THE IDENTITY AND HEAT TOLERANCE OF MICROORGANISMS CAUSING SPOILAGE OF PASTEURIZED SWEET PICKLES

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Edward K. Robbins

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By

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AN ABSTRACT

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The purpose of this study was to identify and determine the heat resistance of microorganisms causing spoilage in pasteurized sweet pickles. Twenty-one cultures of acid-forming bacteria were isolated and identified as Lactobacillus plantarum (3 strains), Lactobacillus brevis (2 strains), and Lactobacillus fermenti (16 strains).

All cultures isolated were screened to determine their approximate heat resistance; and the one strain of each species found to be most resistant selected for thermal death time studies. The thermal death time of L. fermenti (F_{160}=2.5) in phosphate buffer at pH 7.0 showed that this organism was the most heat resistant of the three species. L. brevis (F_{160}=1.19) and L. plantarum (F_{160}=1.08) were not greatly different in heat tolerance. Therefore, L. fermenti was used for all further studies.

Concentrations of sucrose above 30 per cent resulted in a decrease in population of the test organism, even at room temperature. Sucrose solution of 15 and 25 per cent had little or no significant effect at room temperature but exerted a great protective action at pasteurizing temperatures. L. fermenti in 15 and 25 per cent sucrose solutions had F_{160} values of 4.9 and 6.6 respectively.

The thermal death time of L. fermenti was greatly reduced when acetic acid was added to the substrate in which the cells were heated. With 0.5 per cent acid and 25 per cent sucrose, the F_{160} was further reduced to 0.365. The addition of 3 per cent salt (NaCl) to a solution containing 1 per cent acetic acid and 25 per cent sucrose did not greatly affect the thermal resistance of L. fermenti.

These results indicate that the pasteurization of sweet pickles at 160°F for 20 minutes or 165°F for 15 minutes (center of the jar temperature) would be sufficient to eliminate spoilage due to the organisms isolated in this study.

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INTRODUCTION

The manufacture of fresh cucumber pickles has increased from an insignificant volume in 1930 to an amount equal to about 30 per cent of the total pickle production today. The preservation of fermented pickles involves only the addition of sufficient levels of acid and salt, acid and sugar, or lower levels of these agents combined with sodium benzoate. Rarely are these products pasteurized. However, the manufacture of fresh cucumber pickles requires pasteurization. This has created many new problems for the industry.

Many sizes of whole cucumbers and many sizes and shapes of cut cucumbers are packaged. These are packed with many different formulations of acid, sugar, salt, and spices. A large variety of glass containers are used for sales appeal. All of these variables may affect the pasteurization process required for preservation.

There are recommendations in the literature for pasteurizing cucumbers. However, some of these recommendations are not in such a form as to permit their application to continuous pasteurization processes. Also, in some instances, they have failed to take into consideration the many variables between different types of pickles.

The purpose of this study was to isolate and identify microorganisms causing spoilage of fresh pasteurized pickles, and to determine their heat tolerance in various combinations of sugar, acid and salt. This data should provide a basis for determining processes for various types of pickles to insure preservation from microbial spoilage.

REVIEW OF LITERATURE

Organisms of High Acid Foods

High acid foods (pH 3.7 or below) are relatively free from spoilage caused by spore-forming bacteria (26). During pasteurization, the vegetative cells are destroyed leaving spores in the product. The low pH inhibits germination of these spores resulting in dormancy. Such foods as sauerkraut, cucumber pickles, berries, grapefruit, citrus fruit juices, and rhubarb are included in this group.

Etchells and Goresline (11) and Etchells and Ohmer (12) have demonstrated that there are two main groups of organisms responsible for the spoilage of high acid foods. Probably the most economically important spoilage organisms are the gram positive lactic acid bacteria of the genus Lactobacillus. The gas forming Lactobacillus brevis is well known as the causative agent in the spoilage of tomato ketchup, worcestershire sauce and other similar products (3).

Etchells and Ohmer (12), studying the bacteriology of the manufacture of fresh cucumber pickles, found that resistant spore-formers remained after pasteurization. However, during storage these spores did not germinate. If lactic acid bacteria or yeast survive the pasteurization, spoilage of the product usually occurs.

