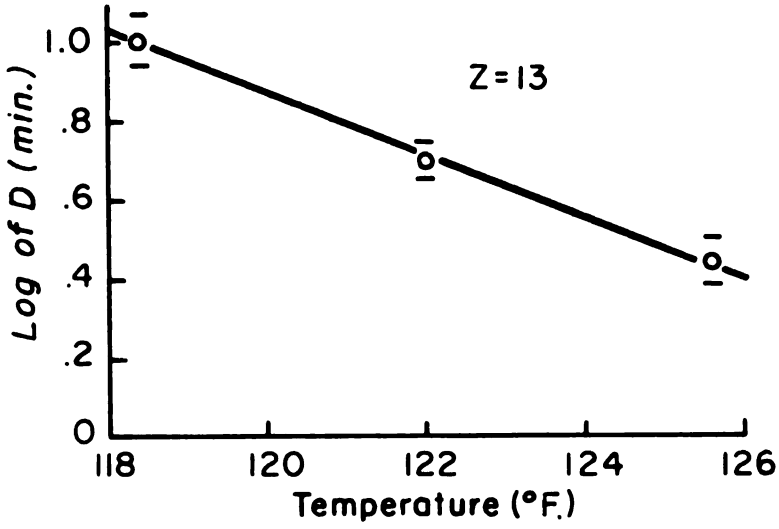


Fig. 5. Thermal resistance curve of P. fragi in half-and-half after growth in half-and-half at 7° C. for 7 days.

Thermal Destruction of P. fragi in Fresh Skimmilk and Cream Containing 20 Per Cent Milk Fat

In these studies the media were prepared in sufficient quantity to permit the use of the same batch throughout the heating trials desired in this section.

Grown and heated in skimmilk. The average number of survivors per milliliter at various time intervals during heating in skimmilk at 48°, 50° and 52° C. is shown in Table 3. These data were used in preparing the survivor curves shown in Figure 6. At 48° C. the initial lag period continued for approximately 25 minutes after which the cells were destroyed in a logarithmic order. The D values at 48°, 50° and 52° C. were 12.5, 6.2 and 1.9, respectively. Figure 7 contains the thermal resistance curve prepared from the D values and a z of 9 was noted. The time required to destroy 99.9999 per cent of the population was 95, 48 and 18 minutes at 48°, 50° and 52° C., respectively. In Figure 8, the thermal death time curve is compared to the milk pasteurization curve. Also included in Figure 8 are the thermal death time curves that were prepared from the data obtained when the cells were grown at 25° C. in half-and-half and grown in cream containing 20 per cent milk fat.

TABLE 3. Number of *P. fragi* per milliliter surviving heat treatment in skimmilk after growth in skimmilk at 25° C. for 20 hours*

Temperature (° C.)					
48		50		52	
Time (Min.)	Survivors	Time (Min.)	Survivors	Time (Min.)	Survivors
0	**	0	**	0	**
8	6,200,000	5	5,700,000	3	6,900,000
16	4,700,000	10	2,800,000	6	2,800,000
24	3,800,000	15	510,000	8	880,000
32	1,100,000	20	210,000	10	210,000
40	230,000	25	13,000	12	14,000
48	46,000	30	1,500	14	690
0	3,500,000	0	3,500,000	0	3,500,000
8	3,300,000	5	3,500,000	3	3,700,000
16	2,200,000	10	1,400,000	6	830,000
24	1,500,000	15	210,000	8	220,000
32	290,000	20	37,000	10	36,000
40	53,000	25	3,800	12	2,300
48	21,000			14	130
		0	9,800,000		
		5	9,000,000		
		10	5,200,000		
		15	1,000,000		
		20	120,000		
		25	30,000		
		30	2,100		

* Counts represent the average of triplicate plates
** Laboratory accident

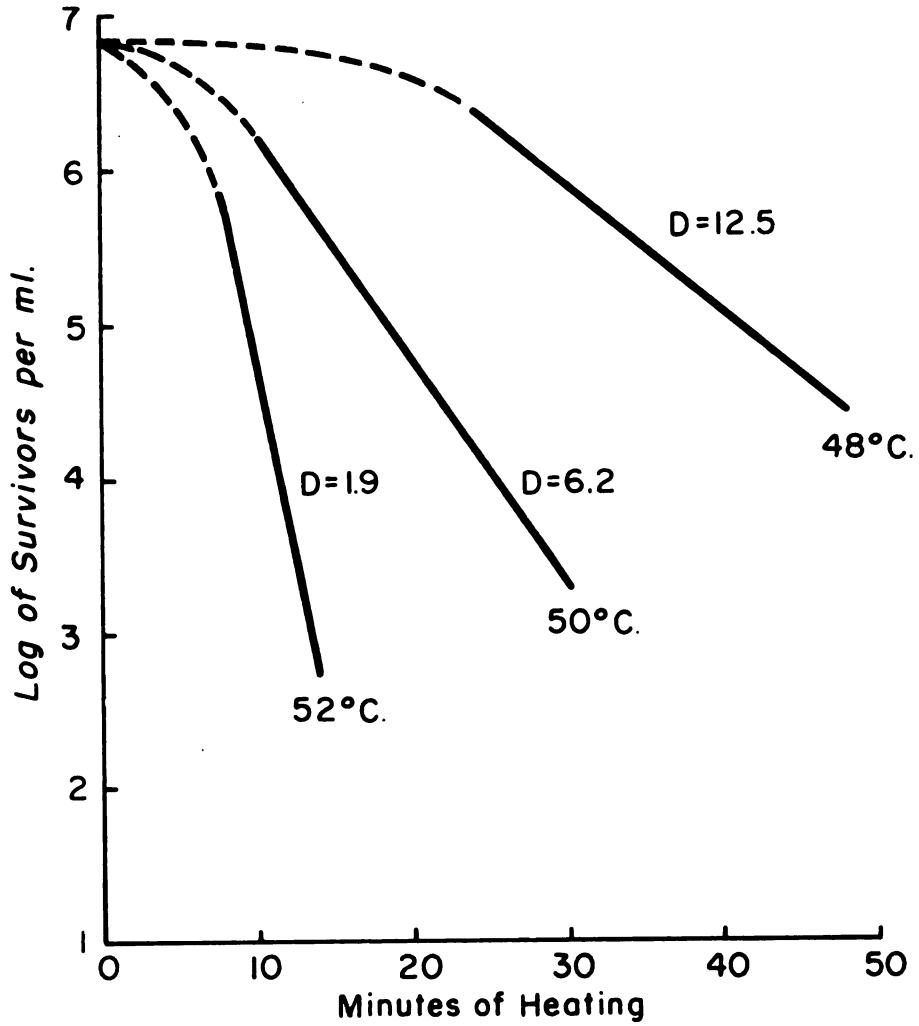


Fig. 6. Rate of destruction of P. fragi in skimmilk after growth in skimmilk at 25° C. for 20 hours.

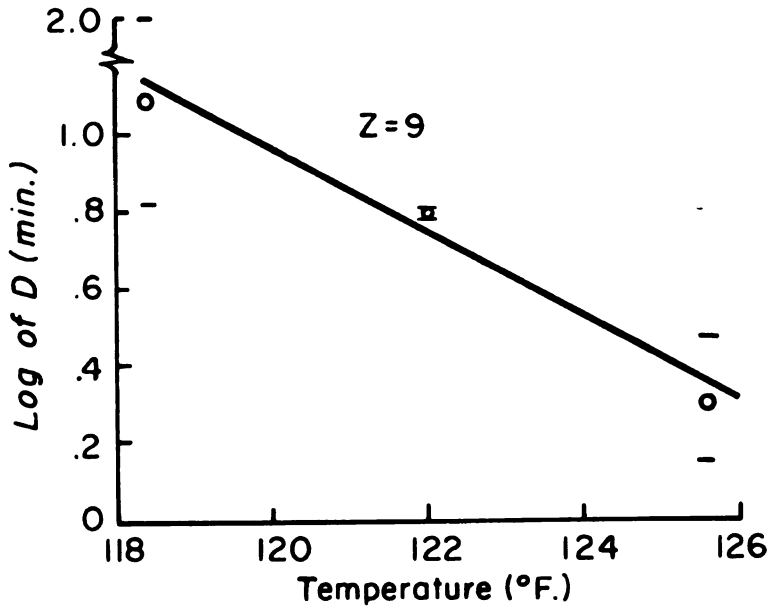


Fig. 7. Thermal resistance curve of P. fragi in skimmilk after growth in skimmilk at 25° C. for 20 hours.

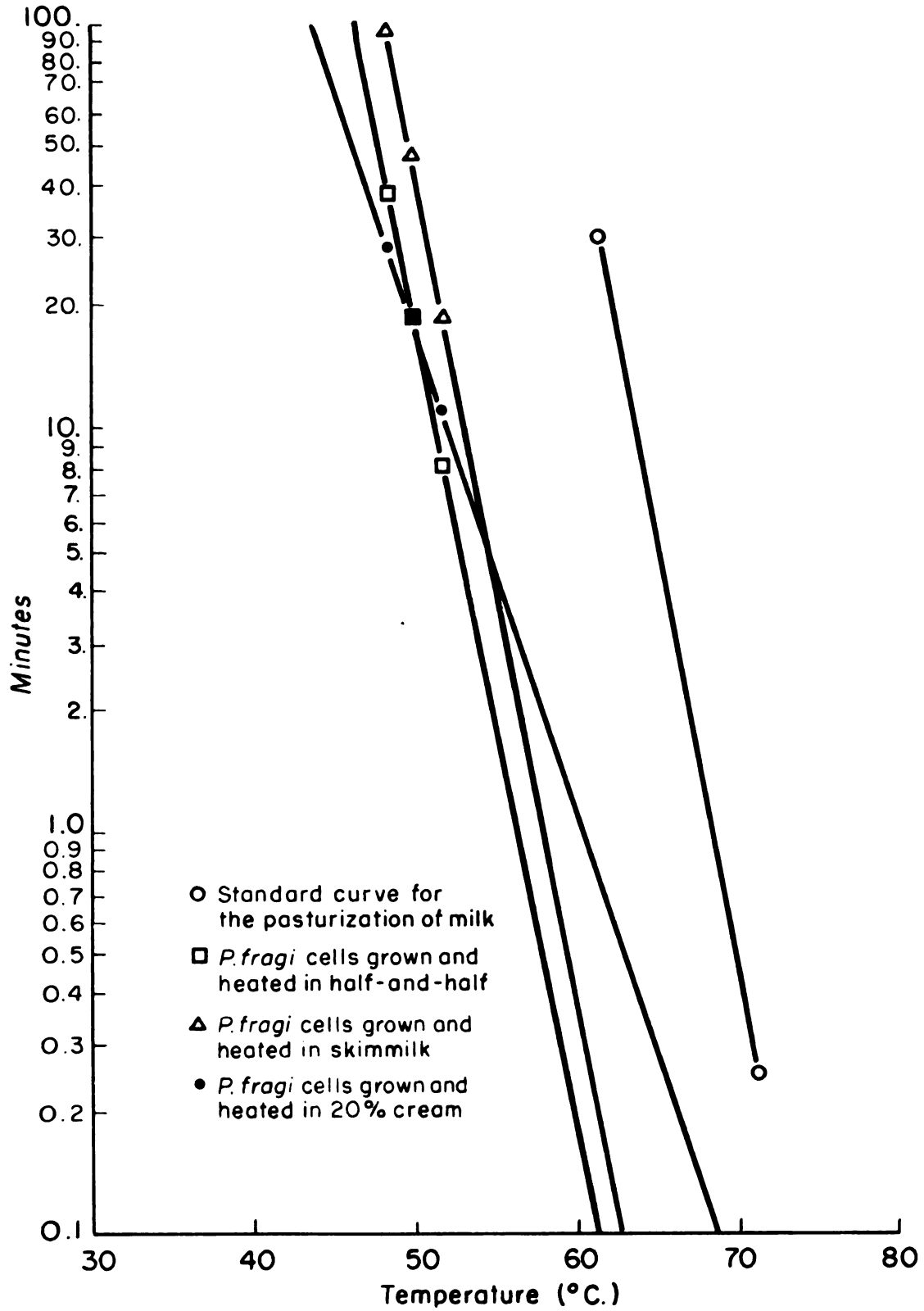


Fig. 8. A comparison of the thermal death time curves of P. fragi in media containing 0, 11 and 20 per cent milk fat with the milk pasteurization curve. (The thermal death time curves are a plot of temperature versus the number of minutes required to bring about a 99.9999 per cent reduction in the original cell population)

Grown and heated in cream containing 20 per cent milk fat. The destruction rate curves shown in Figure 9 were prepared from the data in Table 4. When the cells were heated at 48°, 50° and 52° C. the D values derived from the destruction curves were 4.3, 2.4 and 1.4, respectively. The initial lag periods in these survivor curves are not as long as those obtained with the cells grown and heated in skimmilk. The thermal resistance curve is shown in Figure 10 and a z of 15 was calculated from the slope. At 48°, 50° and 52° C., 99.9999 per cent of the original cell population was destroyed in 28, 18 and 11 minutes, respectively. The thermal death time curve prepared from these times is shown in Figure 8.

Thermal Destruction of P. fragi in Reconstituted Skimmilk, Reconstituted Milk Containing 10 Per Cent Milk Fat and Reconstituted Cream Containing 20 Per Cent Milk Fat

Skimmilk powder and cream containing 42 per cent milk fat were utilized in preparing the following three media: (a) reconstituted skimmilk, (b) reconstituted milk containing 10 per cent milk fat and (c) reconstituted cream containing 20 per cent milk fat.

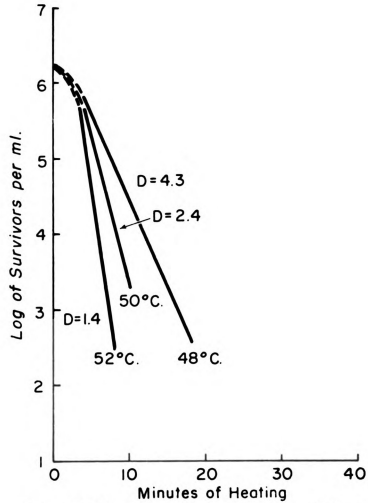


Fig. 9. Rate of destruction of P. fragi in cream containing 20 per cent milk fat after growth in cream containing 20 per cent milk fat at 25° C. for 24 hours.

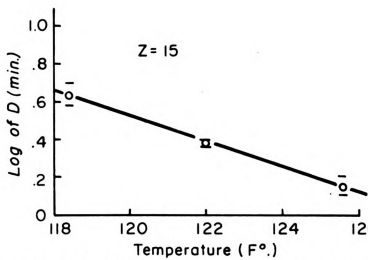


Fig. 10. Thermal resistance curve of P. fragi in cream containing 20 per cent milk fat after growth in cream containing 20 per cent milk fat at 25° C. for 24 hours.

Table 4. Number of P. fragi per milliliter surviving heat treatment in cream containing 20 per cent milk fat after growth in cream containing 20 per cent milk fat at 25° C. for 24 hours*

Temperature (° C.)					
48		50		52	
Time (Min.)	Survivors	Time (Min.)	Survivors	Time (Min.)	Survivors
0	2,400,000	0	2,400,000	0	2,400,000
4	1,300,000	3	1,400,000	3	900,000
8	130,000	6	97,000	4	330,000
12	3,500	8	9,200	5	67,000
16	2,100	10	1,600	6	13,000
20	110			7	3,700
24	70			8	280
0	1,500,000	0	1,500,000	0	1,500,000
4	920,000	3	790,000	3	410,000
8	80,000	6	68,000	4	190,000
12	8,000	8	4,100	5	30,000
16	1,000	10	880	6	3,300
20	320			7	310
24	80			8	50
0	1,700,000	0	2,100,000	0	2,100,000
4	890,000	3	1,500,000	3	870,000
8	110,000	6	230,000	4	240,000
12	6,600	8	34,000	5	41,000
16	690	10	4,500	6	9,600
				7	2,700
				8	420

* Counts represent the average of triplicate plates

Grown and heated in reconstituted skimmilk. The average counts obtained from triplicate plates are shown in Table 5. These data are depicted as destruction rate curves in Figure 11 and the D values at 48°, 50° and 52° C. are 6.2, 3.6 and 1.9, respectively. The z calculated from these D values was 14 (Figure 12). The initial cell populations were reduced by 99.99 per cent in 46, 27 and 12 minutes at 48°, 50° and 52° C., respectively.

