

EFFECT OF pH AND BRINE CONCENTRATION ON
GROWTH AND THERMAL DESTRUCTION OF
PA 3679 IN PROCESSED CHEESE SPREAD

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John A. Jaynes

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This is to certify that the

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John A. Jaynes

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John A. Jaynes

AN ABSTRACT

Submitted to the School for Advanced Graduate Studies of Michigan
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ABSTRACT

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The effect of pH and brine concentration was determined on the growth and thermal destruction of spores of PA 3679 of a known heat resistance in a processed cheese spread. The spores used had a D₂₅₀ of 0.98 minutes and a z of 17.5° F.

Most growth tests were conducted by sealing the inoculated standardized processed cheese spread in TDT-cans and incubating at 37° C. for various periods of time. Growth was evaluated by measuring the expansion occurring in the cans with a dial micrometer.

The amount of skim milk powder used in the production of processed cheese spread effected the growth of the test organism in the product. Profuse growth occurred when a 10.0 per cent concentration was used, but no growth was observed when only 1.0 per cent was used. The amount of spore inoculum, pH, and brine concentration were the same in both lots of processed cheese spread.

Both pH and brine concentration influenced the growth of the test organism in the processed cheese spread. There was no growth at or below a pH of 5.6 and no growth at or above a brine concentration of 7.6 per cent.

The two methods utilized for the recovery of viable spores after the thermal process were the subculturing in stratified liver infusion broth of 0.01 gram of product from each TDT-can, and the incubation of the processed can-

ABSTRACT

JOHN A. JAYNES

ned product with periodical examinations for swells.

Studies conducted to determine the effect of the pH and brine concentration of the processed cheese spread on the thermal process required to destroy the test organism in the product revealed that the pH had an effect, but the brine concentration did not. The average D_{235} values of PA 3679 in the processed cheese spread at pH 5.50, 6.25, and 7.00 were 4.55, 6.90, and 8.25 minutes, respectively. The z values remained constant at 18° F. Tests conducted by incubating the product in the can revealed that as the temperature of the thermal process increased, the minimum number of spores required to initiate growth in the product decreased.

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INTRODUCTION

During the production of processed cheese spreads the batch is usually cooked to approximately 160° F. The finished product is normally packed in hermetic containers and moves through the marketing channels at room temperature to the consumer. Since the cooking temperatures used in the manufacture of the product do not render it sterile, the prevention of spoilage is largely dependent upon the microbial inhibitory nature of the product.

The changes in consumer preference have resulted in an evolution in processed cheese spreads. The pH is higher and the brine concentration lower. These changes in processed cheese spreads have decreased the microbial inhibitory property of the product, resulting in an increased susceptibility to spoilage. A heat treatment to sterilize the product may be utilized to compensate for the increased susceptibility to spoilage.

The heat treatment required to prevent food spoilage by a spore forming organism is a function of the heat resistance and number of bacterial spores in the food, and the rate of heating of the food product. The heat resistance of a specific bacterial spore is a function of the substrate in which it is heated, making it necessary to determine experimentally the heat resistance of the spore in each different substrate.

The objective of this research was to determine the

thermal process requirements of a processed cheese spread for one food spoilage organism under varying conditions of pH and brine concentration. The problem was studied in three parts: (1) The effect of pH and brine concentration on the growth of the spoilage organism; (2) The effect of pH and brine concentration on the thermal destruction of the spoilage organism; and, (3) The effect of pH and brine concentration on the heat treatment required to prevent growth of the spoilage organism. It is hoped that the results of this study will aid industry in the design of thermal processes for processed cheese spreads.

LITERATURE REVIEW

Thermal Processing

Cameron (16) classified foods into two general categories; "acid" foods, pH below 4.5, and "low-acid" foods, pH 4.5 or above. His classification was based primarily upon the premise that Clostridium botulinum would not grow at a pH of less than 4.5. Most pathogenic microorganisms either will not germinate or will not grow in acid foods. Most of the work in the heat preservation of canned foods has been done with the low-acid foods because a relatively severe process is required to reduce the potential spoilage and health hazard of these foods.

Tanner (52) stated that a commercially sterile canned food is one which has been processed by heat in such a manner that it will not spoil under ordinary market conditions, even though it may not be completely sterilized. Design of the thermal process to which a particular canned food should be subjected requires two categories of information, thermal resistance data and heat penetration data.

Bacteria follow a logarithmic order of death when subjected to heat. Rahn (38) pointed out that regardless of the explanation for the existence of the logarithmic order of death, theory permits us to compute death rates and draw conclusions. These death rates make possible the quantitative evaluation of the effect of environmental factors such as pH and brine concentration upon heat

sterilization.

Bigelow and Cathcart (12) described a curve relating time and temperature for the complete destruction of a bacterial population under stated conditions, as a thermal death time (TDT) curve. Bigelow (10) further stated that the plotting of thermal death time data for bacteria on semi-logarithmic paper resulted in a straight line. Ball (7) suggested the phantom thermal death time curve which would relate the time and temperature required to reduce the bacterial population by 90 per cent. Stumbo (49) recommended that the thermal death time curve relate the time and temperature necessary for a known reduction of the bacterial population. Schmidt (45) applied the name of thermal resistance curve to the type of curve suggested by Ball (7).

The time required to reduce a known bacterial population by 90 per cent at a specific temperature is defined as the D value. The D value may be determined by measuring the slope of the survivor curve which relates the number of survivors and time at a specific temperature for a stated set of conditions, or it may be calculated from the results of multiple sample thermal resistance determinations. The methods of Stumbo (50), Stumbo et al. (51), and Schmidt (45) are the methods in general use today for the calculation of D values. Confidence limits can be calculated by the Schmidt method.

Methods of conducting multiple sample thermal resistance determinations in general use today include the mul-

multiple tube method of Bigelow and Esty (13), the TDT-can method of Townsend et al. (2,57), and the thermoresistometer method of Stumbo (50). The bacterial suspension is usually heated in approximately two milliliters of a phosphate buffer solution in the multiple tube method, in approximately 18 g. of the food substrate in the TDT-can method, and in 0.01 ml. of a phosphate buffer solution in the thermoresistometer method.

The design of a thermal process for a canned food necessitates the integration of thermal resistance and heat penetration data. Thermal processes may be calculated by the graphical method of Bigelow et al. (11) and the mathematical method of Ball (5). The nomogram alignment chart method of Olson and Stevens (31) is a solution of the mathematical method of Ball.

Some of the early heat penetration work, as recorded by Thompson (56), was conducted with an apparatus which consisted of a copper-constantan thermocouple located in the center of the can, a cold junction located in a cracked ice bath, and a galvanometer calibrated by reference to a mercury thermometer. A few refinements (8) such as the automatic recording potentiometer and special fittings have taken place since that time, but basically the apparatus remains unchanged.

Townsend et al. (57, 58) determined that the rate of heat penetration was affected by the amount of fill and the head-space. Sognefest and Benjamin (46) conducted heat

penetration tests in TBT-cans and found that the point of slowest heating was "located between the side and center midway between the ends due to the dimple in the bottom of the can". A comparison of heat penetration rates with the can in the flat position and with it on its side revealed a greater f_h , defined by Ball (5), when the can was on its side if, heating took place by convection. When heating took place by conduction, similar f_h values were noted for both positions. These tests were conducted on a 13 g. fill at a retort temperature of 250° F. Sognefest and Benjamin (46) calculated lag correction factors (t_c) which is the time that must be added to the time at retort temperature (U) to obtain the process time (B). The lag correction factors included both the correction for come-up time and the heat penetration lag because timing was started as soon as steam was admitted to the retort. They concluded that a lag correction factor based on a retort temperature of 250° F. could be used at any retort temperature from 230 to 270° F. This conclusion results from the fact that the lag correction factor is a function of the difference between the retort temperature and the initial temperature of the canned product, which is sufficiently great that a change of plus or minus 20 degrees makes very little difference in the resulting lag correction factors.

Spoilage of Canned Cheese

Doane (20) reported that cheese could not be canned

because the product would develop a putrid flavor and odor. The Eighteenth Annual Report of the Council for Scientific and Industrial Research (4) stated that although process cheese packaged in tin normally kept well, even under extreme conditions of temperature, several cases of severe spoilage had been investigated. Palmer and Sly (32) concluded that the cooking temperature in the manufacture of processed cheese was the most important factor in preventing fermentation but Albus and Ayers (1) reported that spore forming anaerobes survived the cooking temperatures. According to Fette and Liebert (34) gaseous fermentation did not occur in processed cheese that was cooked to temperatures greater than 70° C. Ledabyl (26) stated that cooking temperatures in excess of 90° C. were necessary to destroy spore formers. In tests conducted with Clostridium sporogenes in processed cheese spreads Csizar (18) recovered viable organisms after 72 hours at 60° C., 24 hours at 70° C., and 2.5 hours at 80° C.; he was unable to recover viable spores when the cheese spread was subjected to 90° C. for 50 minutes or 100° C. for 6 minutes. He concluded from this work and further investigations (19) that spores of Cl. sporogenes, which produced huffed (swollen by gas) pasteurized cheese, could not be destroyed by temperatures used to make a satisfactory product.

Meyer and McIntire (28) stated that acceptable processed cheese spread could be produced by heat processing the packaged spread in a conventional retort at 240° F. for

75 minutes. Meyer et al. (27) produced a high quality processed cheese spread by heating the product to 292° F. in a heat exchanger, holding for 20 seconds, cooling to 135° F. and aseptically packaging. This procedure resulted in an F_0 of 90 (thermal process equivalent to 90 minutes at 250° F. with a z of 18° F.). High temperature storage failed to produce any spoilage. Ball (6) pointed out that high temperatures have less effect on quality impairment, in proportion to their destructive effect on bacteria, than low temperatures.

Spoilage of Processed Cheese

Numerous incidents of processed cheese spoilage have been recorded. Albus and Ayers (1) reported that processed cheese is a favorable medium for the growth of anaerobes since the product is packaged hot and contains little oxygen. Also, processed cheese contains very few organisms other than spore forming anaerobes because of the cooking temperatures during manufacture. Griffiths (22) isolated an anaerobic spore forming organism from a processed cheese which had developed a serious off-flavor accompanied by a penetrating putrefactive odor within two or three weeks after the cheese was produced.

Hood and Bowen (24) investigated spoilage in processed cheese which contained large gas holes and possessed a very obnoxious, penetrating and putrefactive odor and identified the causative organism as a variant of Cl. sporogenes

Csiszar (17) reported that of the samples of huffed processed cheese examined, 94 per cent were caused by Cl. sporogenes. The Eighteenth Annual Report of the Council for Scientific and Industrial Research (4) attributed huffed and putrid odors in processed cheese to Cl. sporogenes and Cl. welchii. Processed cheese spoilage has also been attributed to Cl. butyricum (34, 42, 64).

Effect of skim milk powder. Skim milk powder is a common ingredient of processed cheese spreads. Templeton and Sommer (55) obtained a satisfactory processed cheese spread by using as high as 10 per cent skim milk powder in the batch. Meyer and McIntire (28) observed that significant levels of carbohydrate (skim milk powder is approximately 50 per cent carbohydrate) reduced the keeping quality of processed cheese spread. Hood and Bowen (24) initiated spoilage in experimental batches of processed cheese by adding nutrients such as skim milk powder, casein digest, and mature cheddar cheese. They found that with each increase in the amount of skim milk powder added, the time required for subsequent spoilage decreased. Van Slyke and Price (61) stated that the addition of skim milk powder decreased the acidity and rendered the cheese spread more favorable for the growth of gas-forming organisms. The Eighteenth Annual Report of the Council for Scientific and Industrial Research (4) pointed out that skim milk powder had been used in the manufacture of all cheese which was spoiled and swollen. The report also stated that the pre-

sence of skim milk powder necessitated lower processing temperatures to avoid a browning reaction of the carbohydrate and that the lower F_0 employed did not destroy the spore forming spoilage organisms present.

Effect of pH. Under the Federal Food, Drug and Cosmetic Act (60), the pH of processed cheese spread may not be less than 4.0. Barker (9) pointed out that the keeping quality of a processed cheese spread is determined by the amount of acid present. Sommer and Templeton (48, 53, 54) recommended acidifying processed cheese spreads to a pH of 5.8 to 6.3 to improve keeping quality. Wearmouth (65) observed that the majority of processed cheese had a pH of 5.9 to 6.6. Pette and Liebert (33) stated that a pH of less than 5.7 was necessary to prevent putrefactive spoilage. Hood and Smith (25) and Ritchie (41) found that putrefactive spoilage was completely eliminated at a pH of less than 5.4.

In 1920 Bigelow and Esty (13) observed that as the pH was decreased the time required for complete destruction of a spore suspension by heat decreased. Bigelow and Cathcart (12) stated that the lower the pH of the food, the lower the F_0 required for sterilization. Sognefest et al. (47) observed that the progressive increase in the resistance of spores to heat was slight from pH 5.5 to 9.0 in comparison to the progressive increase in heat resistance from pH 5.0 to 5.5. Ball and Olson (8) stated that generally, the thermal resistance of spores is greatest at a pH of 7.0. Vas and Proszt (62) attributed the need of a re-

latively small F_0 for the preservation of acid foods to the inhibition of spore germination rather than to the low heat resistance of the spores. Schmidt (44) stated that spores of Cl. sporogenes would germinate at a pH of 5.3 but would not grow.

Effect of salt. Esty and Meyer (21) reported that spores of Cl. botulinum and allied anaerobes exhibited no reduction in heat resistance in the presence of salt until a total concentration of 8.0 per cent was reached. Salt concentrations of 0.5 and 1.0 per cent resulted in greater heat resistance than concentrations of 2.0 and 3.0 per cent. According to Viljoen (63) salt increases the heat resistance of bacterial spores. This protective influence was detectable up to concentrations of 3.5 per cent but the greatest protection was exhibited between concentrations of 1.0 and 2.5 per cent salt. Townsend et al. (59) stated that salt was protective up to concentrations of 4.0 per cent and that higher levels lowered heat resistance.

In studies with comminuted pork Bulman and Ayres (14) showed that when the spore inoculum (PA 3679) remained constant the shelf-life of the product increased with increases in the salt level. They concluded that the critical level of salt in the product for prevention of spoilage was around 4.0 per cent. In experiments with brain-liver-heart medium and processed cheese, Hood and Smith (25) demonstrated the inhibitory effect of salt on the growth of Cl. sporogenes. They concluded that processed cheese would not spoil if it

contained more than 3.5 per cent salt regardless of the pH. Cl. sporogenes was reported to have germinated in 8.0 per cent salt but failed to grow under this condition (44). Yesair and Cameron (68) reported that a comparison of thermal death times of spores of Cl. botulinum in salted and unsalted media demonstrated the inhibitory effect of curing salts, but when the heated media were subcultured there was no destructive influence. The resistance determined by subculturing after heat treatment was approximately the same in the salted and unsalted product.

Putrefactive Anaerobe 3679 as
a Test Organism

PA 3679 is typical of the non-toxic, heat-resistant, mesophilic anaerobes which grow at normal storage temperatures (39) and is widely used by laboratories connected with the canning industry in experiments on canned foods (57).