Anderson et al. (2) isolated several cultures of organisms from spoiled fresh kosher style pickles. Each culture was inoculated into a sound jar of fresh pack pickles and tubes of brine (0.84% acetic acid, 2.5% NaCl). After incubation, only three organisms produced typical

spoilage characteristics. One of these was a yeast and the other two were lactobacilli. No further identification was reported.

The family Lactobacteriaceae was designated by Orla-Jensen (19) to a physiological group of gram positive rods and cocci that ferment carbohydrates to a variety of end products; viz., lactic acid, acetic acid, alcohol, and carbon dioxide. These lactic acid bacteria obtain their energy by fermentation of sugar without the utilization of free oxygen. This necessitates that large amounts of sugar be fermented to obtain sufficient energy for growth (22).

The species of the genus Lactobacillus are separated into two categories; (a) homofermentative and (b) heterofermentative (28). Homofermentative species produce 85 to 95 per cent lactic acid on the basis of sugar fermented and only traces of other end products. The most common organism in this group associated with food fermentation is Lactobacillus plantarum, Orla-Jensen. Probable synonyms of this species are; Bacillus pabuli acidi II Weiss; Bacillus cucumeris fermentati Henneberg; Bacillus wartmanni Henneberg; Bacillus listeri Henneberg; Bacillus maeockeri Henneberg; Bacillus leichmani II Henneberg; Bacillus beijerincki Henneberg; Lactobacillus pentosus Fred, Peterson, and Anderson; Lactobacillus arabinosus Fred, Peterson and Anderson (5, 20).

Results of a study of 400 cultures of L. plantarum demonstrated that the majority of strains formed acid from glucose, fructose, mannose, galactose, arabinose, sucrose, maltose, lactose, raffinose,

and salicin (5, 20). The lactic acid produced is usually optically inactive with only small amounts of acetic acid and carbon dioxide being formed. L. plantarum is microaerophilic having an optimum temperature of 30 to 35°C and a maximum of 40°C. In a recent report, Costilow and Humphreys (6) have demonstrated that some strains of L. plantarum can be separated by their ability to reduce nitrates to nitrites by using indole-nitrate medium (BBL).

Heterofermentative lactics are characterized by the conversion of up to 50 per cent glucose to lactic acid, 20 to 25 per cent to carbon dioxide, and 20 to 25 per cent to ethyl alcohol and acetic acid (28). Heterofermentative lactics are separated into four species on the basis of temperature range of growth and ability or inability to ferment sugars (5, 21).

The most common species of this group Lactobacillus brevis Orla-Jensen, has an optimum temperature range of 30 to 35°C and will grow well from 18 to 40°C with an occasional strain growing at 10° and 45°C. This organism ferments arabinose, glucose, fructose and galactose, but usually shows negative or partial fermentation of lactose, sucrose, mannose, and raffinose (5, 21). Probable synonyms for this organism are: Lactobacillus pentoaceticus Fred, Peterson, and Davenport; Lactobacillus fermentatus Bergey et al.; Lactobacillus lycopersici Mickle; Lactobacillus acidophil-aerogenes Bergey et al.; and Lactobacillus panis Bergey et al.

The second species of the heterofermentative group, Lactobacillus

fermenti Beijerinck, usually does not ferment arabinose or xylose, but does ferment sucrose, lactose, and raffinose (5, 21). Its temperature range for optimum growth is between 35° and 45°C with a maximum of 47° to 50°C; the minimum growth temperature is approximately 20°C. Probable synonyms for this species are: Lactobacillus longus Bergey et al., Lactobacillus gayanii Pederson, and Lactobacillus intermedium Bergey et al.

The third species, Lactobacillus buchneri Bergey et al., ferments arabinose and has a wider range of growth temperatures than the previous two species. Its optimum temperature range is 32° to 37°C, maximum 48°C, and minimum 10°C. Probable synonyms for this species are Bacillus buchneri Henneberg and Bacillus wehmeri Henneberg.

Lactobacillus pastorianus Bergey et al., the fourth hetero-fermentative species, was described by Pederson (21) as long rods which grow at low temperatures. These organisms are less active in the fermentation of sugars than previously described species. Probable synonyms for this species are Saccharobacillus pastorianus var. berolinensis Henneberg, Bacillus lindneri Henneberg, and Bacillus fasciformis Schonfeld and Rommel.