Grown and heated in reconstituted milk containing 10 per cent milk fat. The data in Table 6 show the average number of survivors per milliliter at various time intervals during heating at 48°, 50° and 52° C. The survivor curve prepared from the data obtained at 48° C. has a lag period that continues for approximately 45 minutes (Figure 13) and a total of 75 minutes was required to destroy 99.99 per cent of the initial cell population. At 50° and 52° C., 99.99 per cent of the initial cell population was destroyed in 24 and 11 minutes, respectively. The D_{48}, D_{50} and D_{52} values are 10.5, 5.3 and 2.4, respectively. The thermal resistance curve prepared from these D values is shown in Figure 14 and the z value is 11.

TABLE 5. Number of P. fragi per milliliter surviving heat treatment in reconstituted skimmilk after growth in reconstituted skimmilk at 25° C. for 20 hours*

Temperature (° C.)					
48		50		52	
Time (Min.)	Survivors	Time (Min.)	Survivors	Time (Min.)	Survivors
0	8,200,000	0	8,200,000	0	8,200,000
10	7,800,000	5	7,400,000	3	**
20	**	10	4,400,000	6	1,700,000
30	1,600,000	15	2,200,000	8	810,000
40	110,000	20	750,000	10	210,000
50	1,100	25	100,000	12	32,000
60	43	30	3,300	14	1,100
0	600,000	0	600,000	0	600,000
10	420,000	5	180,000	3	440,000
20	350,000	10	**	6	150,000
30	13,000	15	66,000	8	8,900
40	300	20	5,100	10	500
		25	220	12	20
0	4,100,000	0	4,100,000	0	4,100,000
10	7,100,000	5	2,800,000	3	1,900,000
20	3,300,000	10	750,000	6	210,000
30	130,000	15	150,000	8	78,000
40	4,300	20	54,000	10	6,500
50	90	25	3,600	12	740
		30	94	14	60

* Counts represent the average of triplicate plates
** Laboratory accident

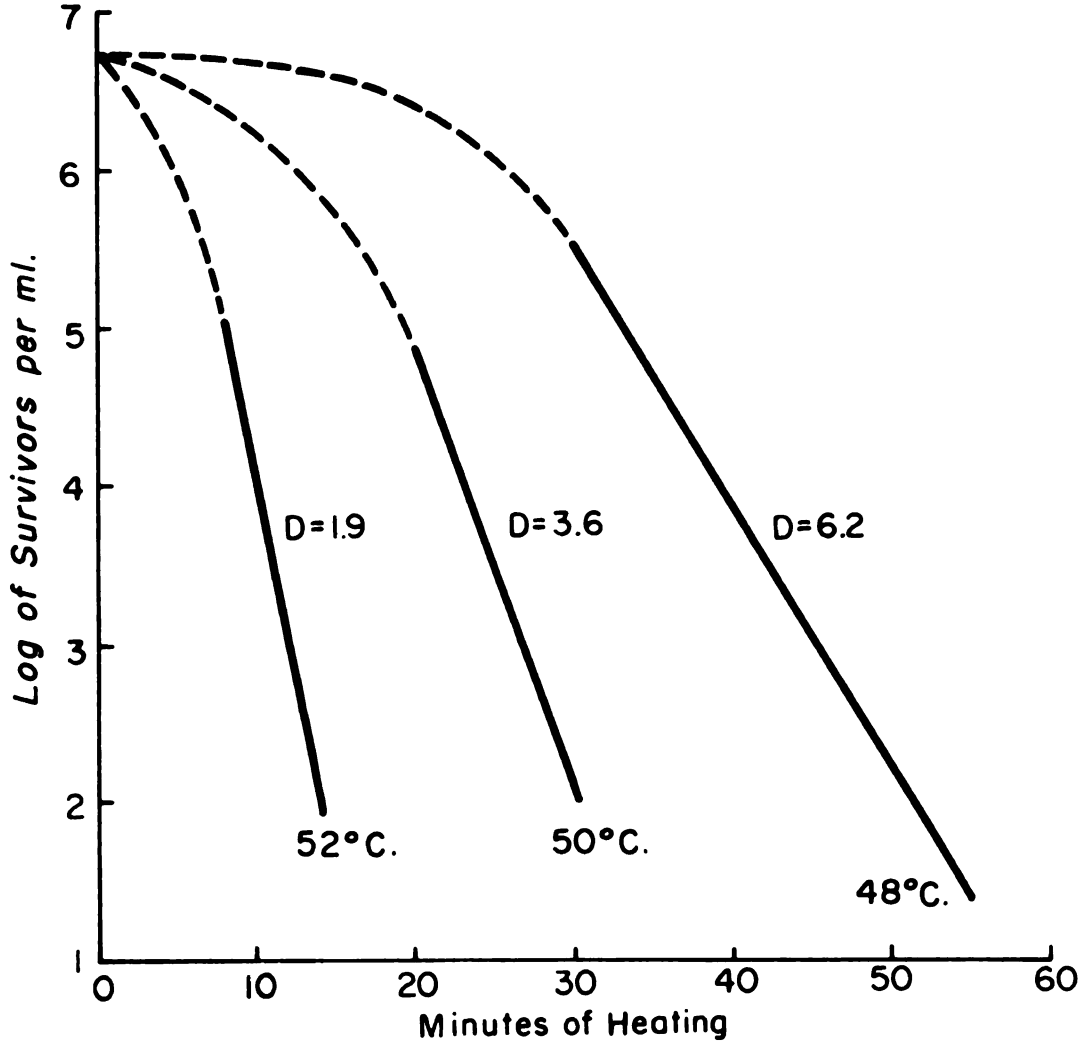


Fig. 11. Rate of destruction of P. fragi in reconstituted skimmilk after growth in reconstituted skimmilk at 25° C. for 20 hours.

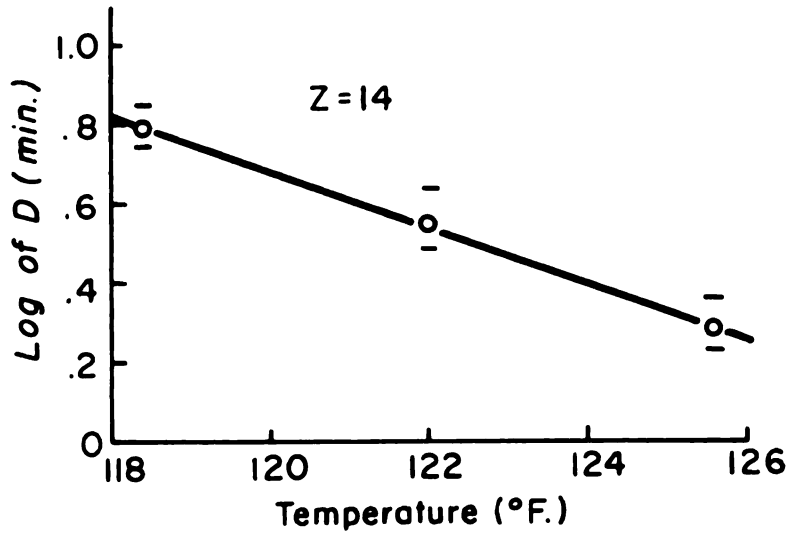
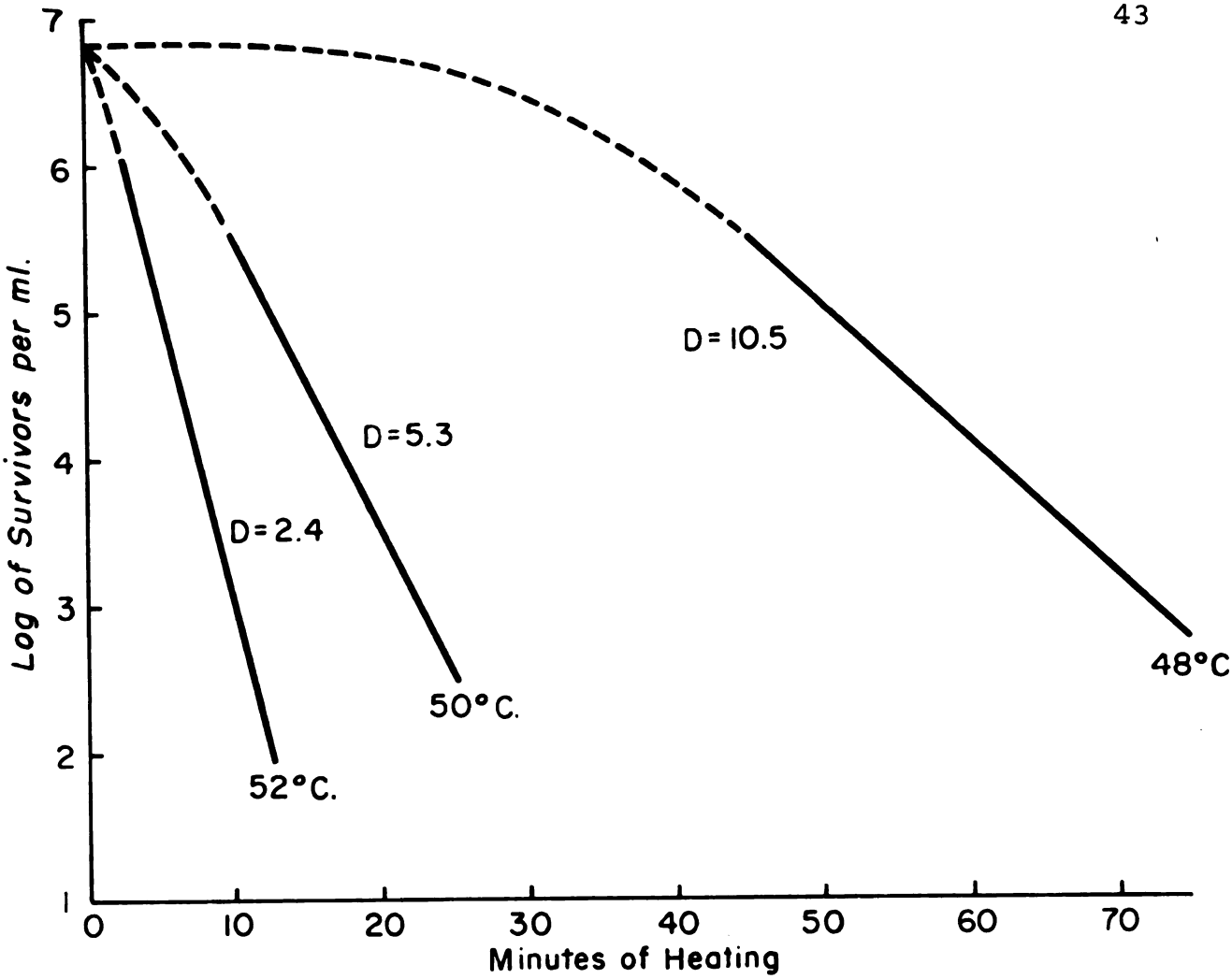


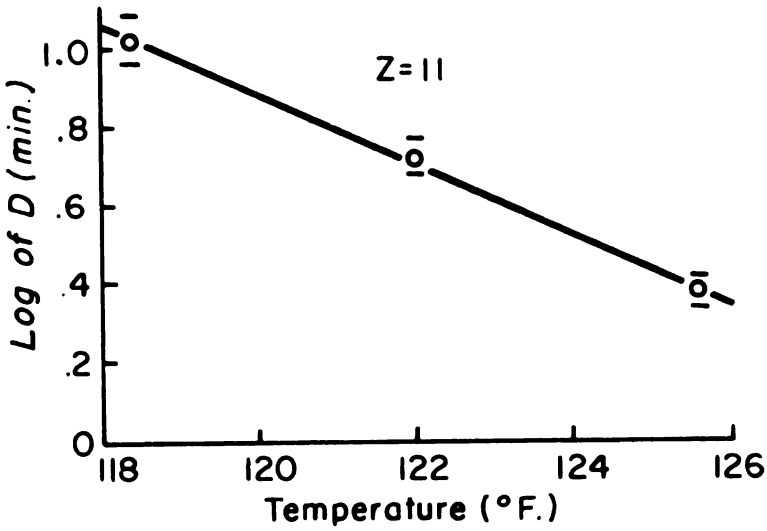
Fig. 12. Thermal resistance curve of P. fragi in reconstituted skimmilk after growth in reconstituted skimmilk at 25° C. for 20 hours.

TABLE 6. Number of *P. fragi* per milliliter surviving heat treatment in reconstituted milk containing 10 per cent milk fat after growth in reconstituted milk containing 10 per cent milk fat at 25° C. for 20 hours*

Temperature (° C.)					
48		50		52	
Time (Min.)	Survivors	Time (Min.)	Survivors	Time (Min.)	Survivors
0	6,500,000	0	5,000,000	0	5,000,000
10	5,800,000	5	1,700,000	3	930,000
20	3,400,000	10	600,000	6	34,000
30	2,400,000	15	64,000	9	3,000
40	640,000	20	3,000	12	200
50	85,000	25	500		
60	5,500	30	60		
0	4,400,000	0	750,000	0	2,700,000
10	8,000,000	4	2,000,000	3	550,000
20	8,300,000	8	640,000	5	54,000
30	5,400,000	12	210,000	7	3,800
40	1,900,000	16	33,000	9	**
50	370,000	20	3,000	11	70
60	30,000	24	750		
0	900,000	0	5,500,000		
15	1,400,000	3	2,000,000		
30	850,000	6	580,000		
45	490,000	8	480,000		
60	6,000	10	230,000		
75	150	12	85,000		
		14	30,000		
0	900,000			0	750,000
15	5,200,000			3	1,100,000
30	5,400,000			6	32,000
45	970,000			9	3,000
60	38,000			12	90
75	990				

* Counts represent the average of triplicate plates
** Laboratory accident


Log of Survivors per ml.
7
6
5
4
3
2
1
0
10
20
30
40
50
60
70
Minutes of Heating
D=10.5
D=5.3
D=2.4
48°C
50°C.
52°C.


Log of D (min.)
1.0
.8
.6
.4
.2
0
118
120
122
124
126
Temperature (°F.)
Z=11

Grown and heated in reconstituted cream containing 20 per cent milk fat. The data presented in Table 7 show the number of cells surviving heating exposures at 48°, 50° and 52° C. The survivor curves shown in Figure 15 were prepared from the data in Table 7. The calculated D_{48}, D_{50} and D_{52} values were 4.2, 2.5 and 2.0, respectively. The initial cell population was reduced by 99.99 per cent in 25, 17 and 12 minutes at 48°, 50° and 52° C., respectively. The z value of P. fragi when heated in the 20 per cent cream was 22 (Figure 16).

In Table 8 a summary is presented of the results reported thus far. In each case the cells were heated in a medium which contained the same concentration of milk fat as the medium in which they were propagated. The media in which the cells were grown and heated were dairy products containing 0 to 20 per cent milk fat. The calculated D values and the calculated z value obtained with each of the media are presented. The 95 per cent confidence limits of the D values also are presented.