The Canned Food Reference Manual (2) states that spores of PA 3679 are more heat resistant than spores of Cl. botulinum and that a process based on the destruction of PA 3679 is more than adequate to destroy spores of Cl. botulinum. Sognefest et al. (47) stated that a process based upon a suspension of PA 3679 having an F_0 of 3.65 to 4.15 minutes in neutral phosphate, should insure a commercially sterile product for non-acid foods. Townsend et al. (57) advocated the comparison of the heat resistance of different suspensions of spores in a standard phosphate buffer

consisting of equal amounts of M/15 Na_2HPO_4 and M/15 KH_2PO_4 mixed to give a pH of 7.0.

Reed et al. (39) reported that a z ($^{\circ}\text{F}$. required for the thermal resistance curve to transverse one log cycle) of 18°F . for PA 3679 was a valid approximation. Reynolds et al. (40) observed z values ranging from 14.5 to 27.5°F . for PA 3679 when the curves were based upon data derived from incubating the food substrate.

Schmidt (44) pointed out that spores which survived drastic killing influences were more exacting in their nutritional requirements; therefore, enrichment was necessary for accurate enumeration of survivors. Sognefest et al. (47) stated that the subculturing of samples frequently resulted in data too erratic to obtain a straight line thermal resistance curve, as contrasted to the straight line curves usually obtained from data from products incubated in TDT-cans. Townsend et al. (59) stated that TDT-cans were difficult to subculture and were not suitable for conducting tests where the test organism did not grow readily in the food. They reported that with uncultured TDT-cans, F values were generally lower and z values were generally different from those obtained by other methods.

Delayed germination was reported to be characteristic of PA 3679 (29). A Laboratory Manual for the Canning Industry (59) suggests that samples containing PA 3679 be incubated at least three months and at least one month after the last positive sample develops. The survival of PA 3679 is usually

determined by noting gas production and characteristic odor in the subculture medium (15). Liver broth is an excellent medium for subculturing PA 3679 (39, 44, 59).

EXPERIMENTAL PROCEDURE

Previous investigations have proven the feasibility of preserving canned processed cheese spreads by heat treating the product. The experimental procedure employed in this investigation was designed to study the effect of pH and brine concentration on the growth and heat destruction of one spoilage organism (PA 3679) in the product.

Preparation of Heat Resistant Spore Suspension of PA 3679

Preparation of growth medium. Ten liters of pork extract were prepared according to the method of Reed et al. (39) for growth of the organism. This procedure is described below:

1. 10 lb. ground lean pork mixed with 10 l. of water.
2. Boil 1 hour.
3. Adjust pH to 7.4 with NaOH.
4. Press out meat and dry for later use.
5. Cool over-night and remove fat.
6. Make up to 10 l. with water.
7. Add 50 g. bacto-peptone, 15 g. bacto-tryptone, 10 g. dextrose and 12.5 g. K_2HPO_4 .
8. Adjust pH to 7.4.
9. Dispense and autoclave 30 minutes at 15 lbs. pressure.

Growth of organism. A culture of PA 3679 of known history was obtained from Wyeth Company in Mason, Michigan. Using the pork extract and the following schedule, six liters

of spores of PA 3679 were grown:

1. Transfer 2 ml. of culture into each of three tubes containing dried pork and 10 ml. of extract. Stratify (two parts mineral oil to one part paraffin) and incubate at 37° C.
2. When the tubes show good growth as indicated by gas production (usually one day) transfer 2 ml. into each of three tubes containing dried pork, two nails, and 10 ml. of extract. Incubate one day at 37° C.
3. Transfer the contents of each of the three tubes into each of three flasks containing dried pork, five nails, and 50 ml. extract. Incubate two days at 37° C.
4. Transfer the contents of each of the three flasks into each of three large flasks containing dried pork, 12 nails, and 2 l. of extract. Incubate one week at 37° C. and then two weeks at 30° C.

Harvesting of organism. The spores were harvested and washed according to the method suggested by Townsend et al. (59) and is briefly described as follows:

1. Filter the material through a layer of fiber glass with cheese cloth on both sides.
2. Fill international centrifuge bottles (250 ml.), centrifuge (30 minutes at 1500 r.p.m.) and decant repeatedly until all spores are concentrated in the bottom of six bottles.
3. Decant, add glass beads, and fill with sterile distilled water. Shake five minutes and centrifuge.
4. Decant, fill bottles one-half full with sterile distilled water, shake five minutes, combine into four bottles, and centrifuge.
5. Decant, fill bottles one-half full with sterile distilled water, shake five minutes, combine into two bottles, and centrifuge.
6. Decant, fill bottles one-half full with sterile distilled water, shake five minutes, combine into one bottle, and centrifuge.
7. Decant, fill bottle three-fourths full with neutral phosphate buffer (equal amounts of M/15 KH_2PO_4 and M/15 Na_2HPO_4), shake five minutes, and centrifuge.

8. Repeat step seven three times except do not centrifuge last time.
9. Store concentrated spore suspension in capped bottle at 40° F.

Standardization and storage of organism. The spore suspension was standardized by determining the most probable numbers (MPN) of spores in the concentrated suspension and then diluting with neutral M/15 phosphate buffer to obtain approximately 10^6 spores per milliliter. The diluted suspension was stored at 40° F. The MPN and all subsequent subculturing was carried out in liver infusion broth (39, 44, 59); stratified with two parts mineral oil to one part paraffin (59). The MPN was conducted using five tubes at each dilution. Two liters of liver infusion broth were prepared by the method of Reed et al. (39) as outlined below:

1. Boil 1000 g. of coarse ground beef liver in 2000 ml. of water for one hour.
2. Adjust pH to 7.2 with NaOH and boil for an additional 10 minutes.
3. Filter material through cheese cloth and cotton.
4. Air dry solid portion and make fluid up to 2000 ml. with water.
5. Add 20 g. peptone and 2 g. K_2HPO_4 .
6. Dispense into tubes, each of which contains a pinch of dried liver.
7. Stratify and autoclave 30 minutes at 15 lb. pressure.
8. Store at 40° F.

Before use, the tubes of liver infusion broth were exhausted by flowing steam in an autoclave for 20 minutes to remove

excess oxygen and melt the stratifying cap. The tubes were then tempered to 45° C. in a water bath and inoculated with the proper dilution of spores. If a LPN was being determined, the tubes were heated for 10 minutes at 80° C. to heat-shock the spores and then incubated at 37° C. Positive tubes were identified by the presence of gas which was indicated by the elevation of the stratifying cap above the surface of the broth. Most positive tubes became apparent within the first five days of incubation.

Thermal resistance of organism. The thermal resistance of the suspension was determined by exposing samples of spores to different heat treatments in a thermoresistometer and then subculturing in tubes of stratified liver infusion broth. The results, in numbers of positive and negative tubes at each time-temperature combination, were used to calculate D values, which in turn were used to plot a thermal resistance curve.

The thermoresistometer used was similar to the one described by Stumbo (51) and Pflug and Esselen (37). It is an apparatus which essentially consists of a pressure chamber which can be maintained at a specified temperature and a series of valves and pistons which introduce small cups containing a known number of spores into the steam chamber for a specified length of time, after which the cups are automatically withdrawn and dropped into tubes of subculture broth. Five cups were tested at a time.

The sample cups (11 mm. outside diameter by 8 mm. deep and formed from tinplate 0.008 in. thick) were soaked and washed in alcohol, placed in petri dishes and sterilized in an autoclave for 15 minutes at 15 lb. pressure. The standardized suspension of spores was shaken 15 minutes and a small portion transferred to a 50 ml. flask. The flask was placed on a magnetic stirrer which kept the suspension constantly agitated. The cups were loaded with spores using a micro syringe-burette which held 0.25 ml. calibrated in 0.0001 ml. The micro syringe-burette was filled from the flask and then clamped to a ring stand. Each cup was removed from the petri dish with sterile tweezers, 0.01 ml. of suspension measured into it, and the cup returned to the petri dish. The calibration of the syringe was checked before using by weighing delivered samples of distilled water on an analytical balance. Twenty-five cups could be loaded each time the syringe was filled. The petri dishes of loaded cups were placed in a desiccator and held at least 24 hours prior to use to remove most of the moisture before the cups were introduced into the high temperature chamber.

The procedure for making a test with the thermoresistometer follows:

1. Adjust pressure controller to obtain desired temperature.
2. Set exposure time on automatic cycle timer.
3. Aseptically place loaded cups and subculture tubes in apparatus. Tubes containing PA 3679 should be stratified and steamed at least 20 minutes.
4. Actuate timing cycle and rotate trip bar 180 degrees.

5. After the cups are automatically withdrawn and dropped into the subculture tubes; remove and flame tubes, insert sterile plugs, and place in a 37° C. incubator.
6. Reset support pins for next trial.

The complete operation, excluding the time the cups are in the chamber, takes approximately one minute. Two trials were made at each time and temperature, resulting in 10 replicate tubes. All tubes were incubated at least three months.

At the end of the incubation period a D value and 95 per cent confidence limits were calculated for each of the test temperatures, using the method described by Schmidt (45). Using this procedure a curve was prepared for each temperature condition by plotting the value of probability (P) as a function of time (U) on arithmetic probability paper and determining the L.D.50 point which is the time (U) at which P equals 0.50, or the time at which one-half of the samples are positive and one-half are negative. The probability (P) was calculated using the equation

$$P = \frac{n + 1}{m + n + 2} \quad (1)$$

where n = cumulated samples not surviving each exposure time.

m = cumulated samples surviving each exposure time.

The D value for each temperature was calculated using the L.D.50 value and the equation

$$D = \frac{\text{L.D.50}}{\log A + 0.16} \quad (2)$$

where A = the initial no. of organisms per tube.

The 95 per cent confidence limits of the L.D.50 value were calculated using the equation

$$95\% \text{ CI} = \frac{1.96 \times 2S}{\sqrt{2N}} \quad (3)$$

where $2S$ = difference between the time (U) when $P = 0.16$ or 16 per cent of the tubes would have been negative, and the time (U) when $P = 0.84$ or 84 per cent of the tubes would have been negative, as determined from the probability curve.

N = total no. of tubes in the groups showing partial survival.

The 95 per cent confidence limits of the D value were calculated using the equation

$$95\% \text{ CL}_D = \frac{\text{L.D.50} \pm 95\% \text{ CI}}{\log A \pm 0.16} \quad (4)$$

Preliminary trials were conducted at 240 and 250° F., the D values calculated, and a preliminary thermal resistance curve plotted using the two points. The final thermal resistance tests were conducted at 240, 245, 250, 255, and 260° F. The range of time over which testing was to be conducted was determined and then the actual testing times (U) were found by dividing the range by units of one-seventh of a log cycle. After incubation the D values and 95 per cent confidence limits were calculated and the thermal resistance curve for the spore suspension was plotted.

Growth of PA 3679 in Processed Cheese Spread

Effect of skim milk powder. A preliminary 5 lb. batch of processed cheese spread was prepared to test the effect of skim milk powder on the growth of PA 3679. The batch was divided into two lots and enough skim milk powder was added to give a 1.0 per cent concentration in one lot and a 10.0 per cent concentration in the other. A beaker of each lot of processed cheese spread was placed in a water bath and tempered to 65° C. to obtain complete fluidity. Enough spores of PA 3679 were mixed into the processed cheese spread to give 1.84×10^5 spores per gram and the pH was adjusted to 6.0.

Initially the pH was determined by using both a glass and a quinhydrone electrode system. These determinations revealed no significant difference in the pH when using the two different electrode systems; therefore, the pH was determined with the glass electrode system only, during the latter part of the project. All pH determinations were made at 65° C.

After the addition of the spores and the adjustment of pH, the processed cheese spread was dispensed into test tubes. To facilitate this step, a metal tube (0.7 cm. inside diameter and 15 cm. long) was soldered to a 2 oz. "dose syringe". This instrument made it easy to fill the tubes to the proper level without incorporating air pockets or smearing the product on the upper inner surface of the tube.

Each tube was stratified, plugged, heated for 10 minutes at 80° C. to heat-shock the spores, tempered to 37° C., and incubated eight days at 37° C. At the end of the incubation period each tube was examined for the presence of gas.

The stratifying plug was melted and decanted from all tubes showing gas production and the odor noted. A Gram stain was made of the processed cheese spread and a loop full of the product subcultured in stratified liver infusion broth which was incubated at 37° C.

After the preliminary test was completed a large batch of processed cheese spread was produced for use throughout the remainder of the project. The ingredients included 43 lb. of aged cheddar cheese, 30 lb. of unsalted green cheese curd, 22 lb. of water, 10 lb. of 35 per cent cream, 10 lb. of skim milk powder, and 3 lb. of sodium citrate. The mixture was cooked to 160° F. in a Mojonnier processing kettle, drawn off into No. 10 cans (603 X 700) and the cans sealed. The cans of processed cheese spread were cooled and stored in a 40° F. refrigerator.

The amount of fat and moisture in the processed cheese spread was determined by the Mojonnier method (30). The salt content was determined by the method outlined by Wilster et al. (67). The equation for brine concentration is:

$$\text{brine conc. (\%)} = \frac{\text{g. salt} \times 100}{\text{g. salt} + \text{g. water}} \quad (5)$$

which is equivalent to:

$$\text{brine conc. (\%)} = \frac{\% \text{ salt} \times 100}{\% \text{ salt} + \% \text{ H}_2\text{O}} \quad (6)$$

To determine the total amount of salt necessary to give a specified brine concentration in the processed cheese spread, equation 6 was rearranged as follows:

$$\text{g. salt} = \frac{\% \text{ brine} \times \text{g. H}_2\text{O}}{100 - \% \text{ brine}} \quad (7)$$

where % brine = brine concentration desired.

The brine concentration in the processed cheese spread was standardized by calculating the amount of salt present in the sample, determining the amount of salt necessary to give the desired brine concentration using equation 7, and then adding the difference because when the brine concentration was standardized, it was always increased. In using equation 7, the grams of water added with the spore inoculum were taken into consideration. Brine concentration throughout the project is defined by equation 6.

Effect of pH and brine concentration. Several tests were conducted to determine the effect of pH and brine concentration of the processed cheese spread on the growth of PA 3679. A sealed can of processed cheese spread was removed from the refrigerator and allowed to temper over-night at room temperature. The can was then placed in a covered water bath at 65° C. for one-half day. The can was opened

and the desired amount of processed cheese spread weighed into a beaker which was placed in the 65° C. water bath. Enough spore inoculum was added to result in 1.84×10^5 spores per gram and the brine concentration standardized. The pH was determined and standardized using NaOH or HCl as necessary.

Growth tests were conducted both in test tubes and in TDT-cans. The tubes were stratified, plugged, heated for 10 minutes at 80° C. to heat-shock the spores, tempered, and incubated at 37° C. The TDT-cans contained 18 g. of the standardized processed cheese spread and 1 ml. of spore suspension. The sealed cans were treated in the same manner as the plugged tubes.

The test tubes and a few of the TDT-cans of processed cheese spread were checked visually for evidence of growth, gas bubbles in the tubes and expansion of the TDT-cans. The expansion of most of the TDT-cans of product was determined at pre-selected intervals by a dial micrometer (Figure 1).

By using the TDT-can and the dial micrometer, the effect of pH and brine concentration on the growth lag and amount of gas produced by the spores was determined. Ten TDT-cans were used for each set of variables and the arithmetic average of the can expansion calculated. All measurements were made in a constant temperature 37° C. walk-in incubator to avoid changes in can thickness due to temperature differences.