Pasteurization of Fresh Cucumber Pickles

Commercial pasteurization studies of fresh cucumber pickles were first reported by Etchells in 1938 (10). Rate of heat penetration was measured by placing thermocouples in the center of 24-oz. containers of

fresh cucumber pickles and water. The increase in container temperatures during specific time intervals was determined and process curves plotted using time and temperature relationships. Results of heat studies showed that 60 minutes was required to reach a temperature of 160°F in containers of fresh cucumber pickles in a water bath at 180°F. Thirty minutes was required for water in the same containers to reach 160°F. An internal temperature of 160°F for 20 minutes was recommended as a process. This was based upon bacteriological studies, but data to this effect were absent.

Etchells and Goresline (11) reported on the bacteriological examination of pasteurized and unpasteurized pickles. Two main groups of organisms were found responsible for the spoilage of the unpasteurized products; viz., acid-forming bacteria and yeasts. Pasteurized products contained spore-forming organisms, but these did not increase during three months' storage.

Etchells and Ohmer (12), in 1941, published the results of a four year bacteriological study of pasteurized pickles using processes of 160°F for 20 minutes or 165°F for 15 minutes. A total of 1,768 jars were examined during the 4 year period. Results indicated that all spoilage organisms were destroyed by these procedures.

Etchells and Jones (13) classified all pasteurized pickle products into three classes: (I) unfermented, (II) partially fermented, and (III) fully fermented. Controlled pasteurization of 73.9°C (165°F) for 15 minutes was used on two representatives of each group to

determine if spoilage organisms would be destroyed. The products were checked for the effect of this heat treatment upon crispness, firmness and flavor. The specific items studied were as follows: (a) unfermented sliced fresh cucumbers, (b) unfermented fresh dills, (c) partially fermented overnight dills, (d) partially fermented genuine dills, (e) fully fermented genuine dills (3-5 grains acetic acid), and (f) fully fermented genuine dills (6-8 grains acetic acid). The acid-forming bacteria and yeasts which were capable of causing spoilage were destroyed. Observations indicated that quality in crispness, firmness, and flavor were retained after eight months' storage. Retention of crispness, firmness, and flavor was reportedly due to using a minimum pasteurization time and temperature.

Extensive work on the thermal resistance of acid-forming bacteria and yeast was carried out by Etchells and Jones (13). Controlled pasteurization of 71.1°C (160°F) for 20 minutes or 73.9°C (165°F) for 15 minutes was necessary in order to destroy the test organisms--a Lactobacillus and two yeasts. The investigation involved the use of already pasteurized sliced pickles containing liquors of these concentrations: (a) full strength brine from original jars (1.5% acetic acid, 16.7° Baume), (b) 1/2-strength brine (1.26% acetic acid, 14°Baume), and (c) 1/4-strength brine (1.07% acetic acid, 12.4°Baume). The per cent acidity and sugar stated above existed in lots (b) and (c) only after heating for 80 minutes and equilization between diluted liquor and pickles had occurred.

Jars of sliced pickles from each lot were inoculated with large populations of acid-forming bacteria and yeasts. Samples from each lot were pasteurized at 120, 130, 140, 150, and 160°F allowing a holding time of 15 minutes after the center of the jar reached the desired temperature. Center of the jar temperature was determined by boring a hole in the center of the cap and inserting a thermometer. The thermometer was placed so as the tip was located in the slowest heating part of the jar. Results indicated that there was a definite relationship between survival counts and acid content of the liquors at low temperatures. The size of the inoculum also played a part in thermal destruction.

Pasteurization studies of fresh whole pickles (8) indicates that the rate of heat penetration varies with the uniformity of the product and the nature of the pack. If the product is loosely packed, free circulation of brine by convection currents occurs while a tight pack impedes circulation. Within certain limits, the proportion of brine to pickles also affects the rate of heat penetration (8). An increase in brine to pickle ratios tends to increase rate of heating.

Etchells and Jones (13) using a pasteurizing temperature of 73°C (165°F) for 15 minutes, pointed out that care should be taken to avoid overheating fresh pickles since this would result in loss of firmness and possible development of cooked flavors. In a recent study (8), it was found that heating fresh whole pickles at 82°C (180°F) for 40 minutes had very little effect upon firmness. Cooked flavors were not

detected even after pasteurizing up to three times.