TABLE 7. Number of P. fragi per milliliter surviving heat treatment in reconstituted cream containing 20 per cent milk fat after growth in reconstituted cream containing 20 per cent milk fat at 25° C. for 24 hours*

Temperature (° C.)					
48		50		52	
Time (Min.)	Survivors	Time (Min.)	Survivors	Time (Min.)	Survivors
0	5,300,000	0	5,300,000	0	5,300,000
5	4,300,000	3	6,000,000	3	5,100,000
10	410,000	6	3,200,000	5	1,900,000
15	16,000	9	650,000	7	340,000
20	400	12	65,000	9	32,000
25	60	15	3,100	11	1,900
		18	140	13	200
0	3,600,000	0	3,600,000	0	3,600,000
5	3,600,000	3	5,200,000	3	3,300,000
10	2,300,000	6	3,000,000	5	1,200,000
15	630,000	9	1,000,000	7	210,000
20	31,000	12	140,000	9	7,400
25	2,300	15	11,000	11	710
30	270			13	41
0	1,900,000	0	1,900,000	0	1,900,000
5	3,700,000	3	4,400,000	3	4,300,000
10	660,000	6	1,800,000	5	2,000,000
15	38,000	9	280,000	7	370,000
20	2,200	12	31,000	9	49,000
25	170	15	12,000	11	6,600
		18	35	13	770
0	7,300,000			0	7,300,000
5	5,200,000			3	6,500,000
10	2,500,000			5	2,400,000
15	260,000			7	360,000
20	8,700			9	60,000
25	600			11	4,000
30	15				

* Counts represent the average of triplicate plates

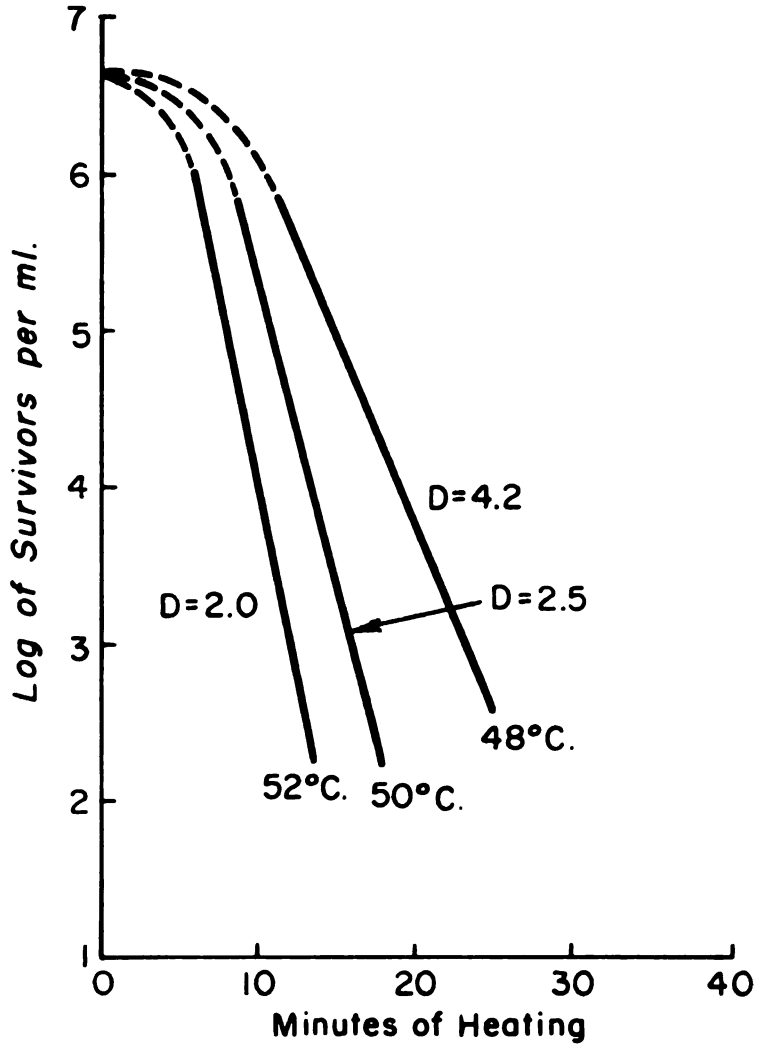


Fig. 15. Rate of destruction of P. fragi in reconstituted cream containing 20 per cent milk fat after growth in reconstituted cream containing 20 per cent milk fat at 25° C. for 24 hours.

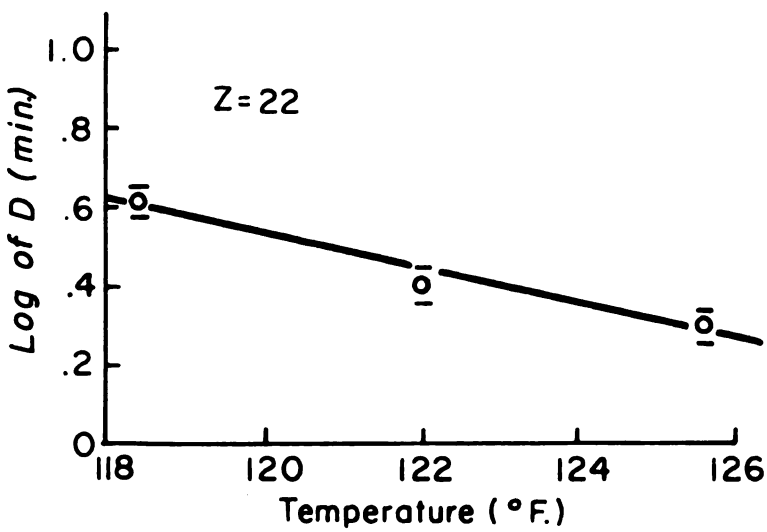


Fig. 16. Thermal resistance curve of P. fragi in reconstituted cream containing 20 per cent milk fat after growth in reconstituted cream containing 20 per cent milk fat at 25° C. for 24 hours.

TABLE 8. Thermal destruction of P. fragi when grown and heated in various dairy products containing 0 to 20 per cent milk fat

Product	D	95% Confidence limits	D	95% Confidence limits	D	95% Confidence limits	z
	48° C.		50° C.		52° C.		(°F.)
Half-and-half[a]	5.4	4.5 - 6.7	3.0	2.6 - 3.7	1.0	.84 - 1.2	10
Half-and-half[b]	10.0	8.7 - 11.8	5.0	4.5 - 5.5	2.7	2.4 - 3.1	13
Fresh[a] skimmilk	12.5	6.6 - 100.0	6.2	6.1 - 6.4	1.9	1.4 - 3.0	9
Cream (20% fat)[c]	4.3	3.8 - 5.0	2.4	2.3 - 2.5	1.4	1.3 - 1.6	15
Reconstituted[a] skimmilk	6.2	5.6 - 7.1	3.6	3.1 - 4.4	1.9	1.7 - 2.3	14
Reconstituted[a] milk (10% fat)	10.5	9.2 - 12.3	5.3	4.8 - 5.9	2.4	2.2 - 2.6	11
Reconstituted[c] cream (20% fat)	4.2	3.8 - 4.5	2.5	2.3 - 2.8	2.0	1.8 - 2.2	22

a P. fragi cells grown at 25° C. for 20 hours.
b P. fragi cells grown at 7° C. for 7 days.
c P. fragi cells grown at 25° C. for 24 hours.

Thermal Destruction of P. fragi Cells in a Medium Containing a Different Concentration of Milk Fat Than the Medium in Which the Cells Were Grown

The data presented in the previous sections were obtained with cells grown and heated in media containing the same concentration of milk fat. In the trials reported in this section the concentration of milk fat in the growth medium was not the same as that of the heating medium.

Grown in reconstituted skimmilk -- heated in reconstituted milk containing 10 per cent milk fat. The data in Table 9 show the average number of P. fragi per milliliter surviving heat treatment in milk containing 10 per cent milk fat. The destruction curves shown in Figure 17 were constructed from these data and the D values obtained were 5.0, 2.3 and 1.4 at 48°, 50° and 52° C., respectively. The z calculated from the slope of the thermal resistance curve was 13 (Figure 18). A 99.99 per cent reduction in population occurred after 40, 20 and 8 minutes of exposure at 48°, 50° and 52° C., respectively.

Grown in reconstituted skimmilk -- heated in reconstituted cream containing 20 per cent milk fat. The curves presented in Figure 19 show the destruction rate of P. fragi when heated in 20 per cent reconstituted cream after propagation in reconstituted skimmilk. The data in Table 10 were utilized in

TABLE 9. Number of P. fragi per milliliter surviving heat treatment in reconstituted milk containing 10 per cent milk fat after growth in reconstituted skim-milk at 25° C. for 20 hours*

Temperature (° C.)					
48		50		52	
Time (Min.)	Survivors	Time (Min.)	Survivors	Time (Min.)	Survivors
0	4,300,000	0	4,300,000	0	4,300,000
6	660,000	4	430,000	3	350,000
12	470,000	8	190,000	5	130,000
18	160,000	12	40,000	6	7,600
24	23,000	16	6,400	7	1,500
30	560	20	150	8	1,100
36	16			9	310
0	3,700,000	0	3,700,000	0	3,700,000
6	380,000	4	150,000	3	160,000
12	160,000	8	61,000	5	2,400
18	40,000	12	7,100	7	200
24	3,000	16	300		
30	100				

* Counts represent the average of triplicate plates

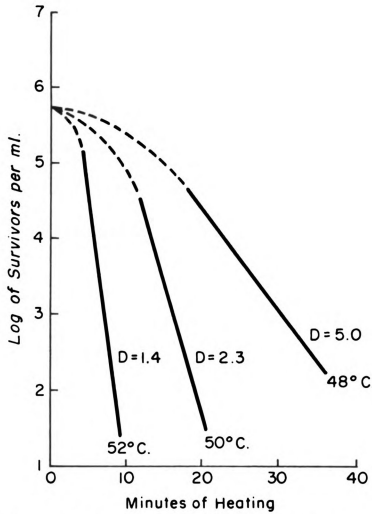


Fig. 17. Rate of destruction of P. fragi in reconstituted milk containing 10 per cent milk fat after growth in reconstituted skimmilk at 25° C. for 20 hours.

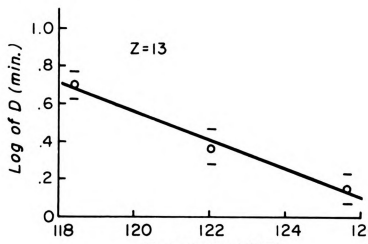


Fig. 18. Thermal resistance curve of P. fragi in reconstituted milk containing 10 per cent milk fat after growth in reconstituted skimmilk at 25° C. for 20 hours.

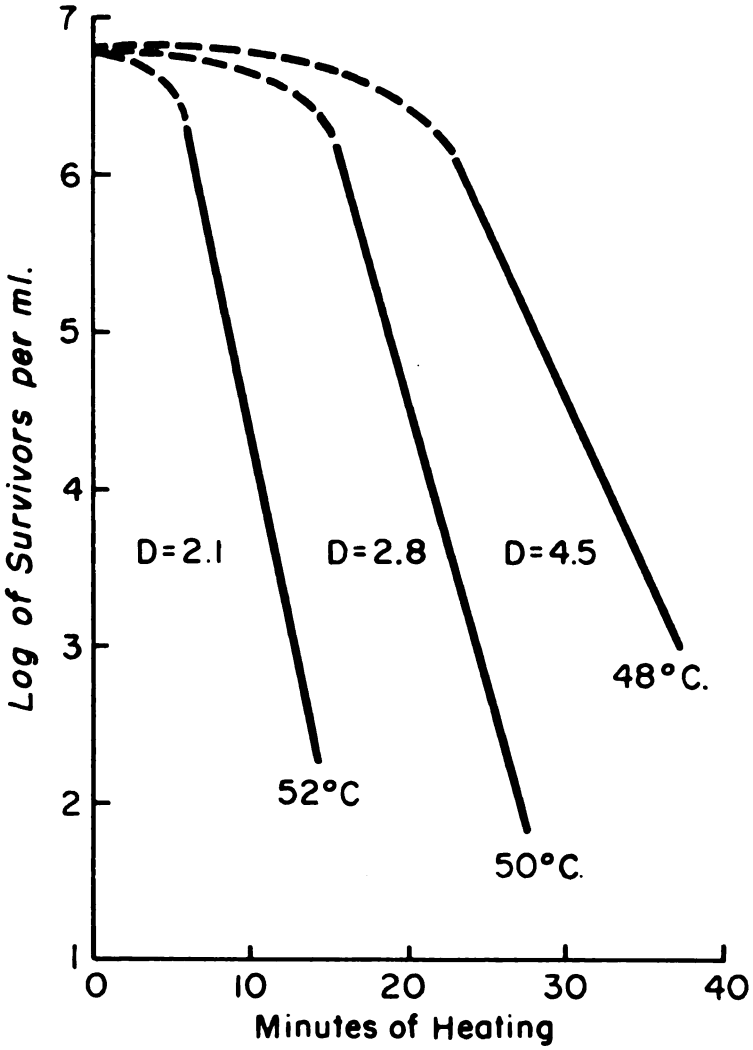


Fig. 19. Rate of destruction of P. fragi in reconstituted cream containing 20 per cent milk fat after growth in reconstituted skimmilk at 25° C. for 20 hours.

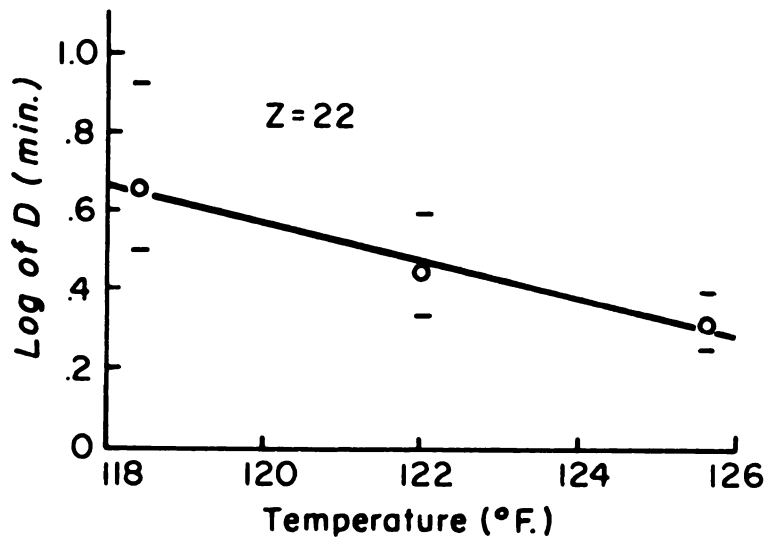


Fig. 20. Thermal resistance curve of P. fragi in reconstituted cream containing 20 per cent milk fat after growth in reconstituted skimmilk at 25° C. for 20 hours.

TABLE 10. Number of *P. fragi* per milliliter surviving heat treatment in reconstituted cream containing 20 per cent milk fat after growth in reconstituted skim-milk at 25° C. for 20 hours*

Temperature (° C.)					
48		50		52	
Time (Min.)	Survivors	Time (Min.)	Survivors	Time (Min.)	Survivors
0	2,200,000	0	2,200,000	0	2,200,000
8	6,600,000	5	5,800,000	3	7,300,000
16	**	10	**	6	1,800,000
25	1,200,000	15	800,000	9	180,000
32	150,000	20	130,000	12	5,100
40	1,800	25	4,200	15	70
48	41	30	90		
0	5,300,000	0	5,300,000	0	5,300,000
7	5,300,000	5	4,100,000	3	5,200,000
14	2,300,000	10	1,100,000	5	1,600,000
21	300,000	15	170,000	6	1,200,000
28	10,000	20	3,400	7	480,000
35	50	25	52	8	**
				9	55,000
				12	900

* Counts represent the average of triplicate plates
** Laboratory accident

preparing these curves and the D_{48}, D_{50} and D_{52} values calculated from the slope of these curves were 4.5, 2.8 and 2.1, respectively. A z of 22 was calculated from the slope of the thermal resistance curve shown in Figure 20. The initial cell population was reduced by 99.99 per cent in 38, 25 and 14 minutes at 48°, 50° and 52° C., respectively. Table 15 contains a summary of the data obtained from cells grown in reconstituted skimmilk and heated in (a) reconstituted skimmilk, (b) reconstituted milk containing 10 per cent milk fat and (c) reconstituted cream containing 20 per cent milk fat.

Grown in reconstituted milk containing 10 per cent milk fat -- heated in reconstituted skimmilk. The destruction rate curves shown in Figure 21 were constructed from the data presented in Table 11. The D values calculated from the straight line portion of these curves were 5.0, 3.0 and 1.2 minutes at 48°, 50° and 52° C., respectively. When the entire survivor curve was considered the populations were reduced by 99.99 per cent in 32, 18 and 8 minutes at 48°, 50° and 52° C., respectively. The z value of the thermal resistance curve shown in Figure 22 is 12.

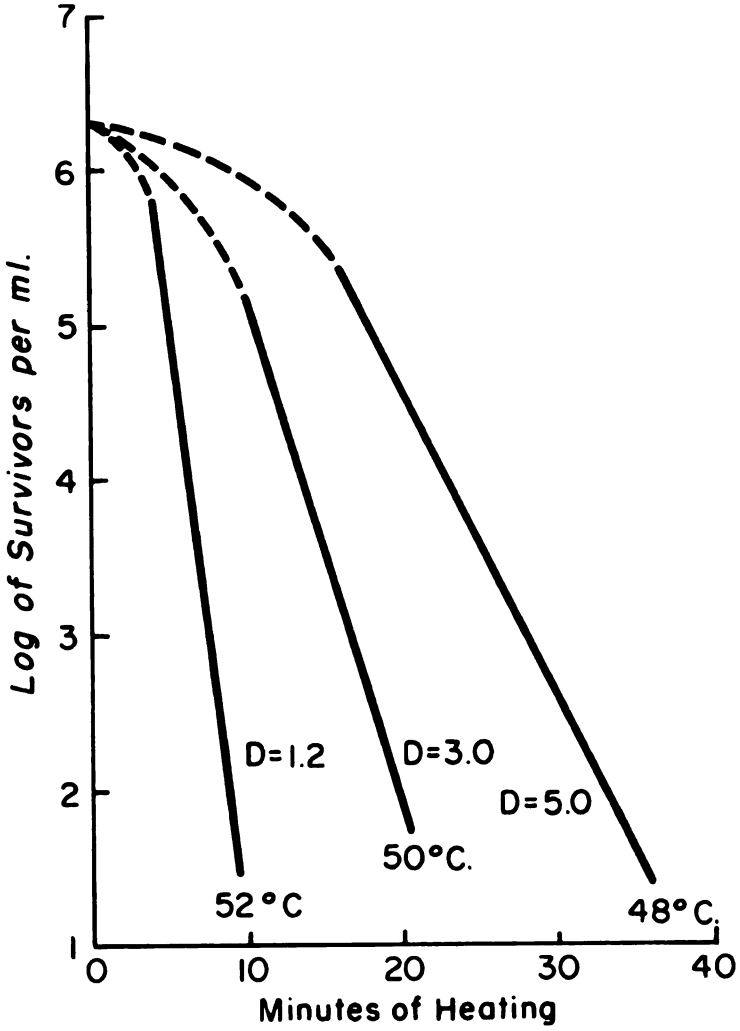


Fig. 21. Rate of destruction of P. fragi in reconstituted skimmilk after growth in reconstituted milk containing 10 per cent milk fat at 25° C. for 20 hours.