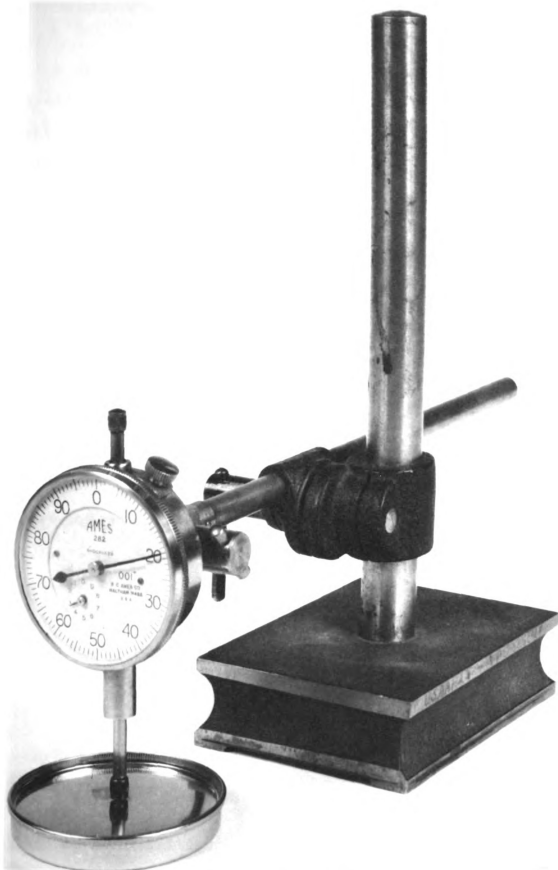


Figure 1. Dial micrometer and TDT-can.

Heat Penetration Studies

The lag correction factor, which is the difference between the total heating time and the time at retort temperature, is evaluated by heat penetration studies. These studies were conducted to determine the effect of fill-weight and the position of the TDT-can on the rate of heating and consequently the lag correction factor of TDT-cans of processed cheese spread. Copper-constantan thermocouples were mounted in 12 TDT-cans (Figure 2). The cans were heated in a miniature retort similar to the one described by Townsend et al. (57). Using the data obtained, heat penetration curves were plotted as described by Ball and Olson (8) and the lag correction factors calculated.

The copper-constantan thermocouples, made from No. 30 B & S gauge wire, were positioned between the side and center midway between the ends of the TDT-can. The thermocouple was held in position by passing the wire through a hole drilled in a No. 00 rubber stopper and the lead wire was brought out through a hole punched in the lower side of the TDT-can. An electrical insulating resin (Epocast) was used to secure the stopper in place and seal the hole where the lead wire was brought out of the can.

Heat penetration data were collected with the TDT-can in the flat position, and with it standing on its edge. Fills of 16, 18, and 20 g. of processed cheese spread were tested in both positions. One milliliter of water was

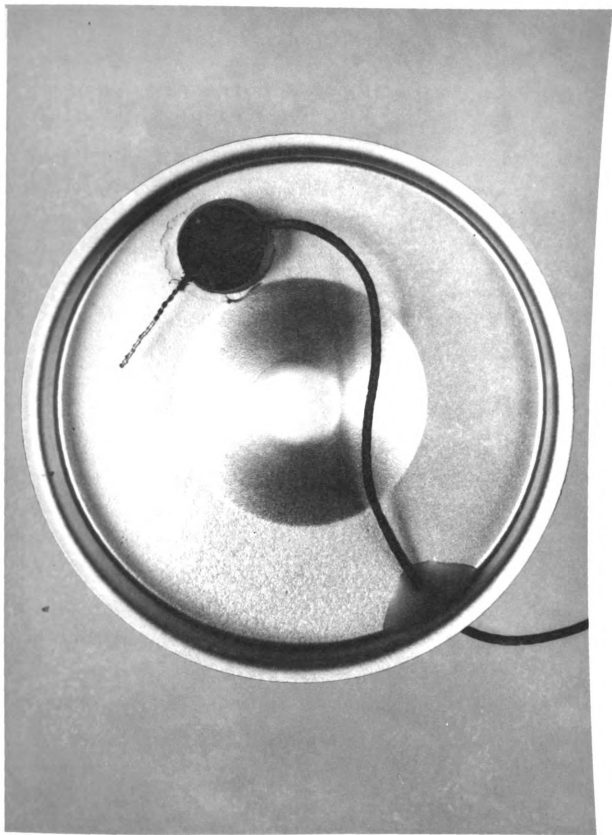


Figure 2. TDT-can and copper-constantan thermocouple.

added to each TDT-can of product of simulate the added spore suspension. The TDT-can of processed cheese spread was placed over a boiling water bath long enough to melt the product and allow it to settle into the can around the thermocouple junction. The TDT-can was sealed at atmospheric pressure and placed in the desired position in the miniature retort. The thermocouple wire was brought out through a stuffing box in the side of the retort and connected to the temperature recording potentiometer. A pressure controller was used to adjust the temperature of a ballast tank to 250° F. and the retort lid was clamped closed.

The following procedure was used in making a test: The vent was opened, the recording potentiometer turned on, and, after waiting 10 to 30 seconds to get an initial temperature reading, the steam valve was opened. The retort was vented for 15 seconds to insure complete air removal. When the temperature recording potentiometer indicated that the contents of the TDT-can had reached retort temperature, the steam was shut off, the vent and drain opened, and the cooling water turned on. When the temperature recording potentiometer indicated that the contents of the TDT-can had reached the cooling water temperature, the water was turned off and the retort opened. The position of the TDT-can was changed and the sequence repeated. Each time the position of the TDT-can was changed, the product was

heated to approximately 210° F. to melt the processed cheese spread and allow it to seek a level position after which it was cooled back to the cooling water temperature before the next trial was started. When the TDT-can was placed on edge the thermocouple junction was located in the bottom half of the can.

The heat penetration curves were plotted and the slope (f_h) and lag factors (j) for each curve were calculated by the method of Ball (5). The f_h and j values obtained from the duplicate tests were used in the calculation of the lag correction factors (t_c).

The lag correction factor (t_c) that must be evaluated is the difference between the total heating time (B) and the lethality (U) at the exposure or retort temperature.

$$t_c = B - U \quad (8)$$

A relatively simple method of calculating lag correction factors from heat penetration data was developed from equation 8 by Fflug and Esselen (37) using Ball's (6) formula.

$$B = f_h (\log jI - \log g) \quad (9)$$

where f_h = slope of heat penetration curve.

j = lag factor.

$I = RT - IT.$ (10)

g = the difference between RT temperature and greatest temperature attained by the point of slowest heating in the can (assumed to be 0.1).

If $g = 0.1$, then the log of $g = -1.0$. Using a log $g = -1.0$

and assuming a z of 18° F. and using the curve in Ball and Olson (8)

$$\frac{f_h}{U} = 0.58 \quad (11)$$

Rearranging equation 11,

$$U = 1.725 f_h \quad (12)$$

substituting equations 9 and 12 into equation 8,

$$t_c = f_h (\log jI - \log g) - 1.725 f_h$$

assuming $\log g = -1.0$ and solving.

$$t_c = f_h (\log jI - 0.725) \quad (13)$$

It was assumed that the TDT-cans of processed cheese spread would be heated from 56 to 250° F. On the basis of this assumption, lag correction factors (t_c) were calculated for use in all subsequent thermal processing studies. The results are shown in TABLE I.

TABLE I

Effect of fill-weight and position of the can on the lag correction factor (t_c) of a processed cheese spread in TDT-cans^a

Fill-wt. grams ^b	Can position					
	Edge			Flat		
	f_h , min.	j	t_c , min.	f_h , min.	j	t_c , min.
16	1.440	1.290	2.41	1.870	0.895	2.83
18	1.740	1.200	2.82	1.995	1.055	3.16
20	1.830	1.205	3.00	2.040	1.120	3.28

a. Assumed $I = RT - IT = 194$, $z = 18^{\circ}$ F.

b. 1 ml. water added to each can.

Ball and Olson's (8) table of P values does not go above a z of 26° F.; therefore, it is necessary to use Ball and Olson's (8) new method, or the method of Pflug (35) which uses Hick's (23) data to calculate a lag correction factor (t_c) for a z of 37° F. The calculation of the lag correction factor (t_c) for a z of 37° F. follows:
From Hicks tables (23)

$$U_h = \frac{f_h}{100} \times 195.7 \quad (14)$$

and rearranging,

$$U_h = 1.960 f_h \quad (15)$$

The ratio of the lethality accumulated during heating and cooling is 0.93 (35).

$$\frac{U_h}{U} = 0.93 \quad (16)$$

Rearranging equation 16 and substituting it into equation 15,

$$U = 2.107 f_h \quad (17)$$

substituting equation 9 on page 30 and equation 17 into equation 8 on page 30,

$$t_c = f_h (\log jI - \log g) - 2.107 f_h \quad (18)$$

assuming $\log g = -1.0$ and solving,

$$t_c = f_h (\log jI - 1.107) \quad (19)$$

By using the same data used to calculate the lag correction factor (t_c) for an 18 g. fill with the TDT-can in the flat position (3.16 minutes, TABLE I) and equation 19,

$$t_c = 1.995 (\log 204.67 - 1.107)$$

$$t_c = 2.40 \text{ minutes}$$

The difference in the lag correction factor (t_c) for a z of 18° F. and a z of 37° F. is

$$3.16 - 2.40 = 0.76 \text{ minutes.}$$

Using equation 2;

$$D = \frac{0.76}{6.68} = 0.11 \text{ minutes}$$

therefore, if the thermal resistance curve exhibited a z of 37° F. instead of 18° F. when the lag correction factor (t_c) used was based on a z of 18° F., the calculated D values would be 0.11 minutes too small.

Effect of pH and Brine Concentration on the Thermal Resistance of IA 3679 in Processed Cheese Spread

To determine the range in which to heat treat the TDT-cans, several lots of processed cheese spread at various pH and brine concentrations were treated at arbitrarily

selected times and temperatures. The first two temperatures were 230 and 240° F. After obtaining these results and assuming a z of 18° F., tests were also made at 220, 225, 235, and 245° F. Two TDT-cans containing 1.84×10^5 spores per gram of processed cheese spread were processed at each temperature and time. These results were used to plot an end-point destruction curve. To construct this curve the temperature was plotted on the arithmetic scale against the time (U) on the log scale of semi-logarithmic paper. The positive and negative results secured from the above procedure were placed on the graph and a straight line drawn above all positive points and below as many negative points as possible. By using this curve as the center of a range, the times (U) at each specific temperature were chosen for the thermal resistance tests. The actual testing times (U) were found by dividing the range by units of one-tenth of a log cycle. The proper lag correction factor (t_c) was added to the times (U) to obtain the heating times (B).

TDT-cans containing 18 g. of standardized processed cheese spread and 1 ml. of spores were used in all tests to determine the effect of pH and brine concentration on the heat destruction of PA 3679 in processed cheese spread. The cans were prepared, heated, incubated over-night, and then 0.01 g. of product from each can was subcultured in liver infusion broth. The 0.01 g. sample was taken by stabbing the center of the opened TDT-can of product in three different places with a calibrated hot loop. After the

third stab the loop of product was introduced into the subculture medium. A subjective evaluation of the color of the product was made when it was subcultured. All tubes were incubated at least three months. Tests for the thermal resistance of PA 3679 were conducted by heating five TDT-cans at each time and temperature. The TDT-cans were prepared as follows:

1. Temper the sealed can of processed cheese spread at room temperature over-night.
2. Place can in 65° C. water bath one-half day.
3. Open can and weigh desired amount into a mix-master bowl which was then placed on the mix-master setting in 65° C. water bath.
4. Mix in the desired amount of spore suspension (1.84×10^5 spores per gram) and salt.
5. Standardize the pH by adding NaOH or HCl as necessary.
6. Weigh 19 g. of mixture into each can and seal.

Batches were standardized at pH 5.50 and a brine concentration of 2.0 per cent, pH 5.50 and a brine concentration of 4.4 per cent, pH 6.25 and a brine concentration of 2.0 per cent, pH 6.25 and a brine concentration of 4.4 per cent, pH 7.00 and a brine concentration of 2.0 per cent, and pH 7.00 and a brine concentration of 4.4 per cent.

D values were calculated for each temperature according to the Schmidt method (45) and the thermal resistance curves were plotted.

Effect of pH and Brine Concentration on the Heat
Treatment Required to Prevent Growth of
PA 3679 in Processed Cheese Spread

The effect of pH and brine concentration on the heat treatment required to prevent growth of PA 3679 in processed cheese spread was determined by sealing 18 g. of standardized processed cheese spread and 1 ml. of the spore suspension in TDT-cans, heat-treating, and incubating the cans at least five months. The number of swollen TDT-cans was noted and the data were used to plot end-point inhibition curves.

Preliminary data were used to aid in the selection of the range to be used in heat treating the product. The times (U) were determined by dividing the range into units of approximately one-fifth of a log cycle. The lag correction factor (t_c) was added to determine the heating times (B). The wide spacing of U values resulted from a shortage of processed cheese spread.

Batches were standardized at pH 6.0 and a brine concentration of 1.6 per cent, pH 6.0 and a brine concentration of 2.4 per cent, pH 7.0 and a brine concentration of 1.6 per cent, and pH 7.0 and a brine concentration of 2.4 per cent. The TDT-cans were heated in miniature retorts at the pre-selected times and temperatures, cooled, and placed in a 37° C. incubator.

Each TDT-can was measured every day for the next three days using the dial micrometer to determine the ini-

tial thickness of the can. All TDT-cans were measured again at the end of 60 days of incubation to determine which ones had expanded due to gas production by PA 3679, and how much. An arithmetic average of the amount of expansion of the five TDT-cans at each time and temperature was computed. To determine if the cans had stopped expanding after 60 days, they were measured once a month for three more months.

Using the data from the positive and negative TDT-cans, end-point inhibition curves were plotted. The number of viable spores remaining in the cans following heat treatment, which showed growth at the longest heating time and no growth at the shortest heating time were calculated for each temperature using equation 2.

The thermal process received by each group of five TDT-cans was equated to the time at 250° F. using the z value obtained from the end-point inhibition curves and the equation for lethality rate (2).

$$F = \frac{U}{\log^{-1} \frac{250 - RT}{z}} \quad (20)$$

where F = equivalent process time at 250° F.

U = time at retort temperature.

RT = retort temperature.

z = slope of the thermal resistance curve.

With the thermal processes placed upon a common reference, the effect of the thermal process and brine concentration on TDT-can expansion at pH values of 6.0 and 7.0 could be compared.

RESULTS

The primary objective of this investigation was to determine the effect of pH and brine concentration upon the growth, thermal resistance, and the heat treatment required to prevent growth of PA 3679 in processed cheese spread. The results of this investigation are reported in the following order: (1) Properties of the spore suspension of PA 3679, (2) Growth of PA 3679 in processed cheese spread, (3) Effect of pH and brine concentration on the thermal resistance of PA 3679 in processed cheese spread, and (4) Effect of pH and brine concentration on the heat treatment required to prevent growth of PA 3679 in processed cheese spread.

Properties of Spore Suspension of PA 3679

The spores from 4 l. of culture media were concentrated to 220 ml. with a MPN of 52.8×10^6 spores per milliliter. This suspension was diluted to a volume of 3520 ml. with M/15 phosphate buffer (pH 7.0) to give a MPN of 3.3×10^6 spores per milliliter. The diluted suspension was stored at 40° F. in large flasks containing glass beads.