Pickles tend to develop an off-flavor during storage if not properly pasteurized. This appears to be related to the presence of peroxidase and related enzymes (2). Therefore it is necessary to base the pasteurization of pickle products upon the heat tolerance of peroxidase, as well as bacterial tolerances. Nebesky et al. (18) reported that peroxidase from commercial packs of fresh whole kosher style dill pickles varied greatly in its thermal resistance. Thermal stability studies on this enzyme at a temperature of 88°C (190°F) indicated that 40 to 45 minutes was required to destroy the enzyme of one lot and a second lot required 150 to 200 minutes. This difference was reportedly due to variation in peroxidase concentration within different lots of the fresh cucumbers. Further studies (2) indicated that after one years' storage at room temperature, residual peroxidase could be demonstrated in quart jars of fresh whole kosher style dill pickles which had been processed 35 minutes at 85°C (185°F). However, after processing 40 minutes, no evidence of peroxidase activity could be demonstrated.

Anderson et al. (2) studying the thermal resistance of microorganisms and peroxidase, found that two lactobacilli and a yeast were capable of causing spoilage of kosher dills. No further identification was made. Only the yeast was capable of surviving one minute at 130°F in brine containing 0.5 per cent acetic acid and 5 per cent salt. Residual bacterial counts remained after pasteurizing at 82°C (180°F) for 40 minutes. These organisms were largely peptonizing spore-formers and

did not increase upon storage. These data were in agreement with the findings of Etchells and Jones (13).

To reduce peroxidase activity completely, more severe heat treatment was found necessary than for microbiological control. However, it was demonstrated that sufficient heat to reduce peroxidase to a low level was adequate to prevent off-flavors (2). The total heating on pasteurization treatment was found to provide an F_{160}* value of 36 minutes. An F_{160} of 27 minutes was obtained by calculation from the data presented by Etchells and Jones (13) for fresh sliced cucumbers in 25-oz. jars (2). This value was based upon their controlled pasteurization of 71°C (160°F) center of the jar temperature for 20 minutes.

Influence of Sugar, Acid and Salt Concentration Upon Heat Resistance of Bacteria

The effect of sugar concentration on microorganisms has been studied by several workers. When sugar is present in sufficient concentrations, protection from heating in most cases is significant. Pederson, Levine, and Buchanan (24) determined that yeast were destroyed more rapidly in distilled water than in syrup when heated at 100°C (212°F). The thermal death time was 6 minutes in syrup at 24°Baume and 28 minutes in 36°Baume syrup.

Braun et al. (4) determined the effect of sugar concentration

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*F_{160} = Total lethality of process expressed in minutes at 160°F.

upon the thermal resistance of bacterial spores associated with spoilage of canned foods. The investigation demonstrated that as sugar concentrations increased so did heat tolerance of Bacillus stearothermophilus. In some cases, heat tolerance was reduced at concentrations of 15 to 50 per cent as typified by the putrefactive anaerobe (PA-3679) and Clostridium botulinum.

Tarkow et al. (27) demonstrated that sucrose solutions above 30 per cent had a significant inhibitive effect on microorganisms. However, results indicated that 40 to 50 per cent glucose solutions were more effective as inhibitors than the same concentrations of sucrose. The apparent difference in inhibitor effect is probably the result of different osmotic pressures. Glucose has an osmotic pressure 1.7 times that of sucrose. Tarkow et al. (27) found that the heat resistance of Saccharomyces cerevisiae was reduced in solutions of 30, 40 and 50 per cent sucrose. However, these data indicate that the spores of Aspergillus niger appeared to be affected very little by heat treatments in either glucose or sucrose solutions.

Etchells and Jones (13) reported that upon inoculation of acid-forming bacteria and yeasts into jars of fresh sliced pickles (1.5% acetic acid, 16.7°Baume' sugar), a great reduction in total population occurred after 1 and 2 hours' incubation at room temperature. This effect was presumably due to acid content of the brine.