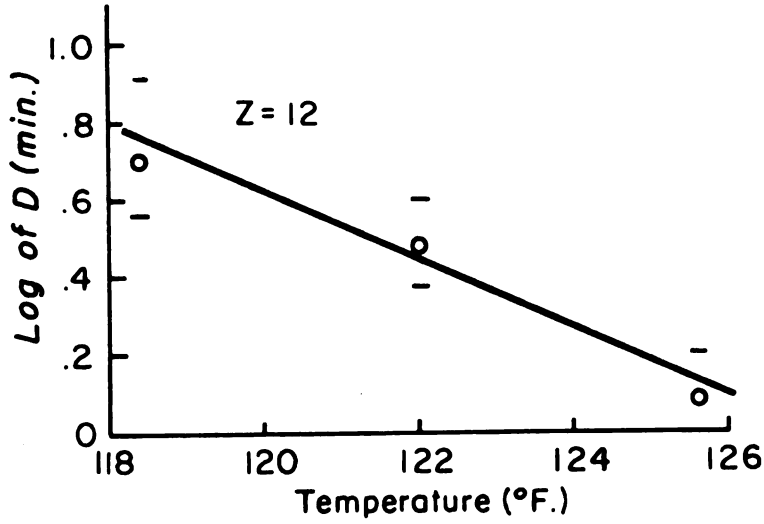


Fig. 22. Thermal resistance curve of P. fragi in reconstituted skimmilk after growth in reconstituted milk containing 10 per cent milk fat at 25° C. for 20 hours.

TABLE 11. Number of *P. fragi* per milliliter surviving heat treatment in reconstituted skimmilk after growth in reconstituted milk containing 10 per cent milk fat at 25° C. for 20 hours*

Temperature (° C.)					
48		50		52	
Time (Min.)	Survivors	Time (Min.)	Survivors	Time (Min.)	Survivors
0	6,000,000	0	6,000,000	0	6,000,000
8	820,000	5	750,000	3	2,400,000
16	360,000	10	110,000	6	49,000
24	14,000	15	3,500	9	100
34	130	20	80		
0	9,100,000	0	9,100,000	0	9,100,000
6	660,000	4	1,700,000	3	2,100,000
12	220,000	8	190,000	5	190,000
18	53,000	12	40,000	7	3,100
24	4,100	16	2,200	9	50
30	100	20	33		
36	22				

* Counts represent the average of triplicate plates

Grown in reconstituted milk containing 10 per cent milk fat -- heated in reconstituted cream containing 20 per cent milk fat. The data in Table 12 show the number of survivors per milliliter in the samples taken during heating at 48° , 50° and 52° C. The D_{48}, D_{50} and D_{52} values obtained from the survivor curves shown in Figure 23 were 3.3, 2.3 and 1.4, respectively. The survivor curves show that 99.99 per cent of the original cell population was destroyed in 25 minutes at 48° C., 15 minutes at 50° C. and 8 minutes at 52° C. The z calculated from the D values was 20 (Figure 24).

A summary of the data obtained with the cells grown in milk containing 10 per cent milk fat is shown in Table 15.

Grown in reconstituted cream containing 20 per cent milk fat -- heated in reconstituted skimmilk. The data in Table 13 were used to prepare the survivor curves in Figure 25. When the cells were heated at 48° , 50° and 52° C. the D values were 5.0, 2.9 and 1.4, respectively. The z value calculated from these D values was 13 (Figure 26). The times required to reduce the initial cell population by 99.99 per cent at 48° , 50° and 52° C. were 20, 15 and 8 minutes, respectively.

TABLE 12. Number of P. fragi per milliliter surviving heat treatment in reconstituted cream containing 20 per cent milk fat after growth in reconstituted milk containing 10 per cent milk fat at 25° C. for 20 hours*

Temperature (° C.)					
48		50		52	
Time (Min.)	Survivors	Time (Min.)	Survivors	Time (Min.)	Survivors
0	2,900,000	0	2,900,000	0	2,900,000
7	1,000,000	4	710,000	3	590,000
14	110,000	8	130,000	5	110,000
21	330	12	2,600	7	6,200
				9	60
0	1,500,000	0	1,500,000	0	1,500,000
6	3,800,000	4	1,600,000	3	1,800,000
12	1,200,000	8	950,000	5	150,000
18	310,000	12	23,000	6	73,000
24	20,000	16	1,000	7	21,000
30	440			8	3,600
				9	2,100

* Counts represent the average of triplicate plates

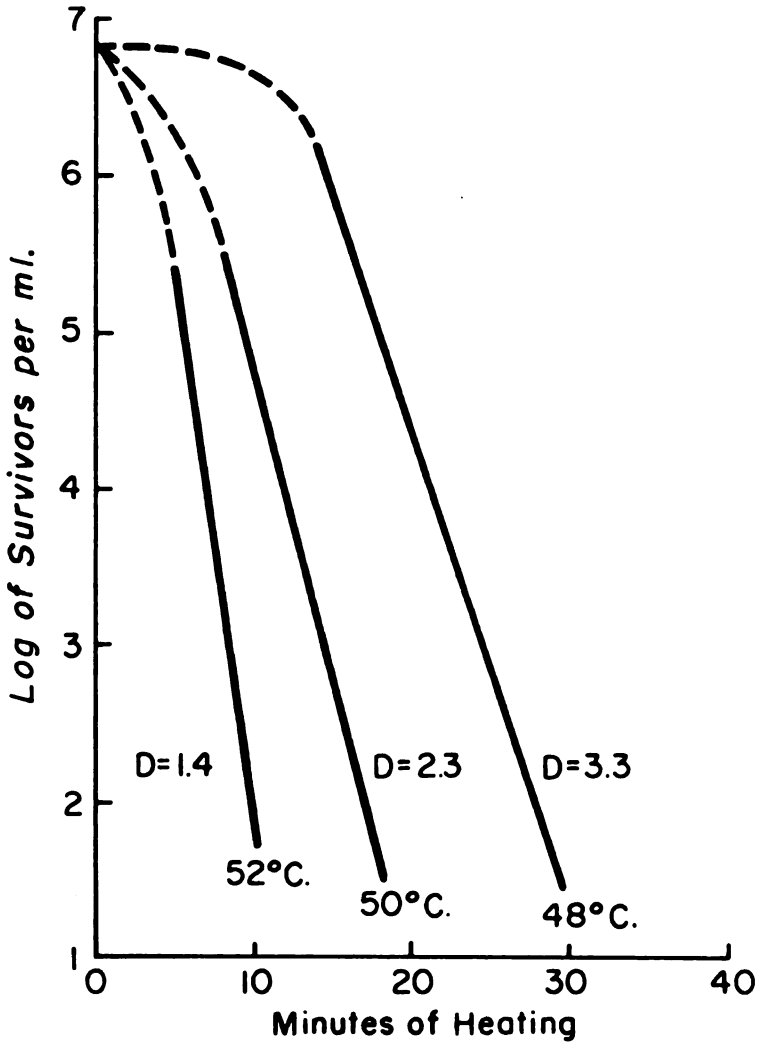


Fig. 23. Rate of destruction of P. fragi in reconstituted cream containing 20 per cent milk fat after growth in reconstituted milk containing 10 per cent milk fat at 25° C. for 20 hours.

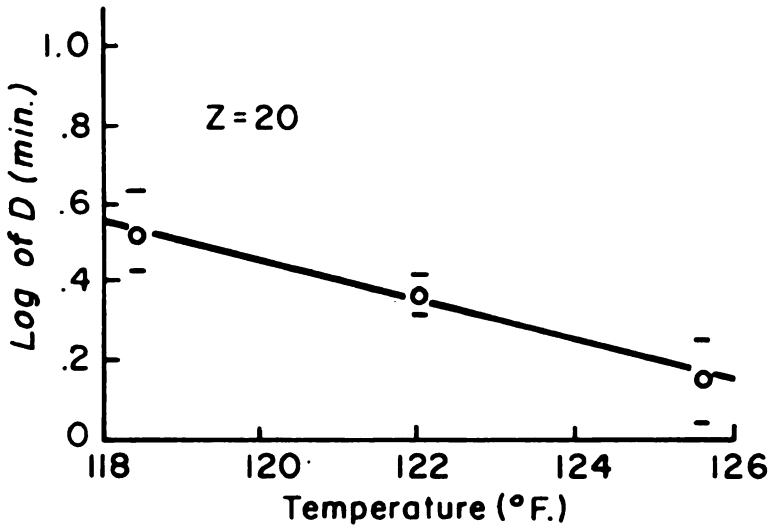


Fig. 24. Thermal resistance curve of P. fragi in reconstituted cream containing 20 per cent milk fat after growth in reconstituted milk containing 10 per cent milk fat at 25° C. for 20 hours.

TABLE 13. Number of P. fragi per milliliter surviving heat treatment in reconstituted skimmilk after growth in reconstituted cream containing 20 per cent milk fat at 25° C. for 24 hours*

Temperature (° C.)					
48		50		52	
Time (Min.)	Survivors	Time (Min.)	Survivors	Time (Min.)	Survivors
0	3,600,000	0	3,600,000	0	3,600,000
8	120,000	5	350,000	3	580,000
16	2,800	10	15,000	6	62,000
24	100	15	200	9	400
32	40				
0	1,400,000	0	1,400,000	0	1,400,000
7	110,000	4	380,000	3	120,000
14	800	8	14,000	5	5,100
21	30	12	200	7	100

* Counts represent the average of triplicate plates

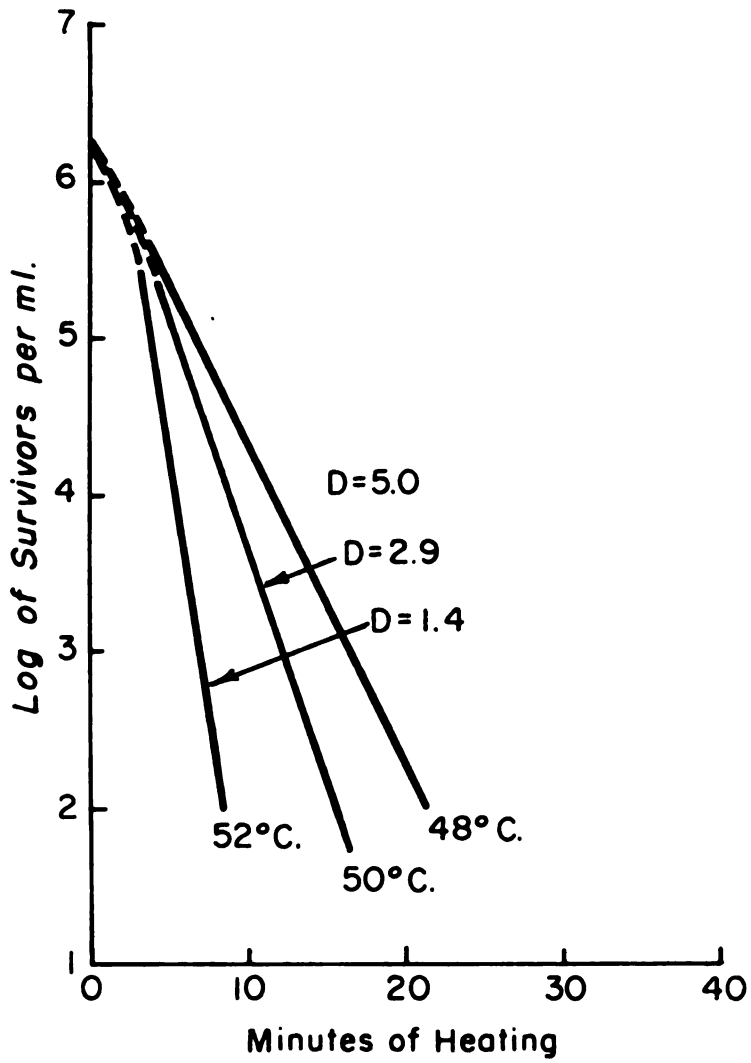


Fig. 25. Rate of destruction of P. fragi in reconstituted skimmilk after growth in reconstituted cream containing 20 per cent milk fat at 25° C. for 24 hours.

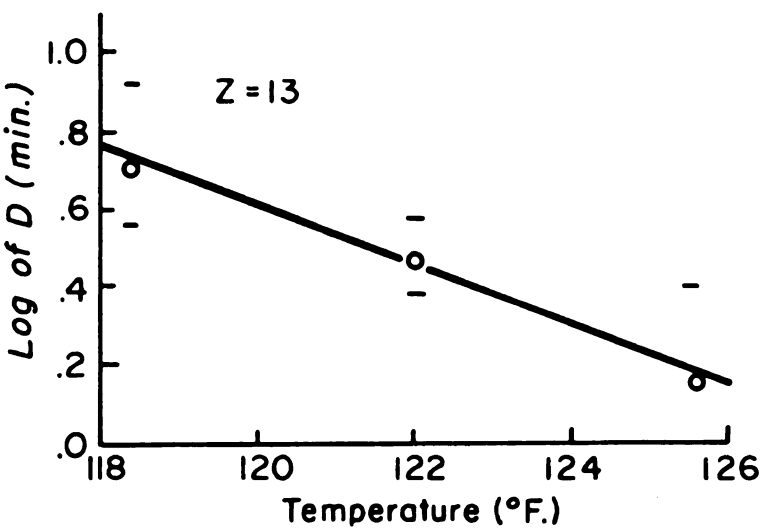


Fig. 26. Thermal resistance curve of P. fragi in reconstituted skimmilk after growth in reconstituted cream containing 20 per cent milk fat at 25° C. for 24 hours.

Grown in reconstituted cream containing 20 per cent milk fat -- heated in reconstituted milk containing 10 per cent milk fat. The data in Table 14 show the average number of P. fragi per milliliter surviving at various time intervals during heating at 48°, 50° and 52° C. Figure 27 shows the survivor curves prepared from these data and the D value of each curve. At 48°, 50° and 52° C. the D values were 3.7, 2.3 and 0.9, respectively. Figure 28 shows the thermal resistance curve prepared with these D values and a z of 11 was calculated from the slope of the curve. A 99.99 per cent reduction in population occurred in 18, 12 and 8 minutes at 48°, 50° and 52° C., respectively.

Table 15 contains the D_{48}, D_{50} and D_{52} values obtained with cells grown and heated in media containing the same concentration of milk fat, and also includes values obtained when the cells were grown and heated in media in which the concentration of milk fat was varied. The z values and the 95 per cent confidence limits of the D values also are included in Table 15.