TABLE II shows the results of preliminary tests of the thermal resistance of spores of PA 3679 as determined by the thermoresistometer technique. The L.D.50 value for each temperature was obtained from the curves in Figure 3. A preliminary thermal resistance curve was constructed

TABLE II

Results of preliminary tests for the thermal resistance of
 spores of PA 3679 in the thermoresistometer (3.3×10^4
 spores per cup, samples subcultured in liver
 broth and incubated at 37°C.)^a

U	No. tubes	No.+	No.-	m	n	P	L.D.50	D
240° F.								
5.0	10	10	0	22	0	0.041	9.3	1.98
7.0	10	8	2	12	2	0.187		
10.0	10	4	6	4	8	0.642		
14.5	10	0	10	0	18	0.950		
250° F.								
1.4	10	8	2	25	2	0.103	3.0	0.64
2.0	10	6	4	17	6	0.280		
2.8	10	6	4	11	10	0.478		
4.0	10	5	5	5	15	0.727		
5.6	10	0	10	0	25	0.962		

a. Data show only the results involved in calculations.

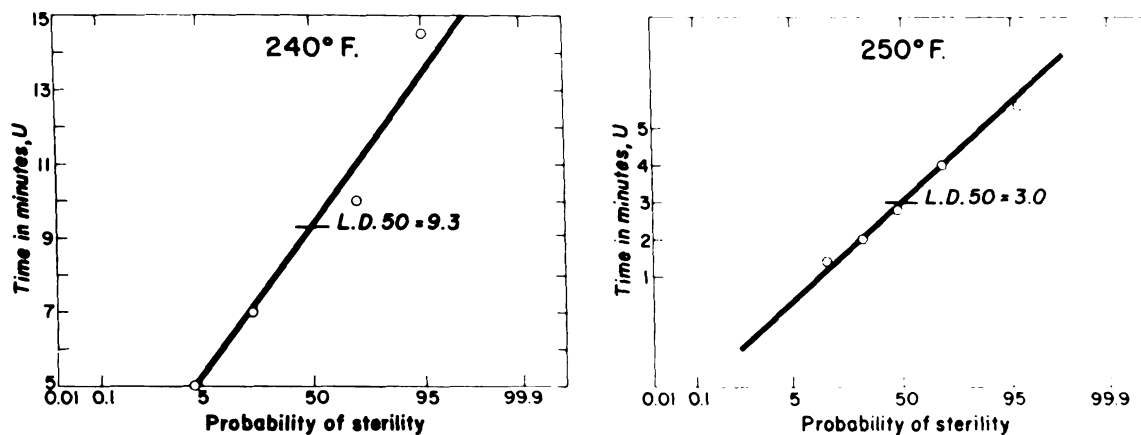


Figure 3. Probability curves for determining L.D.50 time (preliminary test).

using the D_{240} and D_{250} values. The curve had a z value of 20.2° F. and a D_{250} of 0.64 minutes.

The results of the series of tests for the thermal resistance of the suspension are tabulated in TABLE III. The L.D. 50 value for each temperature was obtained from the curves in Figure 4. The D values from TABLE III and their 95 per cent confidence limits were plotted on semi-logarithmic paper and the thermal resistance curve was drawn by eye (Figure 5). This curve has a z value of 17.5° F. and a D_{250} of 0.98 minutes.

TABLE III

Results of tests for the thermal resistance of spores of PA 3679 in the thermoresistometer (3.3×10^4 spores per cup, samples subcultured in liver broth and incubated at 37°C.)^a

U	No. tubes	No.+	No.-	m	n	P	L.D.50	D
240° F.								
5.00	10	10	0	37	0	0.025	17.50	3.74
7.00	10	9	1	27	1	0.066		
10.00	10	9	1	18	2	0.136		
14.00	10	8	2	9	4	0.333		
20.50	10	1	9	1	13	0.875		
29.50	10	0	10	0	23	0.960		
245° F.								
7.00	10	10	0	14	0	0.062	8.50	1.82
10.00	10	3	7	4	7	0.615		
14.00	10	1	9	1	16	0.894		
20.00	10	0	10	0	26	0.964		
250° F.								
2.40	10	10	0	21	0	0.043	4.60	0.98
3.50	10	9	1	11	1	0.142		
4.90	10	2	8	2	9	0.769		
7.00	10	0	10	0	19	0.952		
255° F.								
1.20	10	10	0	23	0	0.040	2.40	0.51
1.70	10	9	1	13	1	0.125		
2.50	10	4	6	4	7	0.615		
3.50	10	0	10	0	17	0.947		
260° F.								
0.61	10	10	0	24	0	0.038	1.22	0.26
0.88	10	8	2	14	2	0.166		
1.20	10	6	4	6	6	0.500		
1.80	10	0	10	0	16	0.944		

a. Data show only the results involved in calculations.

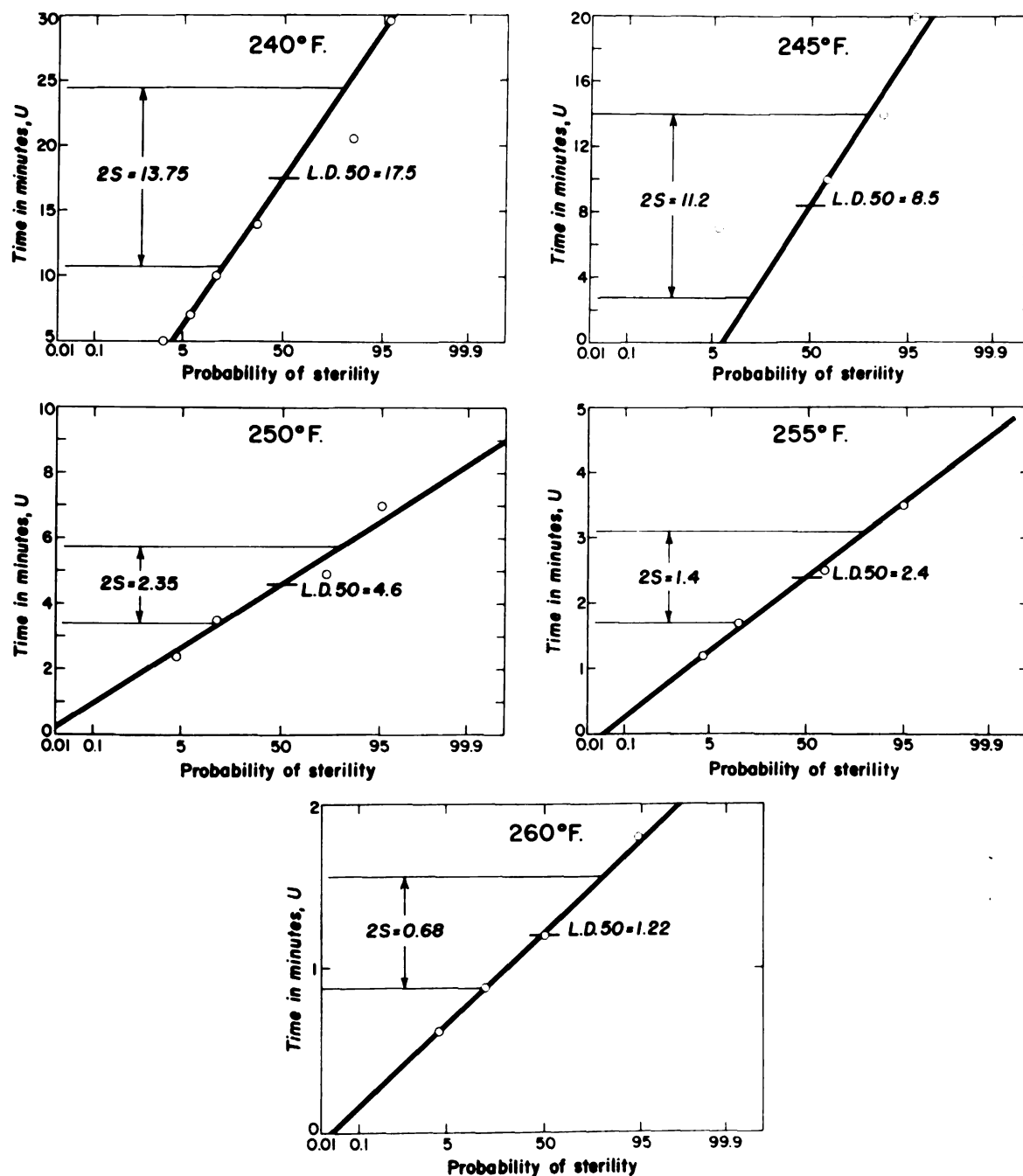


Figure 4. Probability curves for determining L.D.50 time (final test).

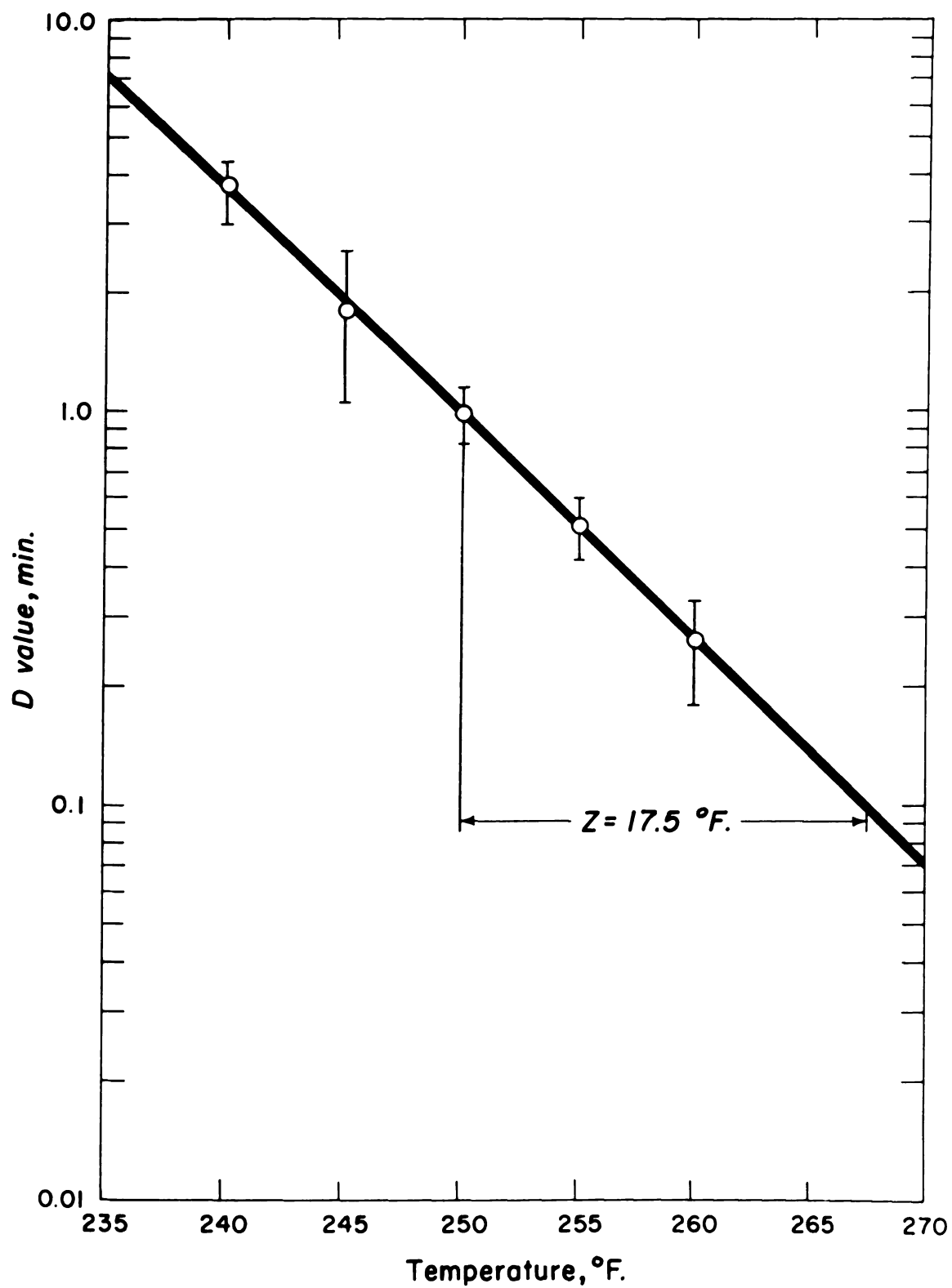


Figure 5. D values with 95% confidence limits and the resulting thermal resistance curve for spores of PA 3679 suspended in neutral phosphate buffer.

Growth of PA 3679 in Processed Cheese Spread

Effect of skim milk powder. In tests to determine the effect of skim milk powder no visually detectable gas was found in the tubes of processed cheese spread containing 1.0 per cent skim milk powder: whereas, the tubes of spread containing 10.0 per cent skim milk powder were honey combed with large pockets of gas (TABLE IV). This gas had a putrid odor typical of PA 3679. A Gram stain of the contents revealed large Gram positive rods. Tubes of stratified liver infusion broth inoculated from these positive tubes of processed cheese spread showed gas production within three days.

TABLE IV

Effect of skim milk powder on the growth of PA 3679 in cheese spread with a pH of 6.0 at the time of inoculation and incubated 8 days at 37° C. (measured by gas production in glass tubes)

Tube no.	Cheese spread plus 1.0% NFDM	Cheese spread plus 10.0% NFDM
1	-	+
2	-	+
3	-	+

After completion of the preliminary test a large batch of processed cheese spread containing 10.0 per cent skim milk powder was produced for use throughout the remainder of the project. This batch of processed cheese

spread had a moisture content of 46.04 per cent, a fat content of 22.34 per cent, a pH of 6.20, and a salt content of 0.775 per cent. The brine concentration was 1.65 per cent.

Effect of pH and brine concentration. TABLE V shows the results, recorded as either positive or negative tubes, of the effect of pH and brine concentration on the growth of PA 3679 in processed cheese spread. There was growth above and no growth at or below pH 5.6 at the brine concentrations tested (2.8 to 6.8 per cent).

In another test where both tubes and TDT-cans were used, the growth of PA 3679 in tubes of processed cheese spread with a pH of 6.2 was completely inhibited at a brine concentration equal to or greater than 7.6 per cent (TABLE VI). The results in TABLE VI indicate that growth which could not be subjectively detected was taking place in the TDT-cans of processed cheese spread. The limitation of subjective evaluation led to the development and use of the dial micrometer for measuring the expansion of TDT-cans.

Figures 6 and 7 show the effect of brine concentration on the gas production by PA 3679 in the processed cheese spread, as measured by the expansion of TDT-cans. An increase in the brine concentration resulted in an increase in the lag time for gas production, a decrease in the total amount of gas produced, a decreased rate of gas production, and an increase in the time during which gas was produced. There was no measureable gas production after 60 days of incubation. The lag time was always greater at pH 6.0 than

TABLE V

Effect of pH and brine concentration on the growth of PA
3679 in a cheese spread incubated 14 days at 37° C.
(measured by gas production in glass tubes)

pH	Brine concentration, %					
	2.8	3.6	4.4	5.2	6.0	6.8
5.2	-	-	-	-	-	-
5.4	-	-	-	-	-	-
5.6	-	-	-	-	-	-
5.8	+	+	+	+	+	+
6.0	+	+	+	+	+	+
6.2	+	+	+	+	+	+

TABLE VI

Effect of brine concentration on the growth of PA 3679 in a cheese spread with a pH of 6.2 when incubated 14 days at 37° C.