The effect of acids in inhibiting the growth of microorganisms may be due to the hydrogen ion concentration or the toxicity of the

undissociated molecule. The toxicity of mineral acids is related to hydrogen ion concentration, but organic acids affect toxicity by the undissociated molecule (3). Fabian and Wadsworth (14) indicated that acetic acid was a better preserving agent than lactic acid for pickle products and that pH was not a reliable indication of preserving ability. Levine and Fellers (16) reported that acetic acid is more effective as an inhibiting agent than lactic acid for bacteria, yeasts, and mold species. Using a synthetic medium and adjusting pH with acetic acid, it was found that bacteria were inhibited at pH 4.9, Saccharomyces cerevisiae at pH 3.9, and Aspergillus niger at pH 4.1. The titratable acidities were 0.04 per cent, 0.59 per cent, and 0.27 per cent acetic respectively. It should be noted that the acidities mentioned relate only to inhibition of a few species in laboratory media and that in commercial application higher concentrations of acetic acid (1.5 to 2 per cent) are needed to preserve a product.

Levine and Fellers (17) reported that the addition of 5 per cent salt and 20 per cent sugar did not affect the amount of acid required to prevent growth and that the toxicity of acetic acid was not due to pH alone. However, an increase in hydrogen ion concentration resulted in a decrease in thermal resistance.

Erickson and Fabian (7) observed that the preserving and germicidal powers of acetic, citric, and lactic acids followed in the order listed when based upon pH. When determined upon per cent of acid present, the order was lactic, acetic, and citric. Yeasts were found

more tolerant to acid than bacteria. For yeasts, toxicity to acids was in the order of acetic, lactic, and citric regardless of pH or concentration of acids present.

EXPERIMENTAL METHODS

Isolation of Spoilage Organisms

A number of 16-oz. jars of fresh sweet sliced pickles were heated in an 180°F water bath for various intervals of time (10, 20, 30, 40, 50, 60, 80, 120 minutes). All jars were stored at room temperature for 21 days. Jars in which spoilage had occurred were characterized by turbidity of the brine or swells. Often, both turbidity and swells occurred simultaneously.

The acid content of the brines was determined by titrating against a standard base. A Beckman line-operated pH meter with a glass electrode was used to determine pH. Sugar concentrations of the brines were determined by direct readings from a hand refractometer. Salt concentrations were determined by titrating with a standard solution of silver nitrate.

Jars which showed turbidity or swells were opened and samples of the brines plated using a standard plating technique (1). V-8 agar (15) was used for the cultivation and enumeration of acid-forming bacteria. Dextrose agar plus 0.5 per cent yeast extract acidified with 4 ml of 5.0 per cent tartaric acid per 100 ml of medium was used for the enumeration of yeast.

Twenty-one cultures of acid-forming bacteria were isolated from plates of the highest sample dilutions. Cultures were maintained in stabs using trypticase sugar agar (BBL) plus 0.5 per cent yeast extract.

Identification of Spoilage Organisms

From the 21 organisms isolated, three separate groups were established. These groups were based on morphological characteristics, gas production, optimum temperature of growth, oxygen requirements, litmus milk reactions, catalase production, nitrate reduction, and ability or inability to ferment certain sugars. These three groups were designated A, B, and C.

Gas production was determined by using a method described by Pederson and Albury (23). As a safeguard, tubes of pasteurized brine were inoculated and sealed with 3 per cent agar to test further for production of gas.

Optimum temperature of growth and oxygen requirements were determined simultaneously using the same tubes. Tubes containing 9 ml of trypticase sugar agar were melted in flowing steam and allowed to cool. These tubes were placed in a water bath at 40°C and inoculated using 0.1 ml of a 24-hour broth culture of each organism. After inoculation, the tubes were shaken and placed in incubators at 10, 20, 30, 35 and 45°C. Optimum temperature of growth was determined by estimating the quantity of growth present in each set of tubes. Oxygen requirements were determined by the level at which most rapid growth occurred in the tubes.

Nitrate reduction (6) was determined by using indole-nitrate medium (BBL). All carbohydrate fermentations were determined by using cystine trypticase agar (BBL) as a basal medium.

Final identifications were according to their morphological and

physiological properties described in Bergey's Manual of Determinative Bacteriology (5) and by Pederson (20, 21).

Determination of Heat Resistance

Pasteurization is based upon the time and temperature necessary to destroy all organisms capable of causing spoilage. Therefore, it is necessary that time and temperature determinations be made upon the most heat resistant spoilage organisms. In order to separate the 21 cultures of acid-forming bacteria into groups based upon thermal tolerance, a screening technique was used. For this purpose, 0.1 ml of a 24-hour broth culture of each organism was inoculated into sterile tubes of trypticase sugar broth. Rubber stoppers were inserted on top of the cotton plugs and the tubes placed in a water bath at various temperatures (130, 140, 150, 160°F). Individual tubes were removed at specific time intervals and cooled in ice water. The heated cultures were incubated for 48 hours. Relative thermal tolerance of an organism at any temperature was determined by noting the time at which negative tubes (no growth) appeared. The most heat tolerant culture from each group was chosen for further study.