TABLE 14. Number of *P. fragi* per milliliter surviving heat treatment in reconstituted milk containing 10 per cent milk fat after growth in reconstituted cream containing 20 per cent milk fat at 25° C. for 24 hours*

Temperature (° C.)					
48		50		52	
Time (Min.)	Survivors	Time (Min.)	Survivors	Time (Min.)	Survivors
0	1,700,000	0	1,700,000	0	1,700,000
7	100,000	4	190,000	3	160,000
14	200	8	2,500	5	10,000
21	20			7	100
0	3,500,000	0	3,500,000	0	3,500,000
6	730,000	4	890,000	3	830,000
12	12,000	8	13,000	5	120,000
18	300	16	16	7	400
				8	70

* Counts represent the average of triplicate plates

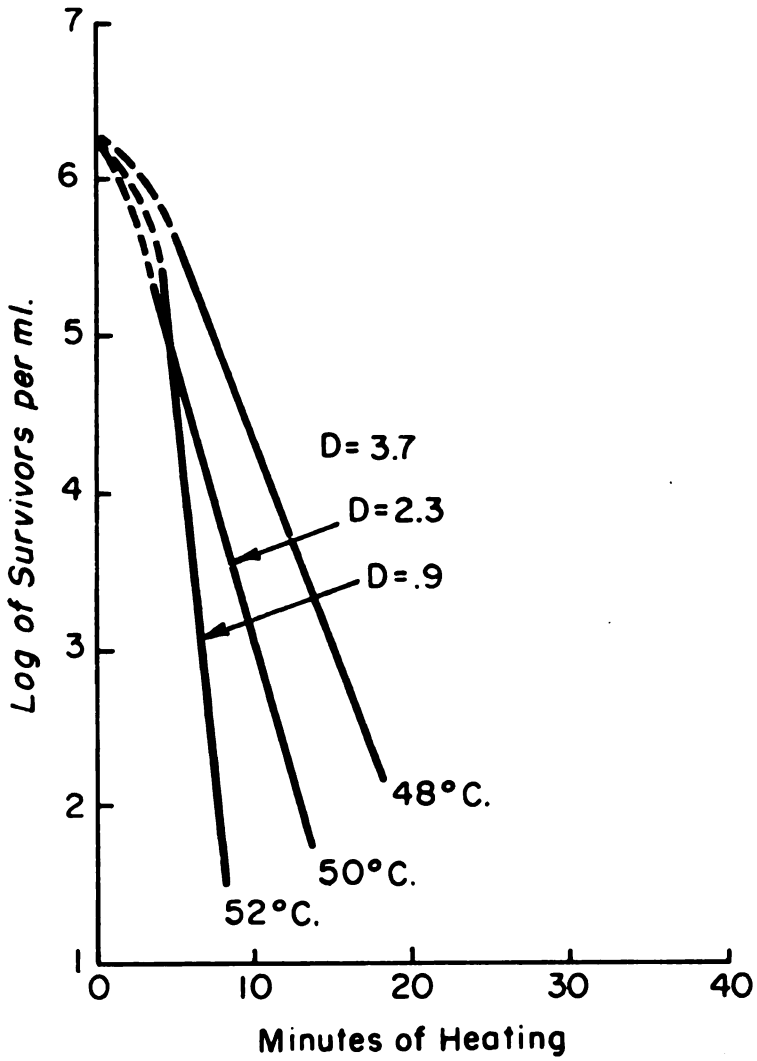


Fig. 27. Rate of destruction of P. fragi in reconstituted milk containing 10 per cent milk fat after growth in reconstituted cream containing 20 per cent milk fat at 25° C. for 24 hours.

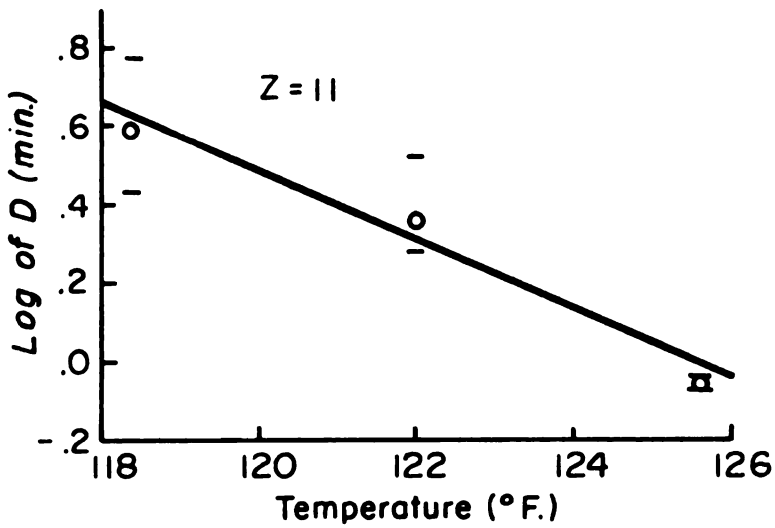


Fig. 28. Thermal resistance curve of P. fragi in reconstituted milk containing 10 per cent milk fat after growth in reconstituted cream containing 20 per cent milk fat at 25° C. for 24 hours.

TABLE 15. Thermal destruction of *P. fragi* in a medium containing a concentration of milk fat which was variant from that in the medium in which the cells were grown

Per cent milk fat in		95% Confidence		95% Confidence		95% Confidence		z(° F.)
growth medium	heating medium	D	limits	D	limits	D	limits	
		48° C.		50° C.		52° C.		
0	0	6.2	5.5 - 7.1	3.6	3.1 - 4.4	1.9	1.7 - 2.3	14
0	10	5.0	4.3 - 5.9	2.3	1.9 - 3.0	1.4	1.2 - 1.7	13
0	20	4.5	3.2 - 8.3	2.8	2.2 - 4.0	2.1	1.8 - 2.5	22
10	0	5.0	3.6 - 8.3	3.0	2.4 - 4.0	1.2	1.0 - 1.6	12
10	10	10.5	9.2 - 12.3	5.3	4.8 - 5.9	2.4	2.2 - 2.6	11
10	20	3.3	2.7 - 4.3	2.3	2.1 - 2.6	1.4	1.1 - 1.8	20
20	0	5.0	3.6 - 8.3	2.9	2.4 - 3.7	1.4	1.0 - 2.5	13
20	10	3.7	2.7 - 5.9	2.3	1.9 - 3.3	.87	.85 - .88	11
20	20	4.2	3.8 - 4.5	2.5	2.3 - 2.8	2.0	1.8 - 2.2	22

Thermal Destruction of P. fragi in Reconstituted Skimmilk Containing Selected Fatty Acids

In these studies the cells were propagated in reconstituted skimmilk at 25° C. for 20 hours and then heated in reconstituted skimmilk which contained 0.1 per cent by weight of an individual fatty acid. The pH of the skimmilk decreased with the addition of the fatty acids, but since the pH did not decrease by the same amount with each of the fatty acids it was necessary to adjust the pH to a common level. A pH of 6.7 was selected since this is normal for skimmilk. Heating trials were also conducted without adjustment of the pH in order to determine what effect the fatty acids would have on the thermal resistance of P. fragi at a pH of less than 6.7.

In reconstituted skimmilk containing lauric acid. A 0.1 per cent concentration of lauric acid in the skimmilk reduced the pH from 6.7 to 6.6. The data in Table 16 show the average number of survivors per milliliter at various time intervals during heating when the pH of the medium was 6.6. The D_{48}, D_{50} and D_{52} values at this pH were 6.7, 3.2 and 1.8, respectively. The survivor curves from which these D values were obtained are shown in Figure 29. The thermal resistance curve prepared from these D values is

TABLE 16. Number of P. fragi per milliliter surviving heat treatment in reconstituted skimmilk containing 0.1 per cent by weight of lauric acid at pH 6.6*

Temperature (° C.)					
48		50		52	
Time (Min.)	Survivors	Time (Min.)	Survivors	Time (Min.)	Survivors
0	12,200,000	0	12,200,000	0	12,000,000
10	8,700,000	5	6,900,000	3	7,000,000
20	4,700,000	10	3,300,000	6	1,200,000
30	890,000	15	660,000	8	170,000
40	100,000	20	100,000	10	65,000
50	1,600	25	9,300	12	4,800
60	41	30	150	14	49
0	11,000,000	0	1,900,000	0	1,900,000
10	7,500,000	5	1,300,000	3	1,800,000
20	2,200,000	10	480,000	6	190,000
30	180,000	15	110,000	8	23,000
40	3,900	20	1,300	10	300
50	120	25	70	12	20
0	1,600,000	0	11,000,000	0	1,600,000
9	5,200,000	5	6,600,000	3	410,000
18	2,400,000	10	2,900,000	5	180,000
27	870,000	15	430,000	7	29,000
36	140,000	20	86,000	9	4,300
45	5,800	25	3,700	11	250
54	240	30	48	13	32

* Counts represent the average of triplicate plates

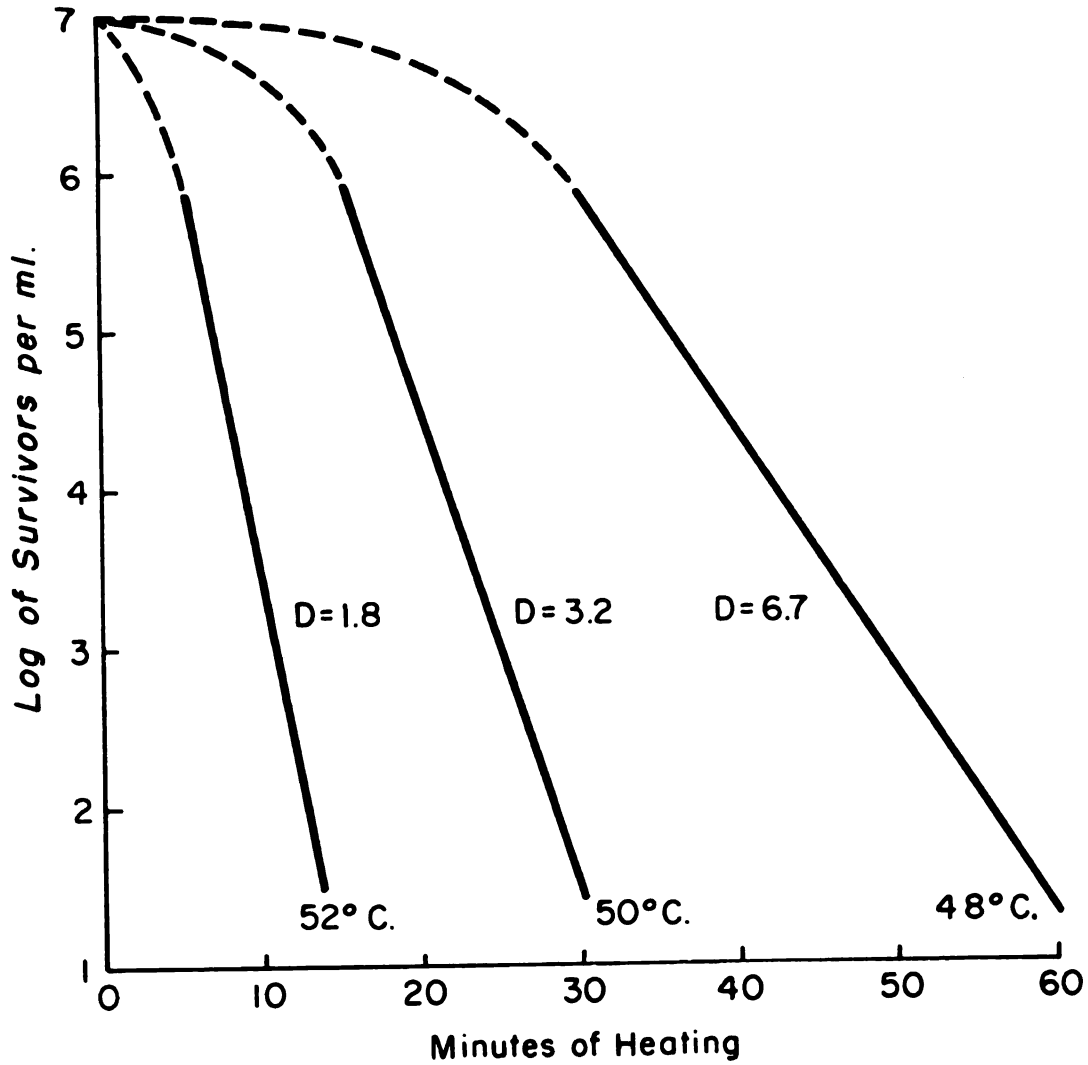


Fig. 29. Rate of destruction of P. fragi in reconstituted skimmilk containing 0.1 per cent by weight of lauric acid at pH 6.6.

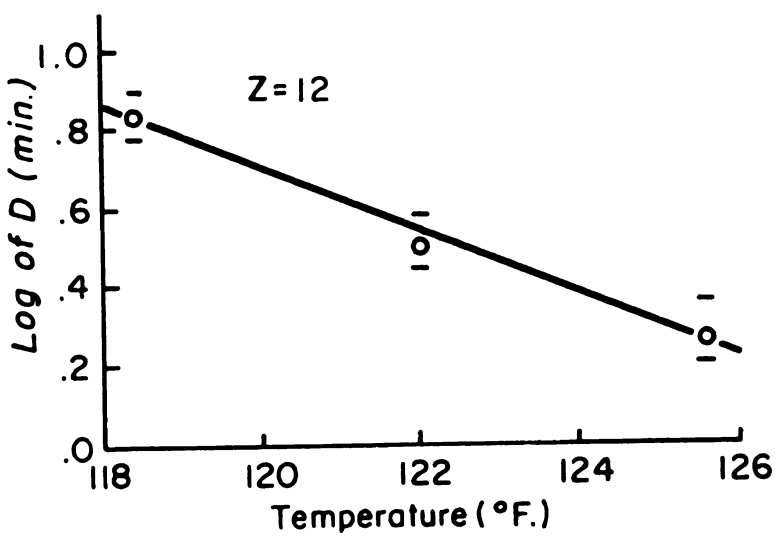


Fig. 30. Thermal resistance curve of P. fragi in reconstituted skimmilk containing 0.1 per cent by weight of lauric acid at pH 6.6.

shown in Figure 30 and the z value was 12. When the pH of the heating medium was 6.6 the cell populations were reduced by 99.99 per cent in 49 minutes at 48° C., 25 minutes at 50° C. and 11 minutes at 52° C.

The data in Table 17 were obtained when P. fragi was heated in skimmilk-lauric acid mixtures at pH 6.7. The destruction rate curves in Figure 31 were prepared from the data in Table 17. The D values of the curves were 6.2, 4.3 and 1.5 at 48°, 50° and 52° C., respectively. In Figure 32 the thermal resistance curve is shown and the z value was 12. The times required to decrease the cell population by 99.99 per cent were 39, 22 and 8 minutes at 48°, 50° and 52° C., respectively.

Summaries of the data obtained at these two pH levels are presented in Tables 22 and 23. Also included for comparison purposes are the data obtained with butyric and oleic acids.