Brine concentration %	Visual examination	
	Glass tubes	TDT-Cans
2.0	++++	+
2.8	+++	-
3.6	++	-
4.4	++	-
5.2	+	-
6.0	+	-
6.8	+	-
7.6	-	-
8.4	-	-

Expansion, inches

0

0

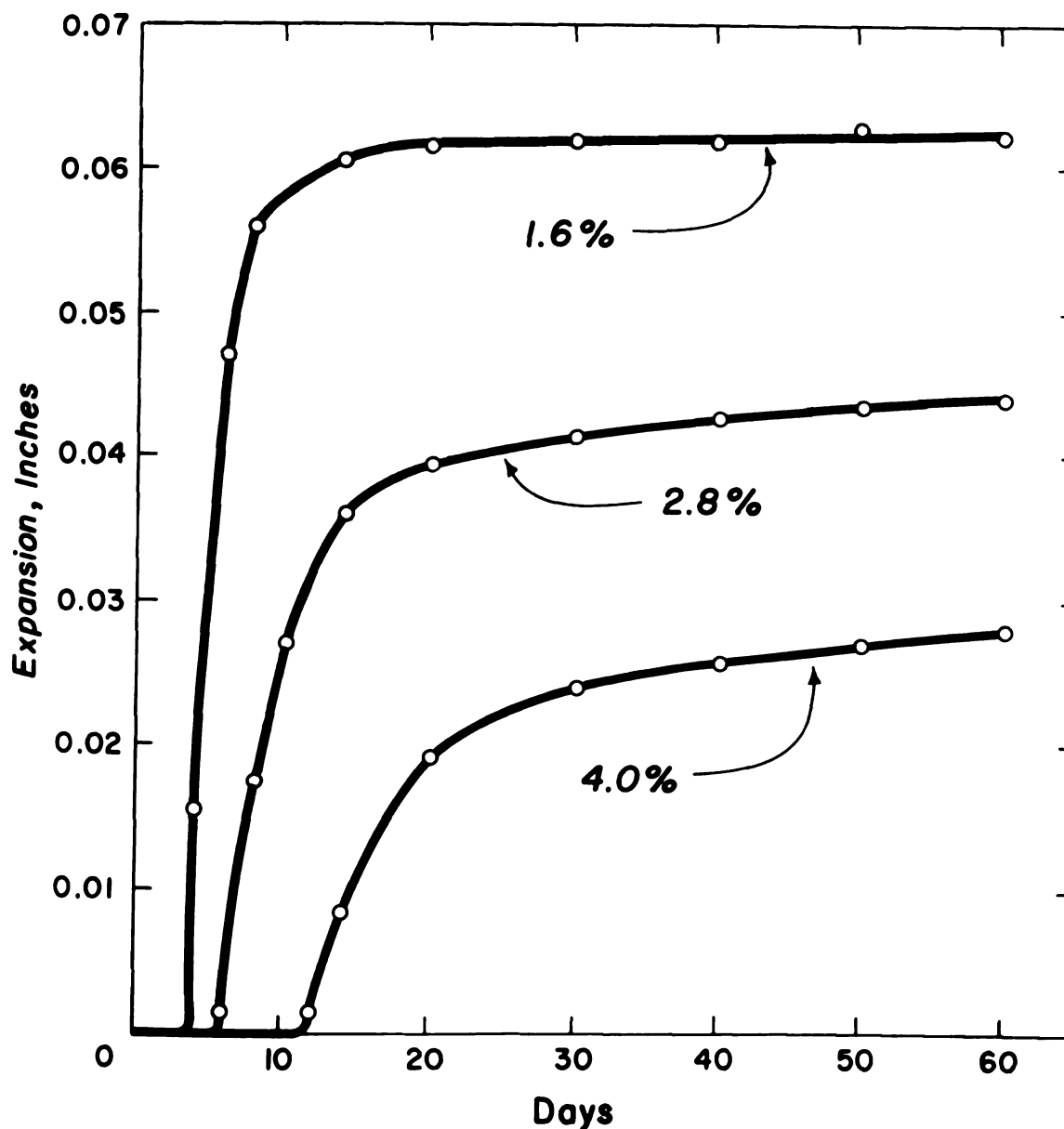


Figure 6. Effect of brine concentration on the gas production of PA 3679 in a cheese spread at pH 6.0, as measured by the expansion of TDT-cans incubated at 37°C. (each value represents an average of 10 cans).

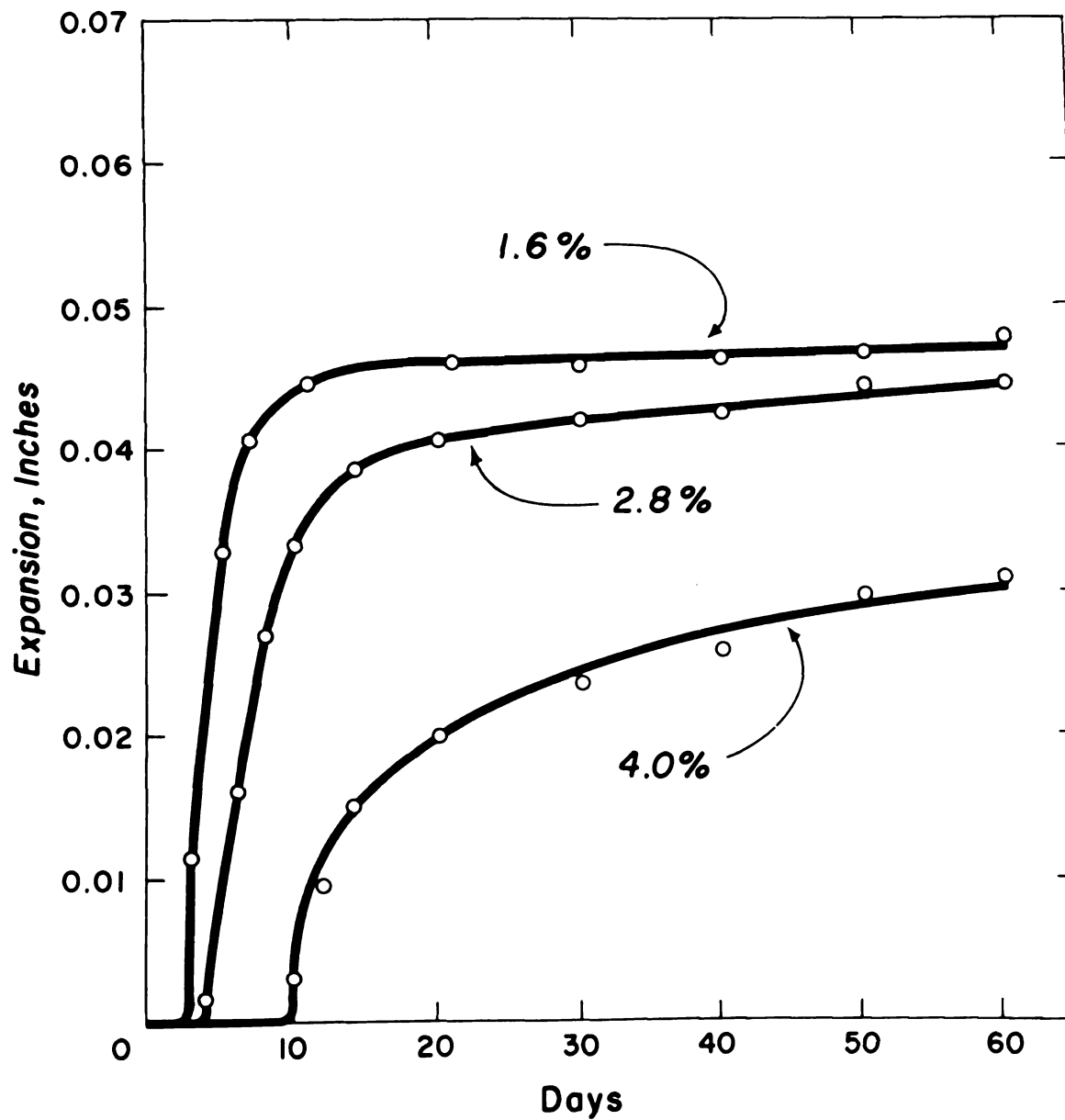


Figure 7. Effect of brine concentration on the gas production of PA 3679 in a cheese spread at pH 7.0, as measured by the expansion of TDT-cans incubated at 37°C. (each value represents an average of 10 cans).

at pH 7.0 (TABLE VII). Figure 8 illustrates the effect of brine concentration at pH 6.0 and 7.0 on the gas production by PA 3679 in processed cheese spread after 60 days of incubation.

**Effect of pH and Brine Concentration on the Thermal
Resistance of PA 3679 in Processed
Cheese Spread**

TABLE VIII shows the results of preliminary tests for the thermal resistance of spores of PA 3679 in the processed cheese spread. The times (U) at the different temperatures were so widely spaced that both duplicates were either positive or negative; the results were the same for all pH and brine concentrations tested. Using data from TABLE VIII, an end-point destruction curve was plotted (Figure 9). The end-point destruction curve was used as the center of the range in the selection of times (U) at specific temperatures for the final thermal resistance tests.

TABLES IX, X and XI show the results of tests for the thermal resistance of spores of PA 3679 in processed cheese spread at pH 5.50, 6.25, and 7.00, respectively, with brine concentrations of 2.0 and 4.4 per cent.

Data from TABLES IX, X, and XI were used to plot Figures 10, 11, and 12, respectively. The D_{235} , F_{235} , and z values from each of the curves in Figures 10, 11, and 12 are tabulated in TABLE XII. The F_{235} values are based on commercial sterility. An increased D_{235} value with each increase in pH was noted. The D_{235} values were essentially

TABLE VII

Effect of pH and brine concentration on the lag phase of PA
3679 in a cheese spread incubated at 37° C.
(measured by the swelling of TDT-cans)

Brine concentration %	Lag time in days	
	pH 6.0	pH 7.0
1.6	4	3
2.0	4	3
2.4	6	4
2.8	6	4
3.2	7	6
3.6	10	7
4.0	12	10
4.4	15	12

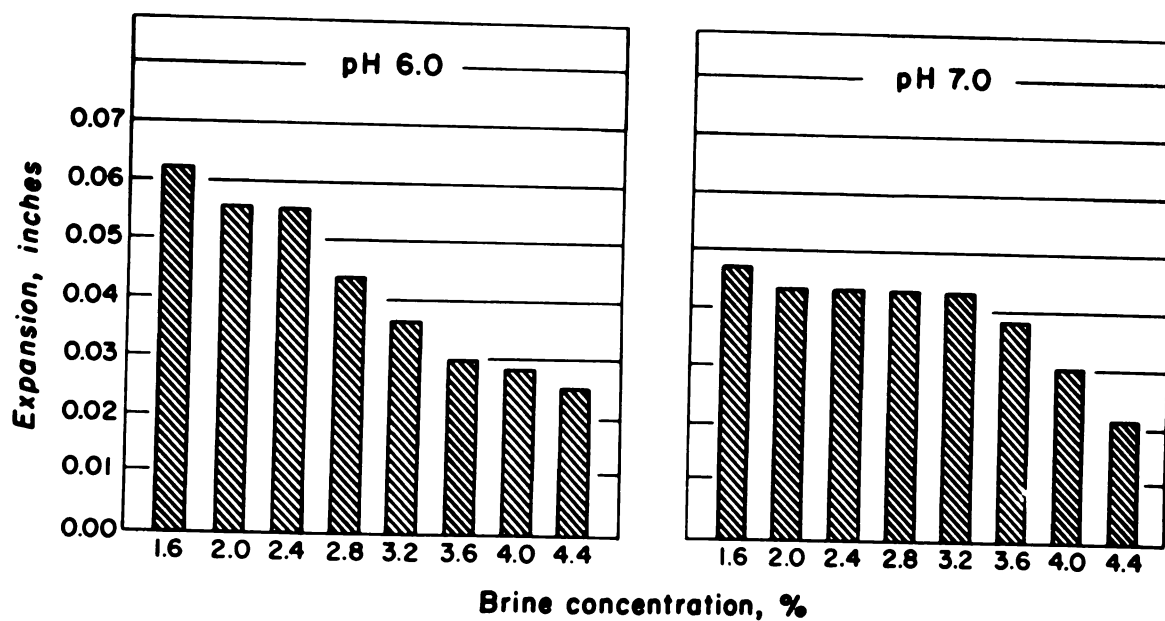


Figure 8. Effect of brine concentration on the gas production of PA 3679 in a cheese spread at pH 6.0 and 7.0, as measured by the expansion of TDT-cans incubated 60 days at 37°C.

TABLE VIII

Results of preliminary tests for the thermal death rate of spores of PA 3679 in a cheese spread (3.3×10^6 spores in each of two TDT-cans per trial, with 0.01 g. from each can subcultured in liver broth and incubated at 37°C.)