The multiple tube method described by Esty and Williams (9) was first employed for determination of thermal death times. Sealed tubes containing a 1.0 ml suspension of cells in phosphate buffer (pH 7.0) were placed in an oil bath at various temperatures (130, 140, 150. 160°F). Five tubes were removed from the bath for each of eight time intervals and

placed in ice water to cool. Contents of the sealed tubes were transferred to tubes of trypticase sugar broth and incubated for 48 hours. For each heating period, positive and negative tubes were plotted on semi-log paper to obtain the thermal death time curve.

The second method of determining the thermal resistance of the cultures was reported by Speck (25) studying the heat resistance of Micrococcus freudenreichii in milk and ice cream mix. Tubes containing 9 ml of phosphate buffer (pH 7.0) were sealed with a rubber diaphragm stopper and placed in a water bath with the water extending 2 in. above the level of the liquid in the tube. Temperature of the water bath was varied to include 135, 140, and 145°F. The temperature of the water bath was regulated so that a variance of only plus or minus 0.5°F occurred. Tubes were inoculated with 1.0 ml of a cell suspension in phosphate buffer. Cells' suspensions were obtained by centrifuging 24-hour broth cultures of each organism and resuspending cells in phosphate buffer (pH 7.0).

Inoculation was performed by using a 5 ml hypodermic syringe and a 17 gauge, 3 in. needle. The needle length allowed the inoculum to be placed in the tubes without splashing. After heating, all tubes were removed and placed in ice water until cool. A standard plating technique was used to determine survivor counts using trypticase sugar agar (BBL) as an enumeration medium. Survivor counts were plotted on semi-log paper resulting in straight line thermal death rate curves for each temperature.

RESULTS

Spoilage Organisms from Pasteurized Fresh Sweet Pickles

Twenty-one cultures of acid-forming bacteria were isolated from jars of spoiled fresh sweet cucumber pickles. The acid-forming bacteria were taken from plates of the highest dilution. Table 1 gives the pH, acid, sugar, and salt concentrations obtained from the spoiled and unspoiled pickle brines. It should be noted that the pH values were lower and the per cent acid correspondingly higher in the spoiled brines than in the unspoiled brines. Differences in sugar, salt, and acid concentrations among the jars of unspoiled pickles were probably due to differences in tightness of pack.

Table 2 gives counts obtained from the spoiled and unspoiled brines using a standard plating technique. Acid-forming bacteria were isolated from the spoiled jars which had been processed for 20 minutes. Therefore, only the most heat tolerant organisms were obtained. Non-acid-forming bacteria were not isolated for further study since the results indicated that they were incapable of growing in this product.

All cultures of acid-forming bacteria isolated were found to be gram positive, non-spore forming, non-motile, rods, which did not produce catalase, were microaerophilic with respect to oxygen, and had an optimum temperature of 30° to 35°C (Table 3). This placed them all as members of the genus *Lactobacillus*.

TABLE 1

pH, ACID, SUGAR AND SALT CONCENTRATIONS
OF BRINES FROM SWEET PICKLES

Jar No.	Process time*	pH	% Acetic Acid	% Sucrose	% NaCl
10**	10	3.35	1.66	18.0	1.11
15**	10	3.10	1.58	17.5	1.07
20**	20	3.42	1.52	15.0	.92
21**	20	3.42	1.47	16.0	.99
35	30	3.85	.81	15.0	.89
44	40	3.73	.97	17.5	1.10
51	50	3.70	1.07	18.5	1.22
62	60	3.74	.91	16.5	1.06
85	80	3.57	1.23	21.5	1.40
125	120	3.75	.94	14.0	1.07