In reconstituted skimmilk containing oleic acid. A 0.1 per cent concentration of oleic acid in the skimmilk decreased the pH to 6.45. The data in Tables 18 and 19 indicate the average number of survivors when the pH of the heating medium was 6.45 and 6.7, respectively. Figures 33 and 35

TABLE 17. Number of P. fragi per milliliter surviving heat treatment in reconstituted skimmilk containing 0.1 per cent by weight of lauric acid and neutralized to pH 6.7*

Temperature (° C.)					
48		50		52	
Time (Min.)	Survivors	Time (Min.)	Survivors	Time (Min.)	Survivors
0	5,400,000	0	5,400,000	0	5,400,000
9	**	4	1,800,000	3	290,000
18	1,200,000	8	570,000	5	120,000
27	74,000	12	180,000	7	9,100
36	400	16	12,000	9	430
45	**	20	290		
		24	48		
0	8,900,000	0	8,900,000	0	8,900,000
9	3,900,000	4	1,500,000	3	850,000
18	710,000	8	290,000	5	95,000
27	140,000	12	51,000	7	5,800
33	17,000	16	4,800	8	1,900
39	1,700	20	1,200	9	460
45	80	24	42	10	61
0	1,600,000	0	1,600,000	0	5,400,000
9	1,100,000	5	1,200,000	3	210,000
18	330,000	10	700,000	5	140,000
27	36,000	15	180,000	7	4,000
36	1,900	20	30,000	8	1,400
45	31	25	1,400	9	210
		30	30	10	55

* Counts represent the average of triplicate plates
** Laboratory accident

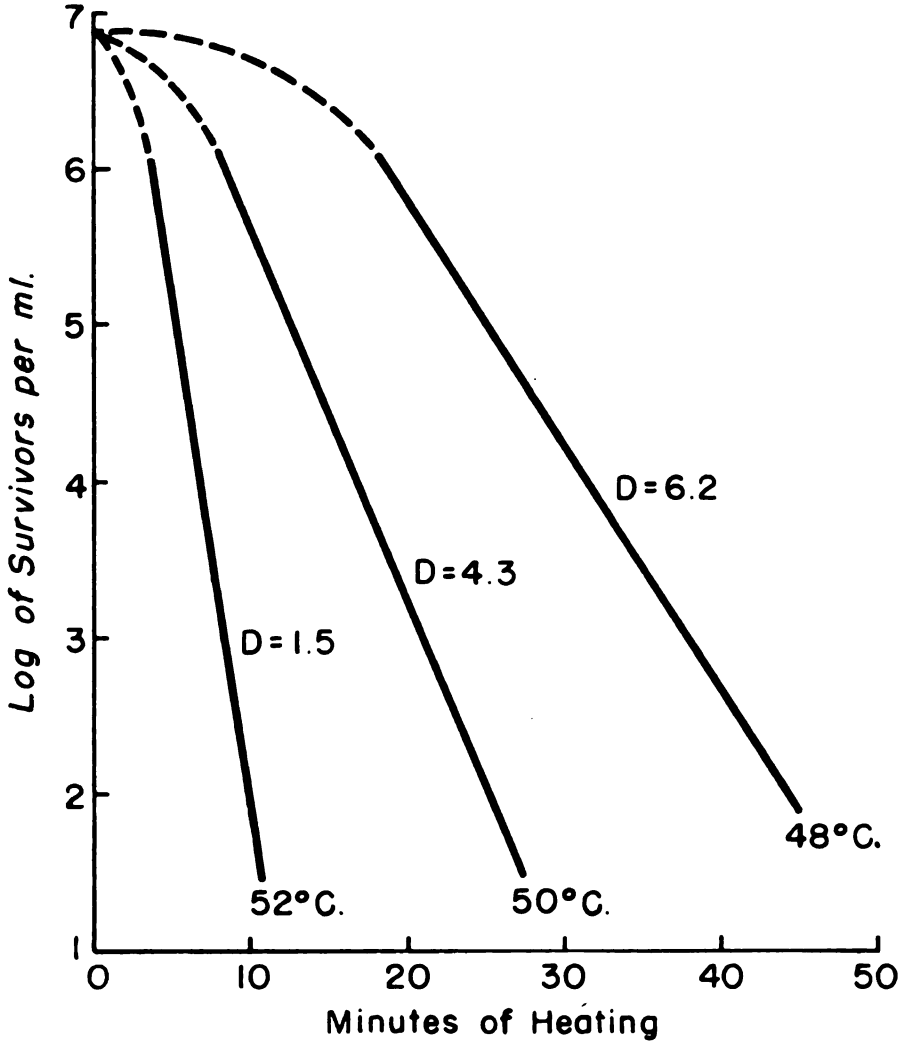


Fig. 31. Rate of destruction of P. fragi in reconstituted skimmilk containing 0.1 per cent by weight of lauric acid and neutralized to pH 6.7.

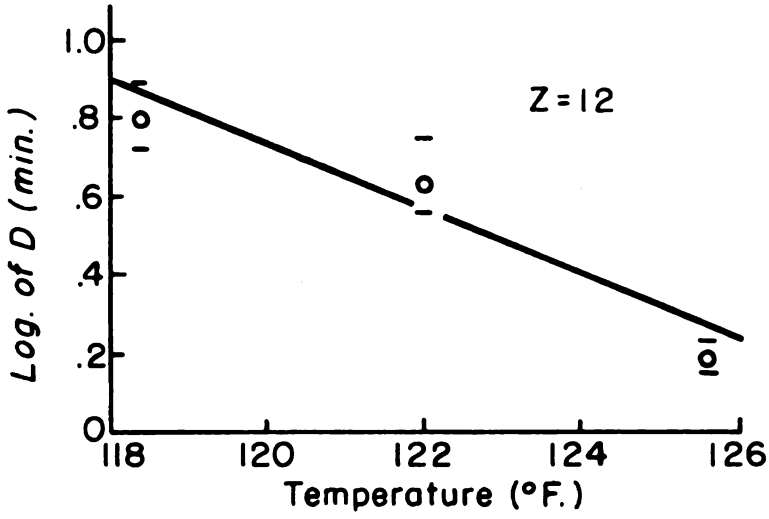


Fig. 32. Thermal resistance curve of P. fragi in reconstituted skimmilk containing 0.1 per cent by weight of lauric acid and neutralized to pH 6.7.

TABLE 18. Number of P. fragi per milliliter surviving heat treatment in reconstituted skimmilk containing 0.1 per cent by weight of oleic acid at pH 6.45*

Temperature (° C.)					
48		50		52	
Time (Min.)	Survivors	Time (Min.)	Survivors	Time (Min.)	Survivors
0	5,500,000	0	5,500,000		
9	530,000	4	310,000		
18	240,000	8	160,000		
27	190,000	12	110,000		
33	63,000	16	63,000		
39	9,100	20	15,000		
45	1,100	24	680		
0	**	0	8,400,000	0	8,400,000
9	1,500,000	5	5,000,000	3	2,200,000
18	720,000	10	3,400,000	6	1,100,000
27	170,000	15	980,000	8	360,000
33	79,000	20	150,000	10	28,000
39	15,000	25	1,900	12	1,200
45	2,400				
0	4,700,000			0	4,700,000
10	1,200,000			3	760,000
20	780,000			6	100,000
30	540,000			8	140,000
40	90,000			10	16,000
50	3,600			12	1,200
60	140			14	59

* Counts represent the average of triplicate plates
** Laboratory accident

TABLE 19. Number of P. fragi per milliliter surviving heat treatment in reconstituted skimmilk containing 0.1 per cent by weight of oleic acid and neutralized to pH 6.7*

Temperature (° C.)					
48		50		52	
Time (Min.)	Survivors	Time (Min.)	Survivors	Time (Min.)	Survivors
0	6,200,000	0	6,200,000	0	6,200,000
10	780,000	5	2,400,000	3	740,000
20	880,000	10	450,000	6	90,000
30	180,000	15	120,000	8	30,000
40	16,000	20	6,300	10	2,700
50	190	25	80		
0	4,400,000	0	4,400,000	0	4,400,000
9	4,200,000	4	710,000	3	90,000
18	760,000	8	300,000	5	10,000
27	110,000	12	100,000	7	1,400
33	18,000	16	4,400	8	450
39	440	20	150	9	430
45	50			10	35
0	6,000,000	0	6,000,000	0	6,000,000
10	1,200,000	5	850,000	3	810,000
20	1,000,000	10	320,000	6	400,000
30	210,000	15	180,000	8	99,000
40	7,600	20	52,000	10	15,000
50	310	25	590	12	710
		30	35		

* Counts represent the average of triplicate plates

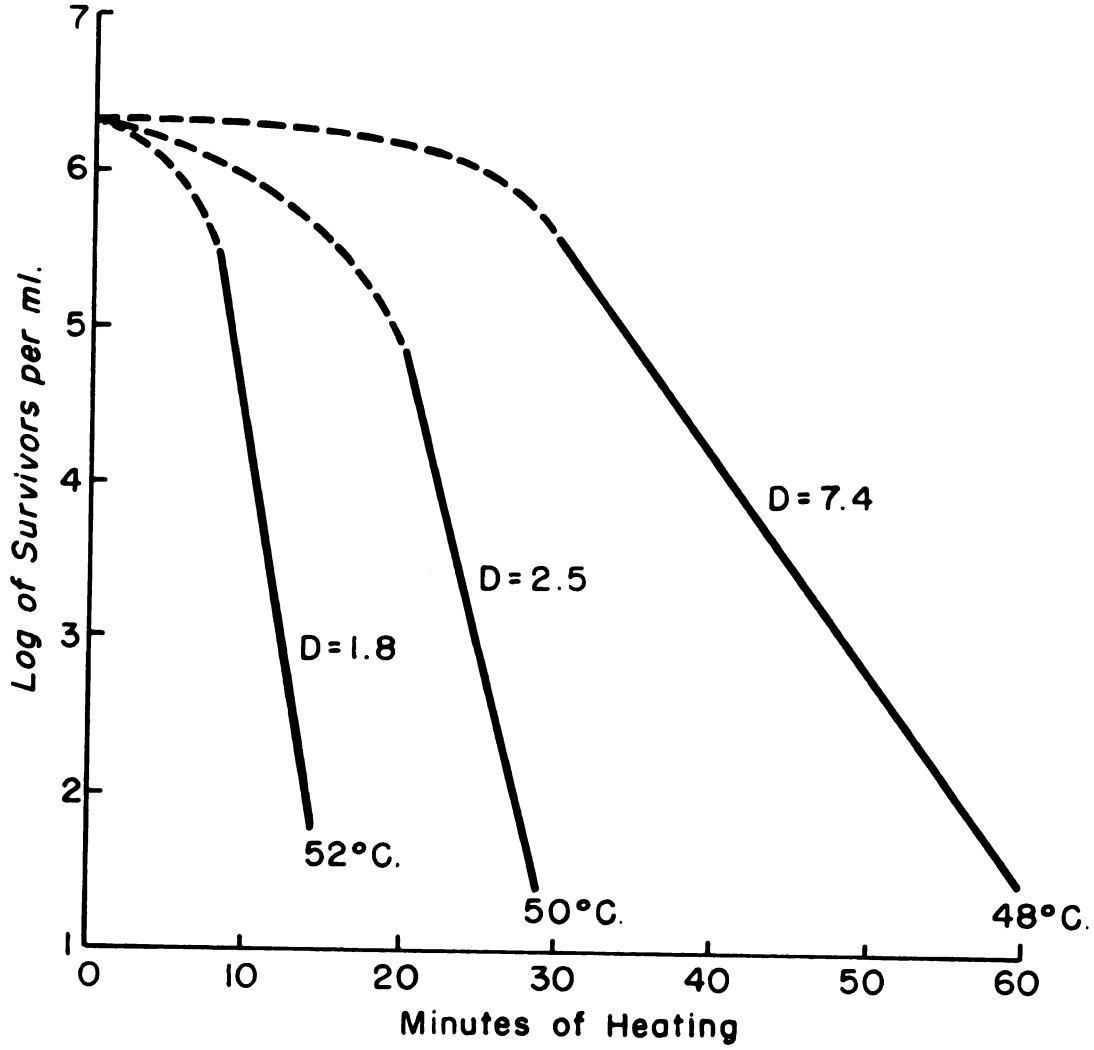


Fig. 33. Rate of destruction of P. fragi in reconstituted skimmilk containing 0.1 per cent by weight of oleic acid at pH 6.45.

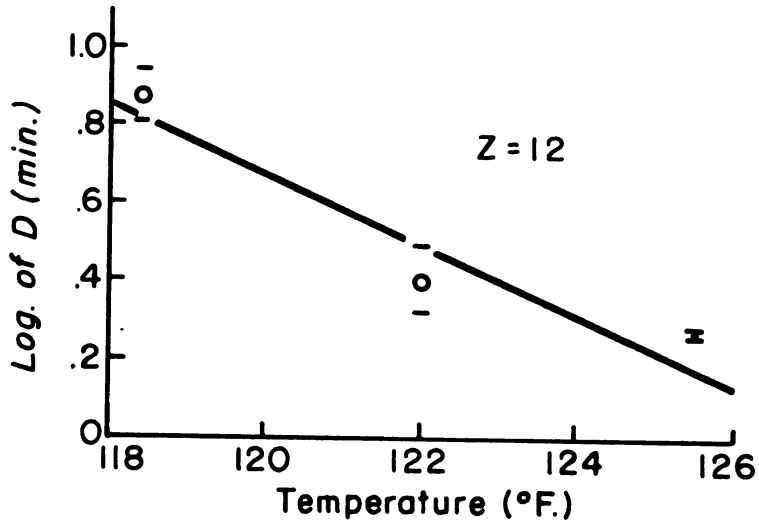


Fig. 34. Thermal resistance curve of P. fragi in reconstituted skimmilk containing 0.1 per cent by weight of oleic acid at pH 6.45.

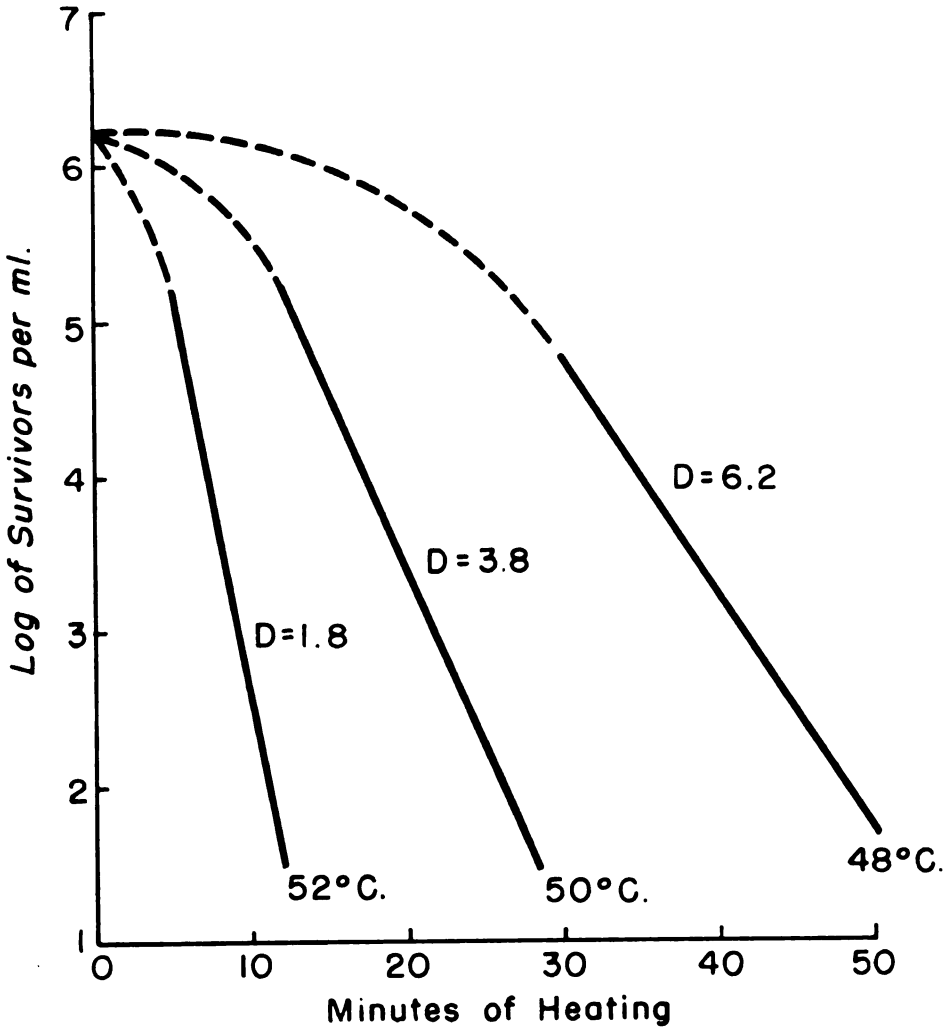


Fig. 35. Rate of destruction of P. fragi in reconstituted skimmilk containing 0.1 per cent by weight of oleic acid and neutralized to pH 6.7.

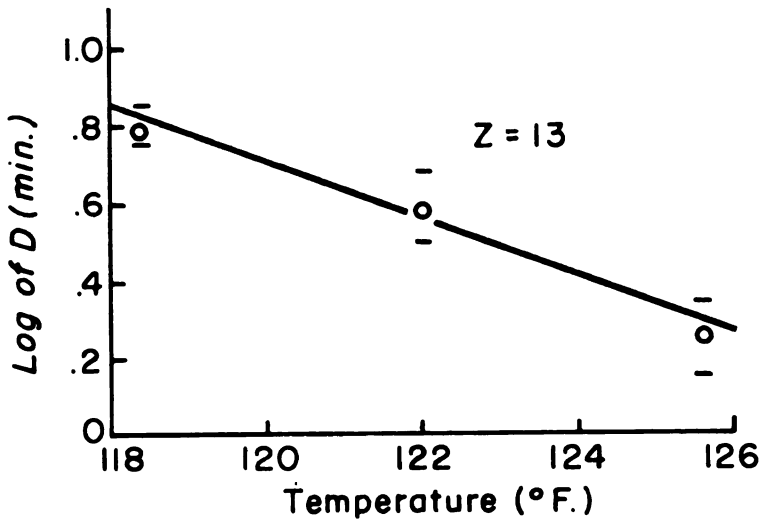


Fig. 36. Thermal resistance curve of P. fragi in reconstituted skimmilk containing 0.1 per cent by weight of oleic acid and neutralized to pH 6.7.

show the survivor curves and D values calculated from the data presented in Tables 18 and 19, respectively. At pH 6.45 the D_{48}, D_{50} and D_{52} values were 7.4, 2.5 and 1.8, respectively. When the pH was 6.7 the D_{48}, D_{50} and D_{52} values were 6.2, 3.8 and 1.8, respectively. At pH 6.45 and 6.7 the z values were 12 and 13, respectively (Figures 34 and 36). The original cell populations were decreased by 99.99 per cent in 53, 26 and 13 minutes at 48°, 50° and 52° C., respectively, when the pH of the heating medium was 6.45. At pH 6.7 the same amount of destruction occurred in 46, 25 and 10 minutes at 48°, 50° and 52° C., respectively.