220° F.		225° F.		230° F.		235° F.		240° F.		245° F.	
U	No.+	U	No.+	U	No.+	U	No.+	U	No.+	U	No.+
pH 5.5 and a brine concentration of 2.0%											
99.8	2	51.8	2	14.0	2	16.8	2	3.7	2	3.7	2
141.8	0	71.8	0	27.0	2	21.8	0	7.1	2	5.2	2
199.8	0	91.8	0	51.0	0	31.8	0	13.5	0	7.0	0
				98.0	0			26.0	0		
pH 5.5 and a brine concentration of 4.4%											
99.8	2	51.8	2	14.0	2	16.8	2	3.7	2	3.7	2
141.8	0	71.8	0	27.0	2	21.8	0	7.1	2	5.2	2
199.8	0	91.8	0	51.0	0	31.8	0	13.5	0	7.0	0
				98.0	0			26.0	0		
pH 7.0 and a brine concentration of 2.0%											
99.8	2	51.8	2	14.0	2	16.8	2	3.7	2	3.7	2
141.8	0	71.8	0	27.0	2	21.8	0	7.1	2	5.2	2
199.8	0	91.8	0	51.0	0	31.8	0	13.5	0	7.0	0
				98.0	0			26.0	0		
pH 7.0 and a brine concentration of 4.4%											
99.8	2	51.8	2	14.0	2	16.8	2	3.7	2	3.7	2
141.8	0	71.8	0	27.0	2	21.8	0	7.1	2	5.2	2
199.8	0	91.8	0	51.0	0	31.8	0	13.5	0	7.0	0
				98.0	0			26.0	0		

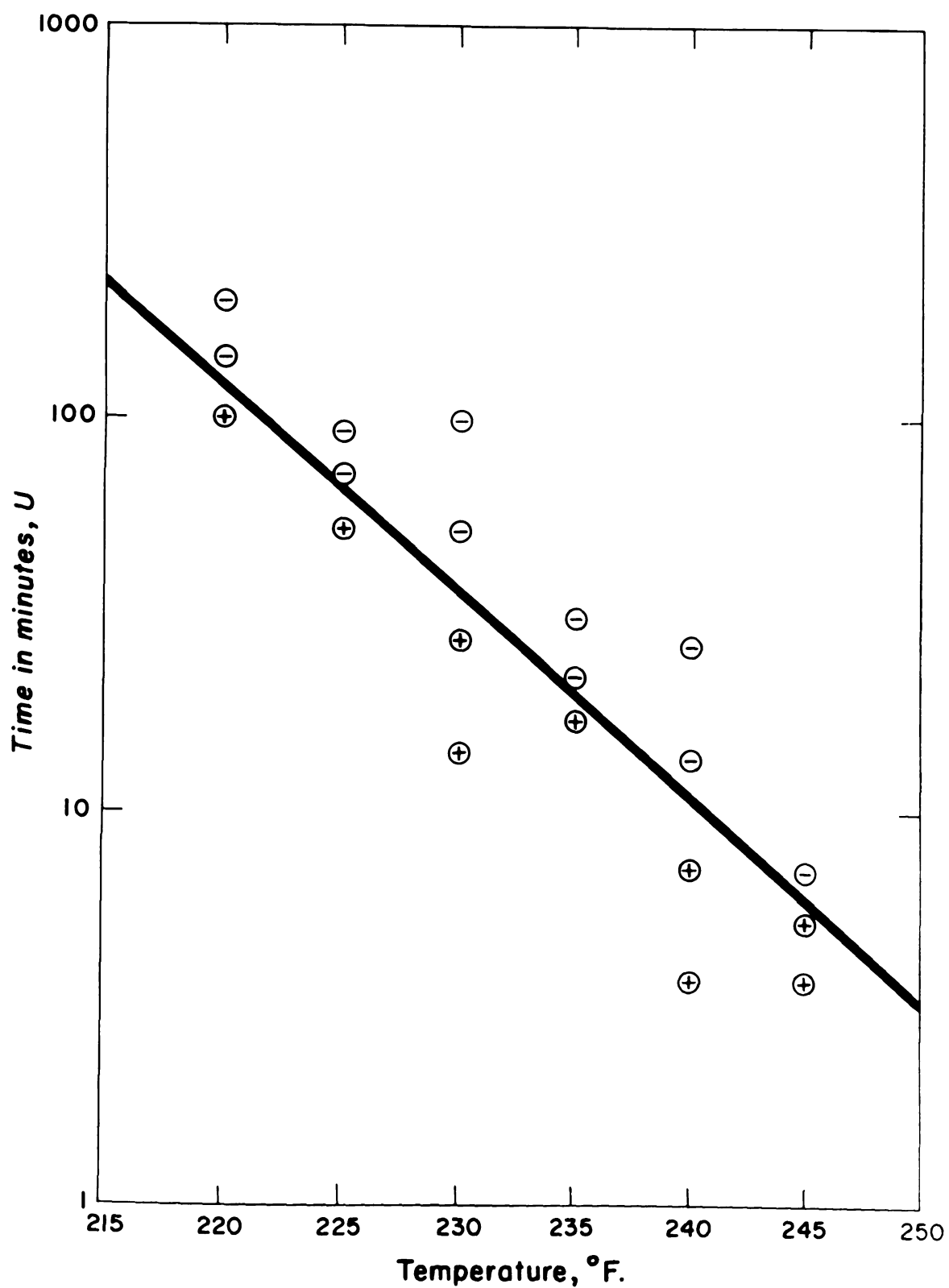


Figure 9. End-point destruction curve of spores of PA 3679 in a cheese spread (each point represents duplicate determinations).

TABLE IX

Results of tests for thermal death rate of spores of PA 3679 in cheese spread at pH 5.50 (3.3×10^6 spores per TDT-can, 0.01 g. from each can subcultured in liver broth incubated at 37° C.)^a

U	No. tubes	No.+	No.-	m	n	P	L.D.50	D
Brine concentration, 2.0%								
225° F.								
38.8	5	5	0	7	0	0.111	48.00	14.03
48.8	5	2	3	2	3	0.571		
59.8	5	0	5	0	8	0.900		
230° F.								
26.8	5	5	0	5	0	0.142	29.80	8.71
32.8	5	0	5	0	5	0.857		
235° F.								
14.3	5	5	0	7	0	0.111	17.50	5.15
17.8	5	2	3	2	3	0.571		
21.8	5	0	5	0	8	0.900		
240° F.								
5.2	5	5	0	9	0	0.090	7.60	2.22
6.8	5	2	3	4	3	0.444		
8.8	5	1	4	2	7	0.727		
11.3	5	1	4	1	11	0.857		
14.3	5	0	5	0	16	0.944		
245° F.								
2.8	5	5	0	12	0	0.071	5.05	1.47
3.9	5	4	1	7	1	0.200		
5.3	5	3	2	3	3	0.500		
6.8	5	0	5	0	8	0.900		
Brine concentration, 4.4%								
225° F.								
31.8	5	5	0	9	0	0.090	41.60	12.16
38.8	5	4	1	4	1	0.285		
48.8	5	0	5	0	6	0.875		
230° F.								
21.8	5	5	0	8	0	0.100	27.50	8.04
26.8	5	3	2	3	2	0.428		
32.8	5	0	5	0	7	0.888		
235° F.								
11.3	5	5	0	7	0	0.111	14.10	4.12
14.3	5	2	3	2	3	0.571		
17.8	5	0	5	0	8	0.900		
240° F.								
6.8	5	5	0	8	0	0.100	9.10	2.66
8.8	5	3	2	3	2	0.428		
11.3	5	0	5	2	7	0.888		
245° F.								
2.8	5	5	0	9	0	0.090	4.90	1.43
3.9	5	3	2	4	2	0.375		
5.5	5	1	4	1	6	0.777		
6.8	5	0	5	0	11	0.923		

a. Data show only the results involved in calculations.

TABLE X

Results of tests for thermal death rate of spores of PA 3679 in cheese spread at pH 6.25 (3.3×10^6 spores per TDT-can, 0.01 g. from each can subcultured in liver broth incubated at 37° C.)^a

U	No. tubes	No.+	No.-	m	n	P	L.D.50	D
Brine concentration, 2.0%								
225° F.								
38.8	5	5	0	14	0	0.062	64.00	18.71
48.8	5	4	1	9	1	0.166		
59.8	5	2	3	5	4	0.454		
71.8	5	2	3	3	7	0.666		
86.8	5	1	4	1	11	0.857		
104.8	5	0	5	0	16	0.888		
230° F.								
39.8	5	5	0	5	0	0.142	44.40	12.98
48.8	5	0	5	0	5	0.857		
235° F.								
17.8	5	5	0	9	0	0.090	23.00	6.72
21.8	5	4	1	4	1	0.285		
26.8	5	0	5	0	6	0.875		
240° F.								
11.3	5	5	0	5	0	0.142	12.80	3.74
14.3	5	0	5	0	5	0.857		
245° F.								
5.3	5	5	0	9	0	0.090	7.30	2.13
6.8	5	4	1	4	1	0.285		
8.8	5	0	5	0	6	0.875		
Brine concentration, 4.4%								
225° F.								
59.8	5	5	0	10	0	0.083	83.00	24.26
71.8	5	4	1	5	1	0.250		
86.8	5	1	4	1	5	0.750		
104.8	5	0	5	0	10	0.916		
230° F.								
32.8	5	5	0	10	0	0.080	45.00	13.15
39.8	5	4	1	5	1	0.250		
48.8	5	1	4	1	5	0.750		
58.8	5	0	5	0	10	0.916		
235° F.								
17.8	5	5	0	6	0	0.125	21.00	6.14
21.8	5	1	4	1	4	0.714		
26.8	5	0	5	0	9	0.909		
240° F.								
11.3	5	5	0	5	0	0.142	12.80	3.74
14.3	5	0	5	0	5	0.857		
245° F.								
6.8	5	5	0	5	0	0.142	7.80	2.28
8.8	5	0	5	0	5	0.857		

a. Data show only the results involved in calculations.

Results of tests for thermal death rate of spores of PA 3679 in cheese spread at pH 7.00 (3.3×10^6 spores per TDT-can, 0.01 g. from each can subcultured in liver broth incubated at 37°C.)^a

U	No. tubes	No.+	No.-	m	n	P	L.D.50	D
Brine concentration, 2.0%								
225° F.								
86.8	5	5	0	5	0	0.142	94.00	27.48
104.8	5	0	5	0	5	0.857		
230° F.								
48.8	5	5	0	6	0	0.125	56.00	16.37
58.8	5	1	4	1	4	0.714		
71.8	5	0	5	0	9	0.909		
235° F.								
26.8	5	5	0	5	0	0.142	29.80	8.71
32.8	5	0	5	0	5	0.857		
240° F.								
11.3	5	5	0	6	0	0.125	14.80	4.32
14.3	5	0	5	1	5	0.750		
17.8	5	1	4	1	9	0.833		
22.3	5	0	5	0	14	0.937		
245° F.								
6.8	5	5	0	5	0	0.142	7.80	2.28
8.8	5	0	5	0	5	0.857		
Brine concentration, 4.4%								
225° F.								
86.8	5	5	0	8	0	0.100	107.50	31.43
104.8	5	3	2	3	2	0.428		
126.8	5	0	5	0	7	0.888		
230° F.								
39.8	5	5	0	10	0	0.083	55.00	16.08
48.8	5	4	1	5	1	0.250		
58.8	5	1	4	1	5	0.750		
71.8	5	0	5	0	10	0.916		
235° F.								
17.8	5	5	0	13	0	0.066	26.20	7.66
21.4	5	4	1	8	1	0.181		
26.8	5	4	1	4	2	0.375		
32.8	5	0	5	0	7	0.888		
240° F.								
11.3	5	5	0	9	0	0.090	15.10	4.41
14.3	5	2	3	4	3	0.444		
17.8	5	2	3	2	6	0.700		
22.3	5	0	5	0	11	0.923		
245° F.								
5.3	5	5	0	6	0	0.125	6.50	1.90
6.8	5	1	4	1	4	0.714		
8.8	5	0	5	0	9	0.909		

a. Data show only the results involved in calculations.

pH 5.50

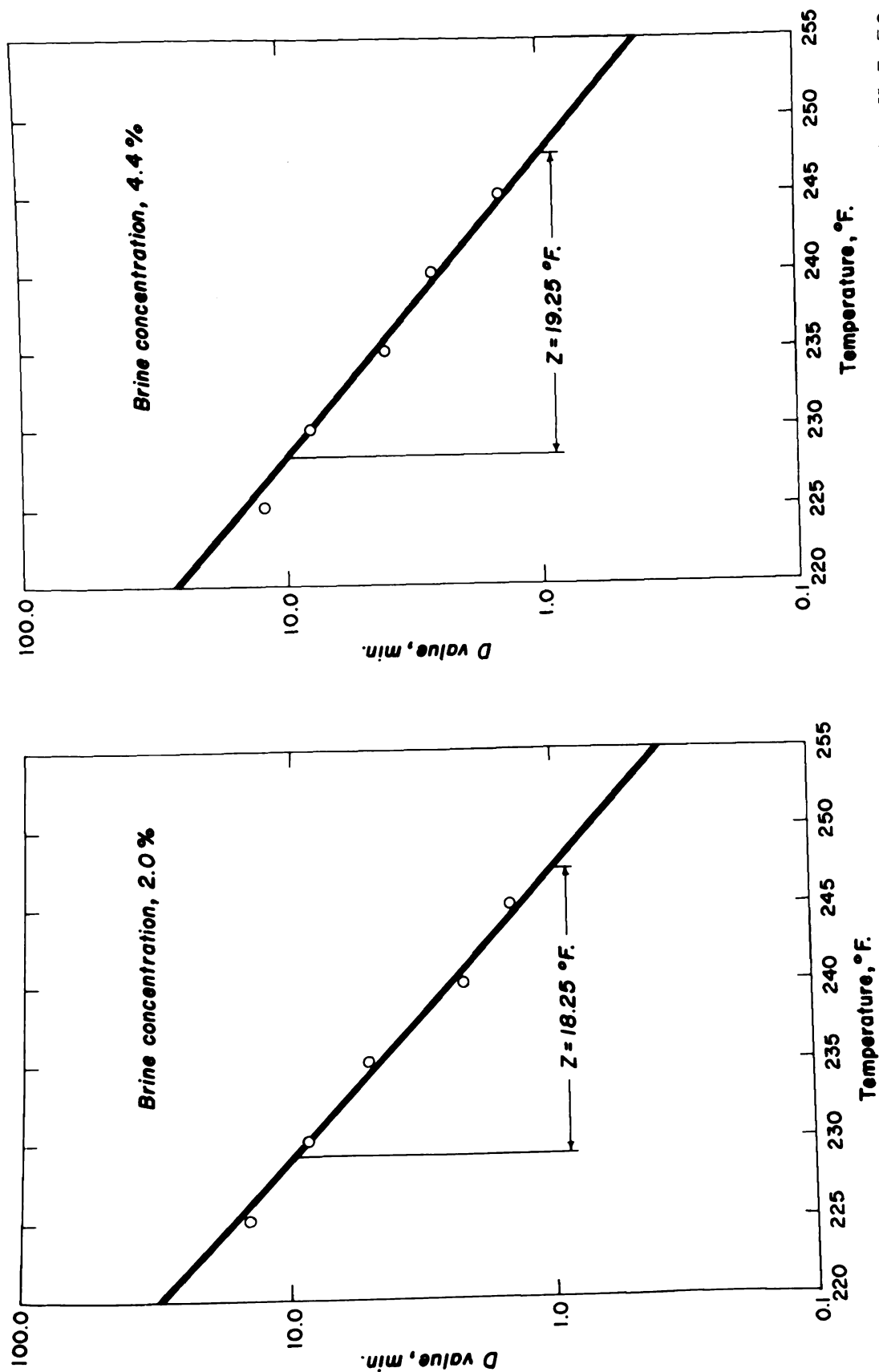


Figure 10. Thermal resistance curves of spores of PA 3679 in cheese spread at pH 5.50.