* Minutes held in 180°F water bath

** Jars in which spoilage had occurred

TABLE 2

POPULATIONS OF BACTERIA AND YEASTS
OBSERVED IN EXPERIMENTAL JARS
OF SWEET SLICED PICKLES

Jar No.	Process time*	Acid-forming bacteria No./ml	Non-acid-forming bacteria No./ml	Yeasts No./ml
10**	10	1.6×10^7	1.0×10^9	0
15**	10	2.2×10^7	8.4×10^7	0
20**	20	5.0×10^9	1.0×10^7	0
21**	20	3.1×10^9	1.0×10^7	0
35	30	< 10	< 10	0
44	40	< 10	< 10	0
51	50	< 10	< 10	0
62	60	< 10	< 10	0
85	80	< 10	< 10	0
125	120	< 10	< 10	0

* Minutes held in 180°F water bath

** Jars in which spoilage had occurred

TABLE 3

MORPHOLOGICAL CHARACTERISTICS OF ORGANISMS STUDIED

GROUP	No. of Cultures	Cell Morphology	Temperature relation	Oxygen Requirements
A	3	Rods: Occurring singly and in short chains, with rounded ends. Show elongation as acid concentration increases. Non-Motile. Gram positive.	Optimum temperature 30°C. Minimum 10-15°C. Maximum 40°C.	Microaerophilic
B	16	Rods: Variable, usually short, in pairs and short chains. Non-motile. Gram positive.	Optimum temperature 35°C. Minimum 20°C. Maximum 45°C.	Microaerophilic
C	2	Rods: Occurring singly, short chains and occasionally in long filaments with rounded ends. Non-Motile. Gram positive.	Optimum temperature 30°C. Minimum 18°C. Maximum 40°C.	Microaerophilic

The Group A cultures were found to be homofermentative and identified as Lactobacillus plantarum. The 16 cultures in Group B and the 2 in Group C all produced gas. However, they could be separated on the basis of carbohydrate fermentations and reduction of nitrates (Table 4). Those in Group B were similar to Lactobacillus fermenti in all respects except for nitrate reduction. However, this may not necessarily exclude them from the species since it has been recently demonstrated that some lactobacilli will reduce nitrates when tested in indole-nitrate medium. Therefore, these cultures were tentatively identified as such.

Group C cultures were identified as Lactobacillus brevis. It was of interest to note that all strains of L. brevis failed to produce gas in glucose broth.

Determination of Thermal Tolerance

In the initial phase of this study, a screening technique was used to determine the heat tolerance of the acid-forming bacteria isolated. This method provided a basis for further heat studies and indicated which organisms would survive higher temperatures at specific time intervals. Table 5 gives the results obtained from the initial screening. One strain (15-2) of Lactobacillus fermenti was seemingly more heat tolerant than any of the other strains studied. However, the remaining strains of the same species did not show the same characteristic. Little difference was noted between strains of Lactobacillus brevis, Lactobacillus

TABLE 4

PHYSIOLOGICAL CHARACTERISTICS
OF ORGANISMS STUDIED

Properties	Group A	Group B	Group C
Fermentation reactions:			
Arabinose	-	-	+
Dextrose	+	+	+
Fructose	+	+	+
Galactose	+	+	+
Lactose	+	+	-
Maltose	+	+	+
Raffinose	+	+	-
Sucrose	+	+	-
Xylose	-	±*	+
Gas production:			
Cucumber brine	-	+	+
Glucose broth	-	+	-
Litmus milk	Acid coagulation	No change	No change
Nitrate reduction	-	+	-
Catalase test	-	-	-

*Majority of strains fermented xylose, but a few did not.