Summaries of the data obtained with oleic acid are presented in Tables 22 and 23.

In reconstituted skimmilk containing butyric acid. A 0.1 per cent concentration of butyric acid in the skimmilk decreased the pH to 5.85, The heating temperatures at this pH were 50°, 52° and 54° C. The data in Table 20 indicate the average number of survivors per milliliter at various times throughout the heating trials with the pH at 5.85. The survivor curves shown in Figure 37 were prepared from the data obtained at pH 5.85. The D values calculated from these survivor curves were 9.1, 5.4 and 1.9 at 50°, 52° and 54° C., respectively. The z calculated from the slope of

TABLE 20. Number of P. fragi per milliliter surviving heat treatment in reconstituted skimmilk containing 0.1 per cent by weight of butyric acid at pH 5.85*

Temperature (° C.)					
50		52		54	
Time (Min.)	Survivors	Time (Min.)	Survivors	Time (Min.)	Survivors
0	1,400,000	0	1,400,000	0	1,400,000
7	800,000	3	430,000	3	180,000
14	370,000	6	150,000	4	86,000
21	150,000	9	84,000	5	14,000
28	33,000	12	25,000	6	5,800
35	3,500	15	3,800	7	2,600
42	700	18	1,000	8	740
0	7,300,000	0	7,300,000	0	7,300,000
7	3,700,000	3	1,100,000	3	**
14	1,000,000	6	230,000	4	150,000
21	150,000	9	60,000	5	61,000
28	51,000	12	12,000	6	7,900
35	5,200	15	**	7	1,800
42	310	18	1,300		
		0	**	0	800,000
		3	3,800,000	3	380,000
		6	1,200,000	4	230,000
		9	410,000	5	64,000
		12	230,000	6	6,900
		15	19,000	7	9,600
		18	5,800	8	2,000

* Counts represent the average of triplicate plates
** Laboratory accident

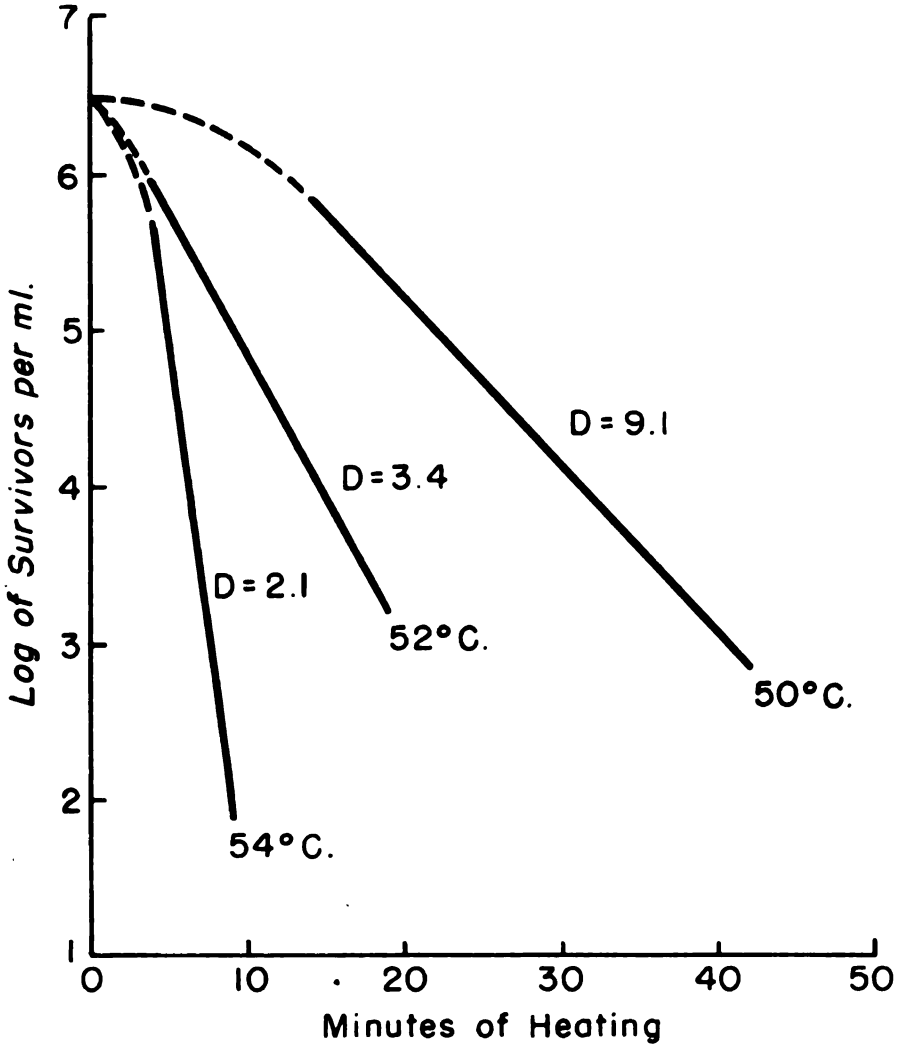


Fig. 37. Rate of destruction of P. fragi in reconstituted skimmilk containing 0.1 per cent by weight of butyric acid at pH 5.85.

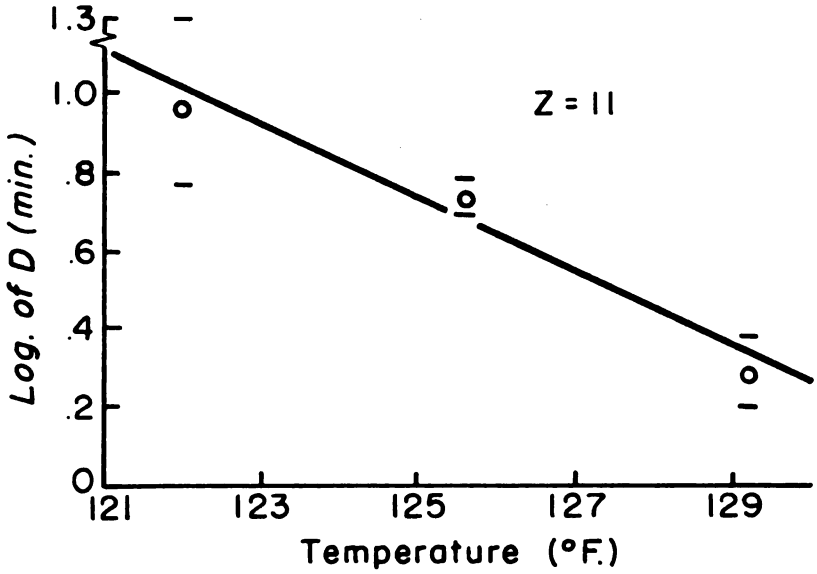


Fig. 38. Thermal resistance curve of P. fragi in reconstituted skimmilk containing 0.1 per cent by weight of butyric acid at pH 5.85.

the thermal resistance curve was 11 (Figure 38). A 99.99 per cent reduction in population occurred in 45, 22 and 8 minutes at 50°, 52° and 54° C., respectively (Table 23).

When the pH of the heating menstrum was 6.7, the heating temperatures were 48°, 50° and 52° C. and the D values at these temperatures were 7.1, 3.4 and 2.1, respectively. The survivor curves from which these D values were derived are shown in Figure 39. Table 21 contains the data which were used in constructing these curves. The slope of the thermal resistance curve gave a z of 14 (Figure 40). The original cell populations were reduced by 99.99 per cent in 43, 20 and 10 minutes at 48°, 50° and 52° C., respectively (Table 23).

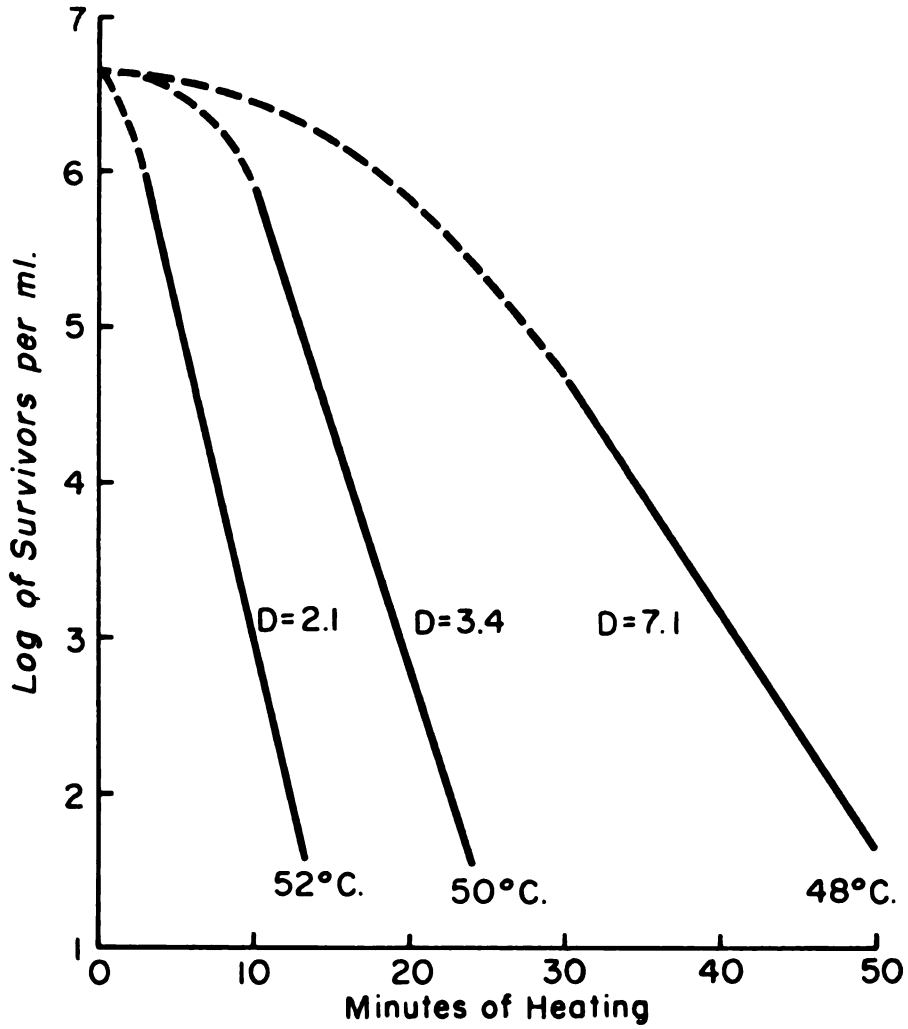


Fig. 39. Rate of destruction of P. fragi in reconstituted skimmilk containing 0.1 per cent by weight of butyric acid and neutralized to pH 6.7.

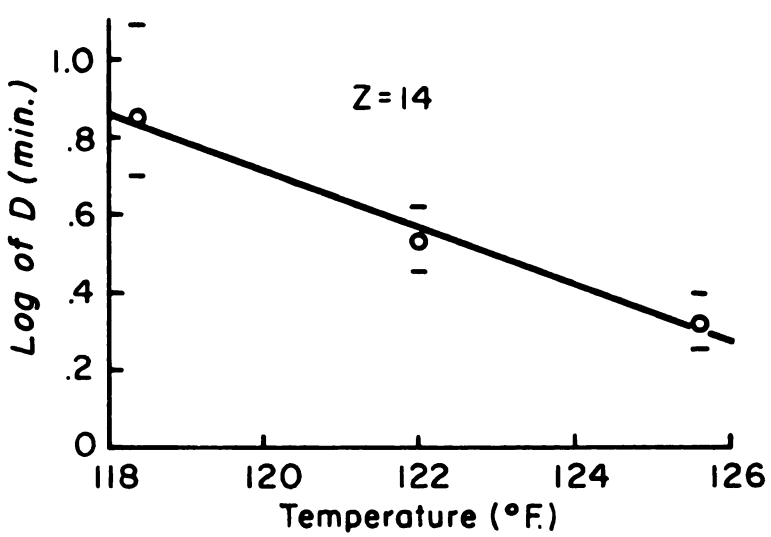


Fig. 40. Thermal resistance curve of P. fragi in reconstituted skimmilk containing 0.1 per cent by weight of butyric acid and neutralized to pH 6.7

TABLE 21. Number of P. fragi per milliliter surviving heat treatment in reconstituted skimmilk containing 0.1 per cent by weight of butyric acid and neutralized to pH 6.7*

Temperature (° C.)					
48		50		52	
Time (Min.)	Survivors	Time (Min.)	Survivors	Time (Min.)	Survivors
0	1,500,000	0	1,500,000	0	1,500,000
10	5,200,000	5	6,000,000	3	3,300,000
20	1,200,000	10	440,000	6	390,000
30	53,000	15	30,000	8	9,500
40	100	20	100	10	1,300
50	10				
0	**	0	**	0	6,700,000
9	1,500,000	4	3,800,000	3	1,600,000
18	550,000	8	460,000	5	280,000
27	130,000	12	140,000	7	160,000
33	11,000	16	32,000	8	62,000
39	320	20	2,400	9	24,000
45	34	24	160	10	4,000
0	6,700,000			0	8,000,000
9	4,900,000			3	3,500,000
18	1,600,000			6	320,000
27	460,000			8	85,000
33	78,000			10	7,100
39	16,000			12	660
45	2,600			14	48

* Counts represent the average of triplicate plates
** Laboratory accident

TABLE 22. Thermal destruction of *P. fragi* after growth in reconstituted skimmilk and heated in reconstituted skimmilk containing 0.1 per cent by weight of butyric, lauric or oleic acid

Skimmilk plus 0.1 per cent of	pH	D	95% Confidence limits	D	95% Confidence limits	D	95% Confidence limits	z(° F.)
		48° C.		50° C.		52° C.		
Oleic acid	6.7	6.2	5.6 - 7.1	3.8	3.2 - 4.8	1.8	1.4 - 2.2	13
Oleic acid	6.45	7.4	6.4 - 8.7	2.5	2.1 - 3.1	1.75	1.7 - 1.8	12
Lauric acid	6.7	6.2	5.3 - 7.7	4.3	3.6 - 5.6	1.5	1.4 - 1.7	12
Lauric acid	6.6	6.7	5.9 - 7.7	3.2	2.8 - 3.8	1.8	1.6 - 2.3	12
Butyric acid	6.7	7.1	5.0 - 12.5	3.4	2.9 - 4.2	2.1	1.8 - 2.5	14
		50° C.		52° C.		54° C.		
Butyric acid	5.85	9.1	5.9 - 20.0	5.4	4.9 - 6.1	1.9	1.6 - 2.4	11

TABLE 23. Time required to destroy 99.99 per cent of the P. fragi cells when heated in the medium and at the temperatures indicated*

Heating medium	pH	Temperature (° C.)			
		48	50	52	54
		Time in minutes			
Skimmilk (control)	6.7	46	27	12	**
Skimmilk plus lauric acid (neutralized)	6.7	39	22	8	**
Skimmilk plus lauric acid (not neutralized)	6.6	49	25	11	**
Skimmilk plus oleic acid (neutralized)	6.7	46	25	10	**
Skimmilk plus oleic acid (not neutralized)	6.45	53	26	13	**
Skimmilk plus butyric acid (neutralized)	6.7	43	20	10	**
Skimmilk plus butyric acid (not neutralized)	5.85	**	45	22	8

* Average of two or three trials
** Not determined

DISCUSSION

Survivor Curves

All of the survivor curves prepared from the data obtained in this investigation had a "lag period" during the initial interval of the heating trials. The duration of this interval depended on the heating temperature and the substrate. The D values were determined by calculating the reciprocal of the slope of the "straight line" portion of the survivor curves. Thus the lag portion of the curve did not influence the D value. Other workers (2, 9, 12, 14, 17, 18, 23, 24, 26, 29, 38, 39, 43, 49, 50) have reported survivor curves with a similar lag period. A few of the reasons offered in explanation for the non-linear survivor curves are: clumping of the cells or spores, poor techniques, erratic data and a non-logarithmic rate of death. The latter reason has been a subject of controversy. Recently Humphrey and Nickerson (18) tested thermal death data for significant non-logarithmic behavior. They analyzed data obtained from heating Bacillus stearothermophilus and found that the spores were not destroyed at a logarithmic rate. They analyzed their data by the method of least squares and also by the second polynomial

($y = a + bx + cx^2$) of least squares. The least squares method determined the position of a straight line; this line represented a logarithmic behavior of death. The second polynomial of least squares determined the position of a curved line through the data; this line represented a non-logarithmic behavior of death. An analysis of variance test was made on the "fit" of these two lines to the data. From the statistical test the hypothesis of linear regression (logarithmic behavior) was rejected and Humphrey and Nickerson (18) concluded that there was significant curvi-linearity (non-logarithmic behavior) in the regression representing the data. On the basis of their data, it would seem that additional research would be necessary to determine whether the second degree polynomial of least squares would apply to all survivor curves. This then raises the question of how one might analyze data obtained from heating bacterial cells and spores. The usual method of determining D and z values is convenient and was used throughout the investigation reported herein.