pH 6.25

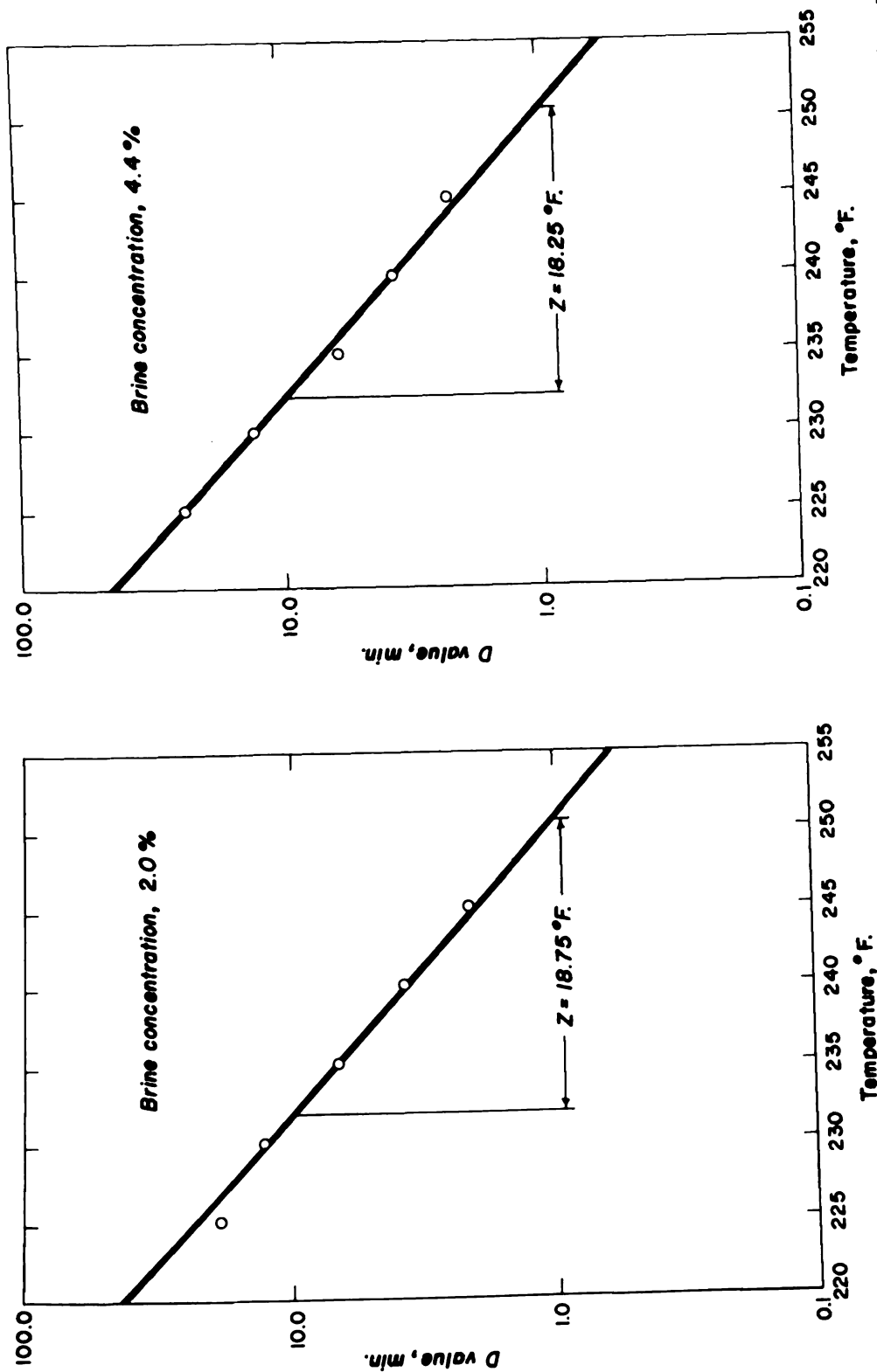


Figure 11. Thermal resistance curves of spores of PA 3679 in cheese spread at pH 6.25.

pH 7.00

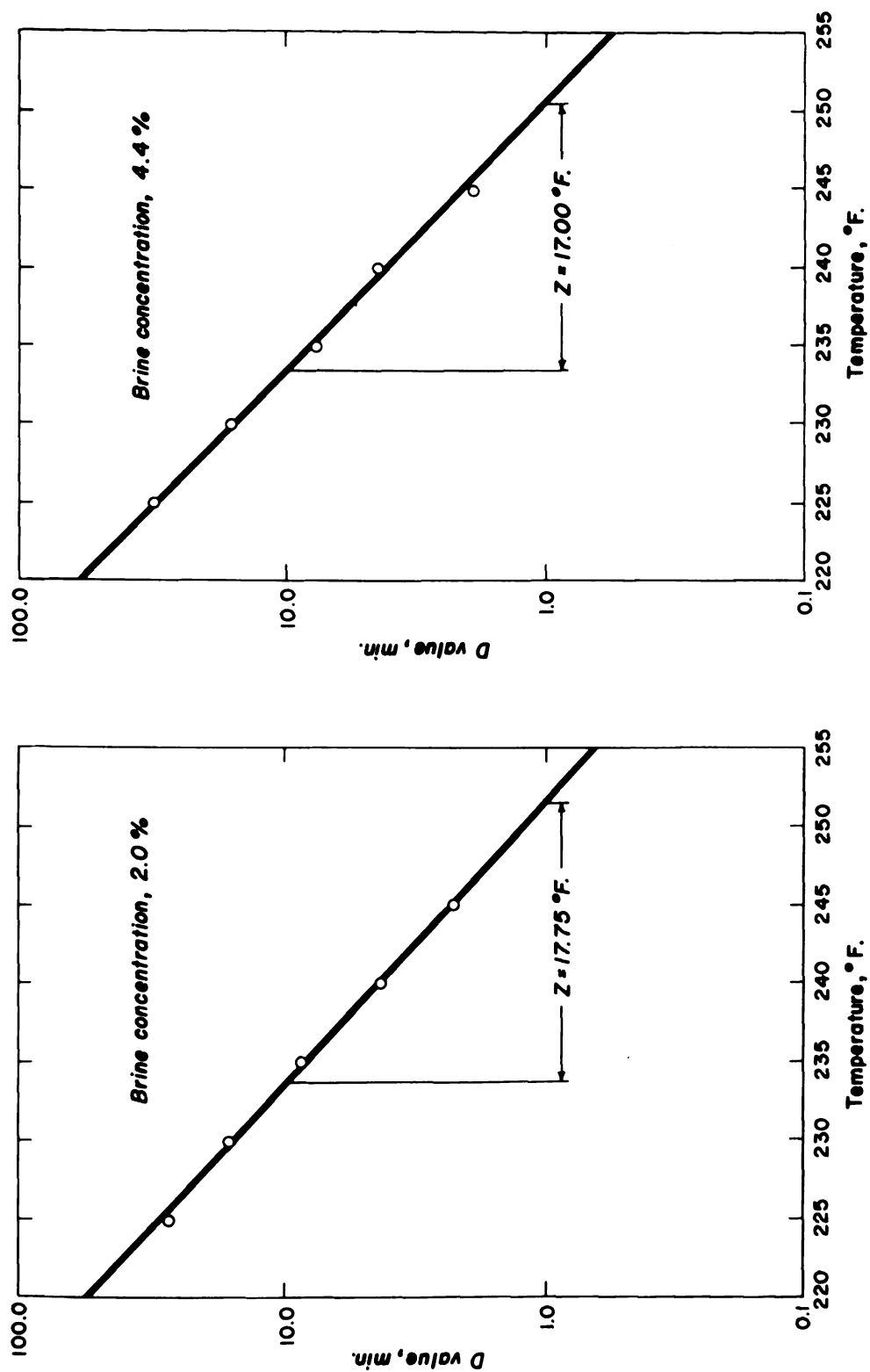


Figure 12. Thermal resistance curves of spores of PA 3679 in cheese spread at pH 7.00.

TABLE XII

Effect of pH and brine concentration on D₂₃₅, F₂₃₅, and z values for TDT-cans of cheese spread containing 3.3×10^6 spores of PA 3679 per can (0.01 g. from each can subcultured in liver broth and incubated at 37° C.)

pH	Brine concentration, %					
	2.0			4.4		
	D ₂₃₅ ,min.	F ₂₃₅ ,min.	z, °F.	D ₂₃₅ ,min.	F ₂₃₅ ,min.	z, °F.
5.50	4.6	24.2	18.25	4.5	23.7	19.25
6.25	6.8	35.8	18.75	7.0	36.8	18.25
7.00	8.4	44.2	17.75	8.1	42.6	17.00

- a. F₂₃₅ values representing commercial sterility calculated using Schmidt's equation (45).

the same at both brine concentrations. The average z value for all pH and brine concentrations is 18.2° F.

The color curve in Figure 14 was obtained from a subjective evaluation of the processed cheese spread in the TDT-cans. TDT-cans of product that were subjected to a time-temperature process above the curve were unacceptably brown while those below the curve possessed an acceptable color.

Effect of pH and Brine Concentration on the Heat
Treatment Required to Prevent Growth of
PA 3679 in Processed Cheese Spread

The results of the effect of different heat treatments on the gas production of PA 3679 in processed cheese spread at several pH and brine concentrations, as measured by the expansion of TDT-cans are shown in TABLE XIII. End-point inhibition curves were plotted from these data. The F_{235} and z values, based on resistance studies of inoculated TDT-cans, are tabulated in TABLE XIV.

The theoretical number of viable spores remaining in the canned processed cheese spread following heat treatment may be calculated using equation 2. The calculated number of spores in the cans showing growth at the longest heating time and no growth at the shortest heating time, at each temperature, are tabulated in TABLE XV.

Figure 13 shows the effect of the sterilizing value of the thermal process (F_{250}^{37}) on the expansion of TDT-cans containing processed cheese spread inoculated with 3.3×10^6

TABLE XIII

Effect of heat treatment (U) at different retort temperatures on the expansion of TDT-cans of cheese spread at several pH and brine concentrations (3.3×10^6 spores of PA 3679 per can and incubated at 37° C.)^a

220° F.		225° F.		230° F.		235° F.	
U	Expan. ^b	U	Expan. ^b	U	Expan. ^b	U	Expan. ^b
pH 6.0 and a brine concentration of 1.6%							
7.6	0.112	5.4	0.100	4.0	0.115	2.9	0.107
12.5	0.095	9.0	0.089	6.6	0.094	4.8	0.098
20.0	0.022	14.5	0.025	10.5	0.051	7.8	0.057
33.0	0.000	24.0	0.000	18.0	0.000	13.0	0.000
pH 6.0 and a brine concentration of 2.4%							
4.6	0.063	3.4	0.061	2.5	0.048	1.8	0.041
7.6	0.031	5.4	0.040	4.0	0.039	2.9	0.029
12.5	0.017	9.0	0.017	6.6	0.015	4.8	0.018
20.0	0.000	14.5	0.000	10.5	0.000	7.8	0.000
pH 7.0 and a brine concentration of 1.6%							
12.5	0.094	9.0	0.103	6.6	0.108	4.8	0.104
20.0	0.073	14.5	0.067	10.5	0.087	7.8	0.087
33.0	0.030	24.0	0.015	18.0	0.036	13.0	0.041
55.0	0.000	40.0	0.000	29.0	0.000	21.0	0.000
pH 7.0 and a brine concentration of 2.4%							
7.6	0.063	5.4	0.057	4.0	0.063	2.9	0.061
12.5	0.045	9.0	0.044	6.6	0.047	4.8	0.044
20.0	0.026	14.5	0.027	10.5	0.017	7.8	0.026
33.0	0.000	24.0	0.000	18.0	0.000	13.0	0.000

a. Data show only the results involved in calculations.

b. Can expansion in inches.

TABLE XIV

Effect of pH and brine concentration on F₂₃₅ and z values for TDT-cans of cheese spread containing 3.3×10^6 spores of PA 3679 per can (cans incubated at 37° C. and examined periodically for swells)^a

pH	Brine concentration, %			
	1.6		2.4	
	F ₂₃₅ .min.	z, °F.	F ₂₃₅ .min.	z, °F.
6.0	7.8	37	4.6	37
7.0	13.0	37	7.8	37

a. F₂₃₅ values based on resistance studies of inoculated TDT-cans.

TABLE XV

Calculated number of spores in cans showing growth (+) at longest heating time and no growth (-) at shortest heating time, as influenced by pH, brine concentration, and processing temperature

Temperature, °F.	No. of spores in thousands	
	Brine concentration	
	1.6%	2.4%

pH 6.0

	-	+	-	+
220	589	1290	1100	1620
225	316	795	759	1290
230	126	490	502	1020
235	43	246	246	661

pH 7.0

	-	+	-	+
220	389	914	912	1510
225	162	537	576	1100
230	48	235	234	693
235	2	74	85	372

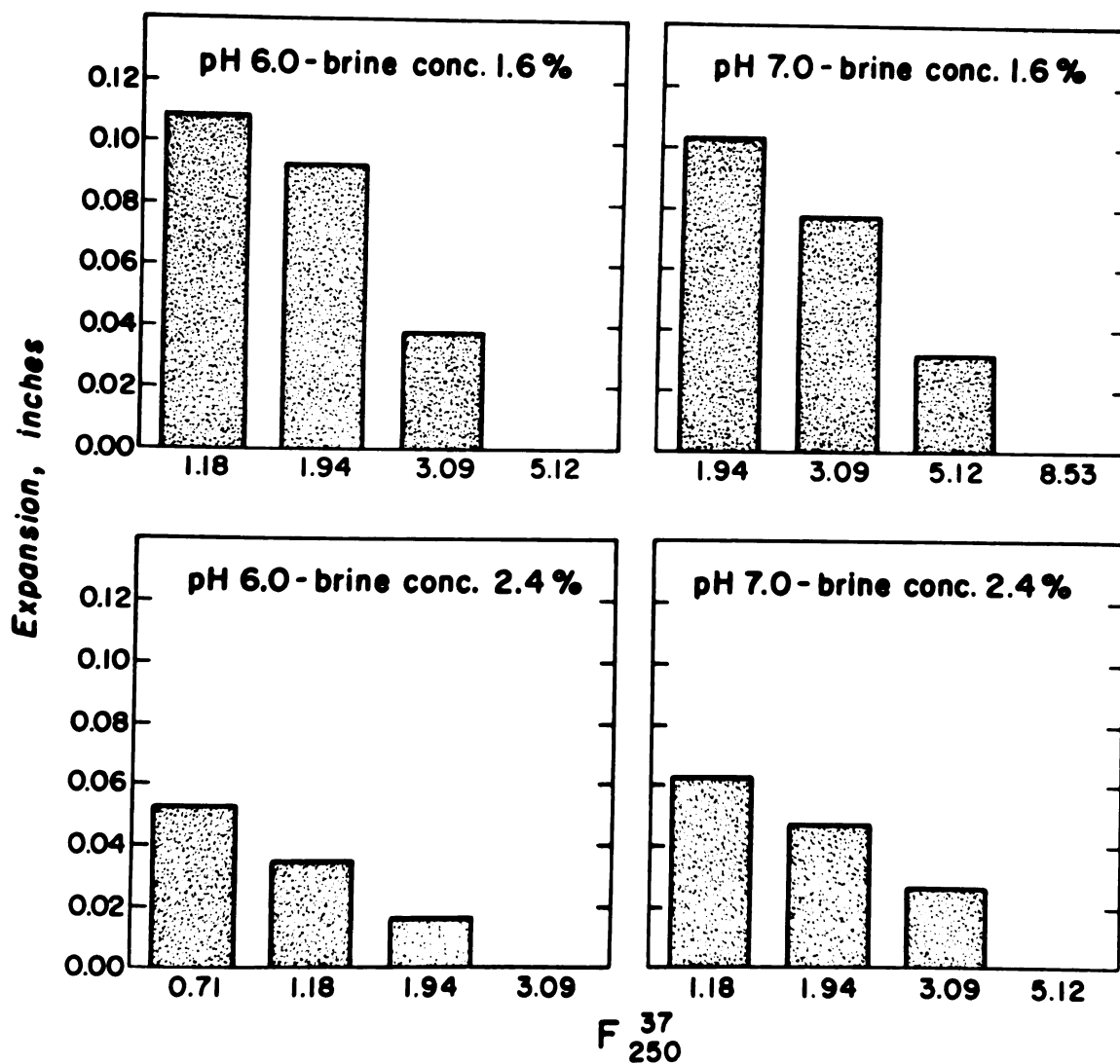


Figure 13. Effect of the thermal process (F_{250}^{37}) on the expansion of TDT-cans containing cheese spread inoculated with 3.3×10^6 spores of PA 3679 and incubated at 37°C . for 60 days.

spores per can and incubated at 37° C. for 60 days.