The survivor curves derived from the data obtained in this investigation showed that the time involved in the lag

period and in the overall heating period decreased as the heating temperature increased. In preliminary analyses of the data during the progress of this research project, survivor curves were prepared by plotting log of survivors per milliliter versus time and drawing a line through the points by the "best fit" method. It was noted that at each of the three heating temperatures a fairly constant ratio was obtained when the time in the lag period was compared to the time required to destroy 99.99 per cent of the initial cell population. This was true only when the same cell suspension was used at all three temperatures. When different cell suspensions were compared a difference in the magnitude of the ratio was noted. Unfortunately, the survivor curves shown in the results section (prepared from the pooled data) do not have a constant ratio. This is attributed to the fact that the original cell populations were not the same. Further investigation would be necessary to determine whether a constant ratio is obtained with other organisms and under different heating conditions.

The P. fragi cells were heated at 48°, 50° and 52° C. which includes the minimum and maximum temperatures which could be used conveniently. Excessively long lag periods

would have been encountered at temperatures of less than 48° C. and even at 48° C. long lag periods were occasionally encountered. For example, in Figure 13, the survivor curve representing the data obtained with reconstituted skimmilk at 48° C. contains a lag of approximately 45 minutes. At temperatures greater than 52° C. the organisms were destroyed at such a rapid rate accurate sampling could not be achieved with the apparatus used.

Thermal Destruction of P. fragi in Half-and-half

This study was undertaken to determine the thermal resistance of P. fragi in a dairy product which contained a higher percentage of milk fat than is normally found in whole milk. The product selected in which to grow and heat the cells was "half-and-half" which contains approximately 11 per cent milk fat.

A comparison of the thermal resistance of P. fragi cells grown at 7° and 25° was included because raw milk and cream held at refrigeration temperatures often contain a high psychrophilic population and it was desired to determine whether a low growth temperature influenced the heat resistance of the organism. According to reports in the literature all psychrophilic organisms are destroyed during

pasteurization, but none of these reports contained data on the thermal resistance of psychrophilic cells that had been propagated at refrigeration temperatures. Work with other organisms has shown that the thermal resistance of cells usually decreases as the growth temperature decreases. However, Lawton and Nelson (28) observed that incubation temperatures of 5° , 10° and 25° C. did not influence the heat resistance of P. fluorescens, P. ovalis, P. geniculata and an unidentified species of Pseudomonas.

When the cells were grown in half-and-half at 7° and 25° C. the z values were 13 and 10, respectively. When the times required to destroy 99.9999 per cent of the original cell population were compared, it was observed that the cells grown at 7° C. required 58, 45 and 42 per cent more heating time at 48° , 50° and 52° C., respectively, than did the cells grown at 25° C. These longer heating times were not due to the lag period since all of the D values obtained with cells grown at 7° C. were approximately twice as large as those obtained with cells grown at 25° C. The reason(s) for the slightly larger z value and the longer heating times obtained with the cells grown at 7° C. is not apparent. Approximately the same number of cells per milliliter were obtained after incubation at 7° C. for 7

days or at 25° C. for 20 hours. The conditions under which the cells were grown and heated were similar except the cells grown at 25° C. were agitated during incubation whereas those grown at 7° C. were not. Therefore, the difference in incubation temperature seems to be the most logical reason for the difference observed in the z values and in the time required to destroy 99.9999 per cent of the original cell population.

Thermal Destruction of P. fragi in Fresh Skimmilk and Cream Containing 20 Per Cent Milk Fat

When P. fragi cells were grown and heated in different batches of half-and-half reproducible results were difficult to obtain. The reproducibility of the curves in replicate experiments improved when the skimmilk and the cream containing 20 per cent milk fat were prepared in sufficient quantities to provide the anticipated needs of the experiments. The data obtained from heating the cells in these two media were compared to the data obtained when the cells were grown in half-and-half at 25° C. and then heated in half-and-half.

When the cells were grown and heated in media containing 0, 11 and 20 per cent milk fat the z values were 9, 10 and 15, respectively. These z values would seem to indicate

that a 20 per cent concentration of milk fat was sufficient to increase the size of the z value whereas a 10 per cent concentration was not sufficient. There was no apparent difference in the z value when the cells were grown and heated in milk containing 0 per cent fat or 10 per cent fat.

A comparison of the times required to destroy 99.9999 per cent of the original cell population showed that the cells grown and heated in skimmilk (Figure 6) required approximately twice as much heating time as did the cells grown and heated in half-and-half (Figure 2) and in 20 per cent cream (Figure 9). These 99.9999 per cent destruction times were used to prepare thermal death time curves for comparison with the milk pasteurization curve (Figure 8). Pasteurization is considered adequate when the thermal death time curve is to the left or below the pasteurization curve. The thermal death time curve shifts toward the pasteurization curve as the required level of destruction increases. However, when the z is approximately 7 the thermal death time curve is almost parallel to the milk pasteurization curve and the margin of safety is adequate although the cells require a long heat treatment. Since the thermal death time curves in Figure 8 are to the left of the pasteurization curve, this would indicate that

pasteurization would normally be sufficient to destroy P. fragi in media containing 0, 11 and 20 per cent milk fat, but the margin of safety would be less when the cells are heated in media containing 20 per cent milk fat.

The z values were nearly the same when the cells were grown and heated in skimmilk (z = 9) and grown and heated in half-and-half (z = 10), but the cells heated in skimmilk required longer periods of exposure to destroy 99.9999 per cent of the original cell population. This was due primarily to the lag period during the initial portion of the heating trials. These two media contained different concentrations of milk fat and also they were not prepared from the same serum solids. Thus the differences observed in the heating exposures required to achieve the same level of cell destruction would seem to be due to either the milk fat or the serum solids. To eliminate the serum solids as a variable, media containing 0, 10 and 20 per cent milk fat were prepared from identical serum solids. The milk fat in the media containing 10 and 20 per cent milk fat was also identical.

Thermal Destruction of P. fragi in Reconstituted Skimmilk, Reconstituted Milk Containing 10 Per Cent Milk Fat and Reconstituted Cream Containing 20 Per Cent Milk Fat

In view of the variation which may have been introduced in preparing media from different batches of product, a carefully controlled study was undertaken to compare z values and the times required to reduce the original cell population by 99.99 per cent. The experimental design of the study involved using standardized media made from the same basic ingredients.

The z values calculated from the data obtained by heating the cells in the media containing 0, 10 and 20 per cent milk fat were 14, 11 and 22, respectively. These z values would seem to indicate that the cells received a limited amount of thermal protection when they were grown and heated in cream containing 20 per cent milk fat. The z values of 14 and 11 would seem to indicate that the medium containing 0 per cent milk fat offered the cells as much thermal protection as did the medium containing 10 per cent milk fat. The z values of 14 and 11 obtained with media containing 0 and 10 per cent milk fat compare favorably with the z of 12.4 obtained by Chaudhary et al. (8) when they heated P. fragi in skimmilk.

A comparison of the times required to destroy 99.99 per cent of the original cell population showed that at 48° C. the longest time was required by the cells grown and heated in media containing 10 per cent milk fat (Figure 13). In this medium approximately 45 minutes of heating time were required at 48° C. before destruction of the cells began to occur at a logarithmic rate. This long lag period was verified by eight different heating trials on eight different days and with eight different cell suspensions. The shortest heating time required at 48° C. was with the cells which were grown and heated in cream containing 20 per cent milk fat (Figure 15). The data obtained by heating the cells at 52° C. indicated that the times required to destroy 99.99 per cent of the initial cell population were nearly the same regardless of the concentration of milk fat in the media in which the cells were grown and heated.

Other workers who have heated microorganisms in dairy products with different concentrations of milk fat are Nichols (33) and Daoust et al. (13). Nichols (33) reported that the heat resistance of Streptococcus thermophilus, Streptococcus durans and four species of Escherichia did not increase as the concentration of milk fat in the heating substrate increased. In some instances the heat resistance

of the cells may have decreased as the per cent milk fat in the heating substrate increased. The decrease in heat resistance could probably be attributed to the increasing concentrations of milk fat in the media in which the cells were heated since all of the cells were grown on agar slants prior to heating. Nichols did not report D or z values, but measured the heat resistance of the organisms by observing the number of positive tubes after exposure for various lengths of time at different temperatures. This method of determining heat resistance would be similar to determining the time required to destroy a certain percentage of the cell population.

Daoust et al. (13) observed that Corynebacterium diphtheriae cells were less heat resistant in 40 per cent cream than in whole milk or ice cream mix. These workers were basing heat resistance on z values. Their observations do not agree with the results reported herein in that the z values obtained when the cells were heated in 20 per cent cream were larger than the z values obtained when the cells were heated in skimmilk and 10 per cent milk. The lack of agreement between the two experiments may be due to the different genera of bacteria which were used as well as the different conditions under which the cells were grown and heated.

On the basis of the data obtained in this investigation and the data presented by others, it is difficult to determine whether milk fat provides thermal protection to non-spore forming bacteria. However, the data obtained in this investigation seem to indicate that the P. fragi cells received a limited amount of thermal protection in the cream containing 20 per cent milk fat.

Thermal Destruction of P. fragi in a Medium Containing a Different Concentration of Milk Fat Than the Medium in Which the Cells Were Grown

This study was undertaken to determine the influence of the growth medium and the heating medium on the z values and the time required to reduce the original cell population by 99.99 per cent. The experimental design of the study involved using standardized media made from the same basic ingredients.

When the cells were heated in media containing 0 and 10 per cent milk fat the z values ranged from 11 to 14, whereas when the heating medium contained 20 per cent milk fat the z values ranged from 20 to 22 (Table 22). The z values did not seem to be influenced by the medium in which the cells were grown prior to heating.

The data obtained by heating the cells at 48° and 50° C. seem to indicate that the growth substrate influenced the time required to reduce the original cell population by 99.99 per cent. Cells grown in 20 per cent cream were reduced to this level in less time than cells grown in reconstituted skimmilk. The medium in which the cells were heated did not seem to influence the times required to destroy 99.99 per cent of the original cell population.

As the time required to destroy 99.99 per cent of the original cell population increased, the length of time involved in the lag period also increased. Humphrey and Nickerson (18) have presented a theory describing why the logarithmic order of death does not begin immediately at the onset of heating. They have suggested that organisms may contain four to ten "heat labile reproduction initiators" and that these "reproduction initiators" would be "heat-induced" during the initial heating, but would eventually be destroyed by further heating, thus the cells would approach a "logarithmic behavior of death." These workers observed that when the straight line portion of the survivor curves was extrapolated back to zero time, the point of intersection was a population that was normally four to ten times larger than the population which

was actually present. This was how these workers obtained the "four to ten" possible "reproduction initiators." If one attempted to relate this theory to the data reported herein, one might postulate that the cells grown in skim-milk may have had a larger number of "reproduction initiators" than the cells grown in the media containing milk fat. Also the possibility exists that the "reproduction initiators" may have been more resistant when the cells were grown in the absence of milk fat. This is strictly a hypothetical observation, but merits mentioning since an explanation for the variation in the time required to accomplish a given amount of destruction is not apparent. In any case, the factor(s) influencing the time required to destroy 99.99 per cent of the original cell population must have been either incorporated directly into the cell during its formation, or perhaps associated with the material surrounding and adhering to the cell. In either case the responsible factor(s) were identified with the cells. This can be substantiated by the fact that the medium in which the cells were grown seemed to exert more influence on the time required to obtain 99.99 per cent destruction than did the medium in which the cells were heated.

Thermal Destruction of P. fragi in Reconstituted Skimmilk Containing Selected Fatty Acids

P. fragi is very lipolytic (1, 19, 32), therefore, some of the free fatty acids or other lipid residues in the growth medium may have been responsible for the differences observed in the time required to destroy 99.99 per cent of the original cell population. The free fatty acids and other lipid residues could have been carried along with the cells when they were transferred into the medium in which they were heated. In an attempt to determine whether free fatty acids influenced the time required to destroy 99.99 per cent of the original cell population, cells were heated in reconstituted skimmilk which contained 0.1 per cent by weight of a selected fatty acid. The fatty acids selected were butyric, lauric and oleic. These fatty acids were selected so that a comparison could be made to show the influence of carbon chain length and saturation versus unsaturation on the destruction rates of P. fragi.

Each fatty acid was added to the skimmilk on a per cent basis rather than a molar concentration, thus the addition of butyric acid resulted in a greater change in pH than did the addition of lauric or oleic acid. To compensate for differences in pH on the thermal resistance

of the cells, heating trials were conducted with and without adjustment of the pH to 6.7 with trisodium phosphate.

Since very little difference exists in the z values obtained with the three fatty acids (11 to 14) it would seem that the carbon chain length and the characteristics of saturation or unsaturation of the fatty acids has little influence on the resultant z values. Also the pH levels encountered did not influence the z values obtained. When the cells were heated in reconstituted skimmilk without the fatty acids the z value was 14, which is within the range of 11 to 14 encountered when fatty acids were added to the skimmilk. Therefore, it appears that the presence of the acids in the skimmilk had little influence on the z values. Perhaps a higher concentration of each fatty acid in the skimmilk would have exhibited a more noticeable effect.

A comparison of the times required to destroy 99.99 per cent of the original cell population showed that with lauric and oleic acids the heating times required were approximately the same when the pH of the skimmilk-fatty acid mixture was 6.7 and less than 6.7 (Table 23). However, when the skimmilk contained butyric acid the times required at pH 5.85

were longer than those required at pH 6.7. There is no apparent explanation for the longer heating times required when the pH was 5.85.

SUMMARY AND CONCLUSIONS

The destruction rates of Pseudomonas fragi were determined on cells heated in media containing different concentrations of milk fat and in reconstituted skimmilk containing selected fatty acids.

All of the survivor curves contained a lag period during the initial interval of the heating trials. When cells from the same suspension were heated at 48°, 50° and 52° C., the time involved in the lag period of the survivor curves appeared to be a fairly constant proportion of the time required to obtain a given level of destruction.

The P. fragi cells grown in half-and-half at 7° C. for 7 days and at 25° C. for 20 hours had z values of 10 and 13, respectively. The cells grown at 7° C. required longer periods of heating to reduce the initial cell population by 99.9999 per cent than did the cells grown at 25° C. These longer heating time requirements were attributed to the incubation temperature.

When cells were heated in media containing 0, 10 and 20 per cent milk fat, the largest z values were obtained with media containing 20 per cent milk fat. The z values were approximately the same when the cells were heated in media

containing 0 and 10 per cent milk fat. The cells grown and heated in media containing 20 per cent milk fat required less time to obtain a 99.99 per cent reduction in the initial cell population than did the cells grown and heated in media containing 0 per cent milk fat.

Data obtained from heating trials performed with media prepared from the same basic ingredients indicated that the z values were influenced by the medium in which the cells were heated, but the z values were not influenced by the medium in which the cells were grown. The largest z values were observed when the cells were heated in cream containing 20 per cent milk fat. The concentration of milk fat was the primary difference in the heating media, thus it would seem that the milk fat was responsible for the larger z values. Apparently the cells were influenced by the medium in which they were grown and this in turn influenced the times required to destroy 99.99 per cent of the original cell population. The cells grown in media containing 0 per cent milk fat required the longest heating times. The medium in which the cells were heated did not influence the times required to destroy 99.99 per cent of the original cell population. The differences observed in the destruction times were attributed to the concentration of milk fat in the growth medium.

Little if any difference in the z values was noted when P. fragi was heated in reconstituted skimmilk which contained 0.1 per cent by weight of butyric, lauric or oleic acid. A comparison of the times required to destroy 99.99 per cent of the initial cell population showed that with lauric and oleic acid the times were approximately the same when the pH of the skimmilk-fatty acid mixtures was 6.7 and less than 6.7. The times required when the pH of the skimmilk-butyric acid mixture was 5.85 were longer than those required when the pH was 6.7. An explanation for these differences in destruction time was not apparent.

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