A graphical summary of the thermal death time, inhibition, and color curves is shown in Figure 14. The thermal death time curves are based upon the data in TABLE XII; the inhibition curves are based upon the data in TABLE XIV; and the color curve is based upon a subjective evaluation of the processed cheese spread after heat treatment.

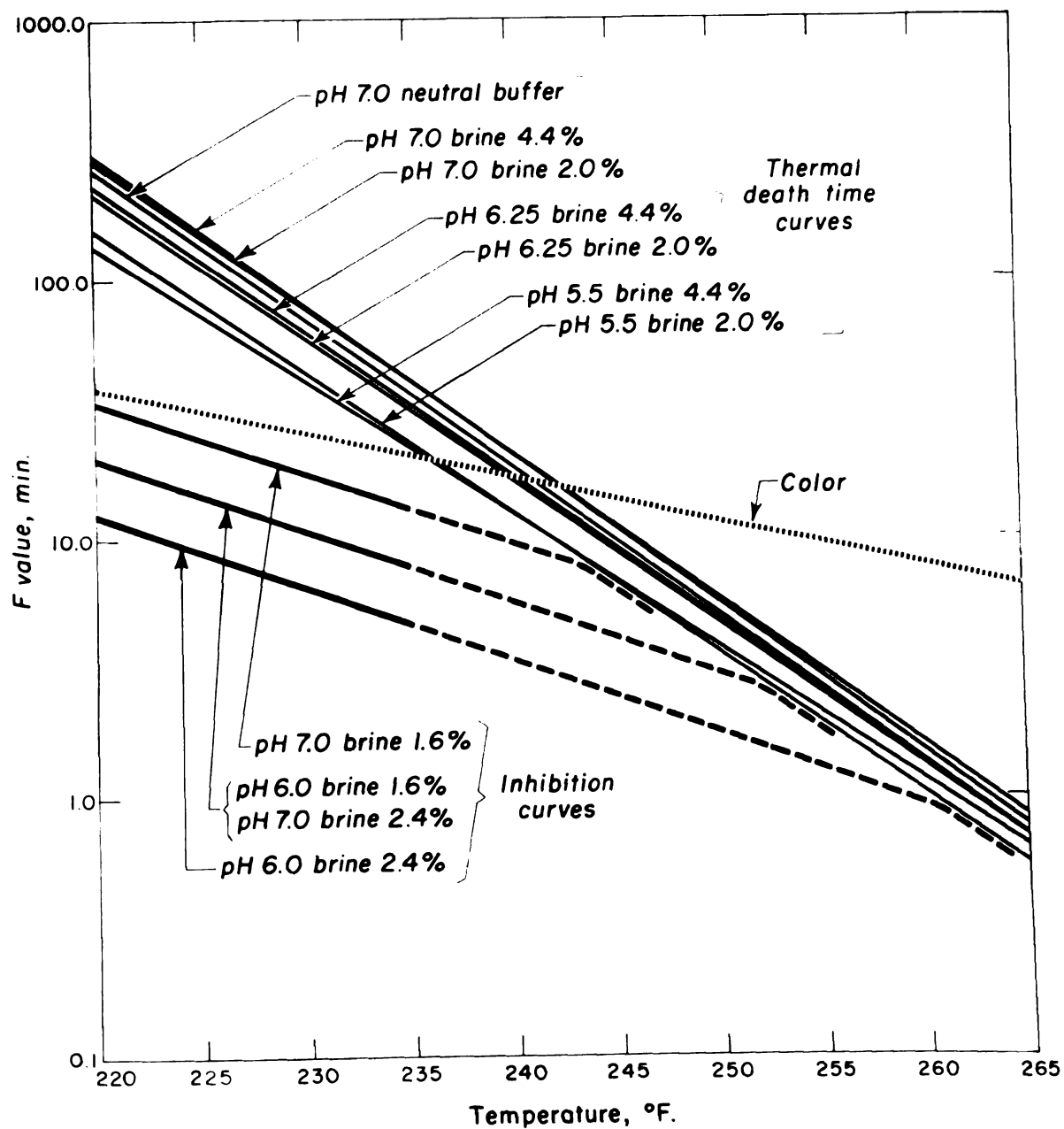


Figure 14. Graphical summary showing thermal death time, inhibition, and color curves.

DISCUSSION

Test Organism

The suspension of PA 3679 possessed a D₂₅₀ of 0.98 minutes with a z value of 17.5° F. The F₂₅₀ value, based upon a lethality of five D values, is 4.9 minutes. Townsend et al. (59) stated that the ideal suspension of PA 3679 should have an F₂₅₀ of 3.6 to 4.2 minutes but the F₂₅₀ of most suspensions of this organism is 5.0 to 5.6 minutes. According to Sognefest (47), a process based upon a suspension of PA 3679 with an F₂₅₀ of 3.65 to 4.15 minutes should insure a commercially sterile product for low-acid foods. The z value of 17.5° F. for the spore suspension agrees in general with the findings of many authorities (39, 45, 46, 47, 57, 59).

Growth of PA 3679 in Processed Cheese Spread

Effect of skim milk powder. The results of the preliminary test shown in TABLE IV indicate a relationship between the amount of skim milk powder used in the production of processed cheese spread and the ability of PA 3679 to grow in the product. Profuse growth was obtained in the product containing 10.0 per cent skim milk powder while no growth was observed in the product containing 1.0 per cent skim milk powder. The difference between growth and no growth in the product cannot be attributed to an increase

in pH resulting from the addition of skim milk powder as reported by Van Slyke and Price (61); because, in the work reported herein, the processed cheese spread was adjusted to pH 6.0 prior to incubation regardless of the amount of skim milk powder used. There may be some factor associated with skim milk powder which stimulates the growth of PA 3679 in processed cheese spread. Further work should be done to elucidate this phenomenon.

The Eighteenth Annual Report of the Council for Scientific and Industrial Research (4) states that the use of skim milk powder in processed cheese necessitates lower cooking temperatures, which fail to destroy the spore forming spoilage organisms present. Cooking temperatures and times used in the production of processed cheese spread do not destroy spores responsible for putrefactive spoilage. If the TDT curve labeled "pH 6.25 brine 2.0 per cent" in Figure 14 is extrapolated to 160° F. (the temperature normally attained in cooking processed cheese spreads), the F₁₆₀ (commercial sterility) would be 450,000 minutes or 7,500 hours. These results confirm the conclusion of Csiszar (19) that spores of Cl. sporogenes, which produce huffed pasteurized cheese, were not destroyed by temperatures used to make a satisfactory product.

Effect of pH and brine concentration. A decrease in pH from 7.0 to 6.0 resulted in an increased lag time for growth and growth was completely inhibited at a pH of 5.6 or below. Most investigators (5, 33, 48, 53, 54) agree

that decreasing the pH of processed cheese increases the keeping quality. Hood and Smith (25) and Ritchie (41) reported that putrefactive spoilage is completely eliminated at a pH of less than 5.4. The acidification of the processed cheese spread used in this project to a pH of less than 5.7 resulted in an objectionably crumbly body and texture.

End-point results in the form of either growth or no growth of PA 3679 in replicate samples of processed cheese spread may be correlated with expansion of TDT-cans of the inoculated product. It is impossible to correlate the amount of growth with the amount of gas production and the amount of gas production with the amount of TDT-can expansion at different pH values because of variation in the solubility of gas in the product. As the pH of the product increases the solubility of the gas increases; therefore, if the same amount of gas was produced at two different pH levels, the TDT-can of product at the lowest pH would show the greatest expansion. Carbon dioxide is approximately ten times more soluble in water at pH 7 than in water at pH 6. The solubility of a gas is also a function of the pressure; more gas will be dissolved in the product at high pressures than at low pressures. TDT-cans with the greatest internal pressure have the largest amount of gas dissolved in the product. Correction for this phenomenon would make the differences in the amounts of expansion of different TDT-cans even greater than the actual measured differences.

The difference in expansion of cans observed at 6.0 compared to pH 7.0 (Figures 6, 7, and 8) cannot be used as a criterion of differences in gas production since differences in expansion due to the greater solubility of gas in the product at pH 7.0 as compared to the solubility at pH 6.0 must be considered.

Each increase in the brine concentration of the product resulted in an increased lag time, a decreased rate of gas production, and a decreased amount of gas production. Growth of PA 3679 was completely inhibited at a brine concentration of 7.6 per cent or greater. The effect of brine concentration on gas production at a specified pH may be determined by the expansion of TDT-cans because salt has very little effect on the solubility of gas. The inhibition of the growth of clostridium organisms by salt is widely recognized but very few comparisons can be made because most of the salt concentrations reported in the literature are recorded as the per cent in the whole sample rather than brine concentration.

Effect of pH and Brine Concentration on the Thermal Resistance of PA 3679 in Processed Cheese Spread

The results in TABLE XII indicate that D₂₃₅ values decrease with reductions in the pH and that brine concentration does not affect the D₂₃₅ values of PA 3679. There is a significant difference in the thermal resistance of spores at pH 7.0 compared to pH 5.5, as determined from the

95 per cent confidence limits. There appears to be a significant difference in the thermal resistance of spores at pH 6.25 compared to 5.5. Differences in the thermal resistance of the spores between pH 6.25 and 7.0 are not statistically significant. There would probably be a significant difference in the thermal resistance of the spores at all three pH levels with a larger number of replications.

Thermal resistance curves vary with the methodology used in obtaining them. The error involved in removing 0.01 g. of sample from each TDT-can for subculturing is estimated to be about plus or minus 20 per cent. Since logarithms are used in the calculations there would be practically no change in the calculated D values unless the subcultured samples were either at least ten times greater or ten times smaller than 0.01 g. In addition to the error in sample weight, errors due to spore distribution and the determination of the most probable numbers are inherent.

The decrease in the thermal resistance of the spores at reduced pH values verified the findings of other investigators (8, 12, 13, 44, 47). However, Vas and Proszt (62) attributed the adequacy of a mild heat process for acid foods to the inhibition of spore germination, rather than to the low heat resistance of the spores in the acid substrate. Since the samples in this study were subcultured in liver infusion broth after heat treatment, any subsequent inhibition of spore germination was removed; therefore, the decreased D values definitely were due to the decreased heat

resistance of the spores at the lower pH values. The lack of influence of brine concentration on the thermal resistance of the spores substantiated the results of Yesair and Cameron (68) who reported that the thermal resistance of spores of Cl. botulinum was unaffected by different concentrations of salt when the samples were subcultured in an optimum growth medium. Salt did not show any protective influence in this study which is contrary to work reported by some other workers (21, 59, 63).

The relative position of the color curve and the F curves in Figure 14 indicate that the thermal processes at higher temperatures for shorter times are preferable to low-temperature-long-time processes for producing a commercially sterile product with an acceptable color. Under conditions of this study the minimum temperatures that could be used to commercially sterilize the processed cheese at pH 5.50, 6.25, and 7.00 and still maintain an acceptable color were 237, 240, and 243° F. respectively. This is true for high-temperature-short-time sterilization and not true for sterilization in the container.

The above results establish the necessity for using high temperatures for short times to obtain a commercially sterile processed cheese spread with an acceptable color. This conclusion agrees with the observation of Ball (6) who pointed out that, in proportion to their destructive effect on bacteria, higher temperatures have less effect on quality impairment than lower temperatures.

The results of the work reported herein show that an F_0 of approximately 5 minutes was required for commercial sterility, which is considerably less than an F_0 of 90 minutes reported by Meyer et al. (27). It would be difficult to control the process to obtain an F_0 of approximately 5 minutes when using a temperature of 292° F. A 0.1 minute holding time at 292° F. would result in an F_0 of approximately 17 minutes which does not take into consideration the lethality accumulated during heating and cooling.

Straight line thermal resistance curves (Figures 10, 11, and 12) are not routinely obtained when plotting data derived from subculturing the sample in a liquid medium. Sognefest et al. (47) were unable to obtain straight line thermal resistance curves from data obtained by subculturing vegetable purees after heat processing.

Effect of pH and Brine Concentration on the Heat
Treatment Required to Prevent Growth of
PA 3679 in Processed Cheese Spread

A comparison of the thermal death time and inhibition curves in Figure 14 reveals that the F values for inhibition are less, and the z values for inhibition are greater than the F and z values for thermal death time. The results agree generally with the results of Reynolds (40) who observed z values ranging from 14.5 to 27.5° F. for PA 3679 when the curves were based on the results of food substrate incubation tests. The results also agree with the observations of Townsend et al. (59) to the extent that F values

are lower and z values are different when the spores are incubated in a food substrate which is slightly inhibitory to growth, as compared to the incubation of the spores in an optimum growth medium.

This phenomenon is explained by the fact that a relatively large number of spores may be necessary to initiate growth in the inhibitory food substrate (see TABLE XV). The statistical number of spores necessary to get 50 per cent positive and 50 per cent negative samples is 0.69 when using an optimum subculture medium. If the food substrate is inhibitory to the growth of the organism, the number of spores necessary to produce 50 per cent positive and 50 per cent negative samples may be several hundred-thousand.

An examination of TABLE XV reveals that fewer spores are required to initiate growth in processed cheese spread at pH 7.0 and a brine concentration of 1.6 per cent than at pH 6.0 and a brine concentration of 2.4 per cent. Another interesting phenomenon is the fact that with an increase in the temperature of heat treatment the number of spores necessary to initiate growth in the product decreases. This may be related to the availability of nutrients.

SUMMARY

The effect of pH and brine concentration was determined on the growth, thermal destruction, and the heat treatment required to prevent growth of spores of PA 3679 of a known heat resistance in a processed cheese spread. Determination of the thermal resistance of the spore suspension revealed a D_{250} of 0.98 minutes and a z of 17.5° F.

The amount of skim milk powder used in the production of processed cheese spread had an effect upon the growth of the test organism in the product. When a 10.0 per cent concentration was used, profuse growth took place, but when a 1.0 per cent concentration was used, no growth was observed. The pH, brine concentration, and amount of spore inoculum were the same under both conditions.

A decrease in pH from 7.0 to 6.0 resulted in an increased lag time and growth was completely inhibited at a pH of 5.6 or below. The acidification of the processed cheese spread used in this project to a pH of less than 5.7 resulted in an objectionably crumbly body and texture.

Each increase in the brine concentration of the product resulted in an increased lag time, a decreased rate of gas production, and a decreased total amount of gas production. Growth was completely inhibited at a brine concentration of 7.6 per cent or more.

Heat penetration studies were conducted using TDT-cans of processed cheese spread and copper-constantan

thermocouples. It was found that the lag correction factor increased with each increase in the fill-weight and was greater when the TDT-can was processed while setting in the flat position than when it was processed setting on its edge.

Studies conducted to determine the effect of the pH and brine concentration of the processed cheese spread on the thermal process required to destroy the test organism in the product revealed that the pH had an effect, but that the brine concentration did not. The average D_{235} values of the test organism in the processed cheese spread at pH 5.50, 6.25, and 7.00 were 4.55, 6.90, and 8.25 minutes respectively. The z values remained constant at 18° F.

A comparison of thermal death time and inhibition curves revealed that the difference in results stemmed from the difference in the number of survivors necessary to produce 50 per cent positive and 50 per cent negative samples when calculating D values. It was also found that as the temperature of the heat treatment increased the minimum number of spores necessary to initiate spoilage in the product decreased.

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