TABLE 5

RESULTS OF SCREENING TESTS FOR THERMAL TOLERANCE OF BACTERIA FROM SPOILED PICKLES

Species	Culture No.	No. of Tubes Positive After Various Heat Treatments*																	
		130°F Minutes					140°F Minutes					150°F Minutes					160°F Minutes				
		20	40	60	80	120	5	10	15	20	30	1	5	10	15	20	1	2	3	5	10
L. plantarium	10-1**	2	2	2	0	0	2	2	0	0	0	2	0	0	0	0	0	0	0	0	0
	10-2	1	2	2	0	0	2	2	0	0	0	2	0	0	0	0	0	0	0	0	0
	15-1	2	2	2	0	0	2	2	0	0	0	2	0	0	0	0	0	0	0	0	0
	211	2	2	0	0	0	2	2	0	0	0	2	0	0	0	0	0	0	0	0	0
L. brevis	15-3**	2	2	2	0	0	2	1	2	0	0	2	2	0	0	0	1	0	0	0	0
	15-4	2	2	2	0	0	2	2	2	0	0	2	2	0	0	0	0	0	0	0	0
L. fermenti	15-2**	2	2	2	1	0	2	2	2	2	0	2	2	2	0	0	2	1	1	0	0
	20-1	2	2	1	0	0	2	1	2	0	0	1	1	0	0	0	0	0	0	0	0
	20-2	2	2	2	0	0	2	2	2	0	0	2	2	0	0	0	0	0	0	0	0
	20-3	2	2	1	0	0	2	2	1	0	0	2	2	0	0	0	0	0	0	0	0
	20-4	2	2	2	0	0	2	2	2	0	0	2	2	1	0	0	0	0	0	0	0
	20-5	2	2	2	0	0	2	2	1	0	0	2	2	0	0	0	0	0	0	0	0
	20-7	2	2	2	0	0	2	2	2	0	0	2	2	0	0	0	0	0	0	0	0
	21-1	2	2	1	0	0	2	2	1	0	0	2	2	0	0	0	0	0	0	0	0
	21-2	2	2	2	0	0	2	2	2	0	0	2	2	0	0	0	0	0	0	0	0
	21-3	2	1	1	0	0	2	2	2	0	0	2	2	0	0	0	0	0	0	0	0
	21-4	2	2	2	0	0	2	2	2	0	0	2	0	0	0	0	0	0	0	0	0
	21-5	2	2	2	0	0	2	2	2	0	0	2	0	0	0	0	0	0	0	0	0
	21-6	2	2	2	0	0	2	2	1	0	0	2	0	0	0	0	0	0	0	0	0
	212-1	2	2	2	0	0	2	2	0	0	0	2	2	0	0	0	0	0	0	0	0
	212-2	2	2	1	0	0	2	1	1	0	0	1	0	0	0	0	0	0	0	0	0

* 2 tubes were subjected to each time-temperature exposure and incubated 48 hours.

** Cultures which were used for further heat studies.

plantarum, and Lactobacillus fermenti with exception of strain (15-2). Due to the small number of tubes used, "skipping," and apparent similarity of all strains to heat tolerance, this method could not be considered quantitative. In order to make a comparative study of these organisms, one culture of each species was selected for study. Selection was based upon ability of the organisms to withstand higher temperatures. Therefore, strain 15-2 of L. fermenti and 15-3 of L. brevis were selected due to their slight tolerance over other strains. No differences were observed between strains of L. plantarum, but one strain was selected for comparative purposes.

The multiple tube method described by Esty and Williams (9) was abandoned in the initial phase of this study. Considerable error occurred due to "skipping" and contamination from air-borne spore-forming bacteria. The time and equipment necessary were considered impractical for further work.

The heating of the cell suspensions was conducted according to the method of Speck (5) and survivor counts determined at various times. These counts were plotted on semi-log paper to obtain thermal death rate curves. The thermal death rate constant (k) was calculated for each of these curves as follows:

$$k = \frac{1}{t_2 - t_1} (\log N_1 - \log N_2)$$

k = thermal death rate constant

t_1 = time at first reading

t_2 = time at second reading

N_1 = population at t_1

N_2 = population at t_2

The lower the k values for any specific temperature, the slower the thermal destruction of cells.

Thermal death time curves were constructed by determining a point on the thermal death rate curves for each temperature and menstrum at which 99.999 per cent destruction of cells had occurred. These points were plotted on semi-log paper (log time vs. temperature) to give a straight line thermal death time curve.

The time required at 160°F to destroy 99.999 per cent of the cells present in a suspension was designated as the F_{160} value. The F_{160} value was determined by extending the thermal death time curve until it crossed 160°F on the temperature scale and reading the number of minutes required to destroy 99.999 per cent of the cells at this point.

"z" values were expressed as the number of degrees Fahrenheit on the temperature scale over which the line passes in traversing one logarithmic cycle on the time scale. "z" values indicate the slope of the thermal death time curve.

The results of the studies on the three species of lactic acid bacteria in phosphate buffer (pH 7.0) are given in Table 6 and Figure 1. L. fermenti was decidedly the most heat tolerant of the organisms studied. Results indicate that k values for the three species were similar at 135°F, but at 140, 145°F L. fermenti had much smaller values than the other two. This resulted in a high z value for this species and an F_{160}

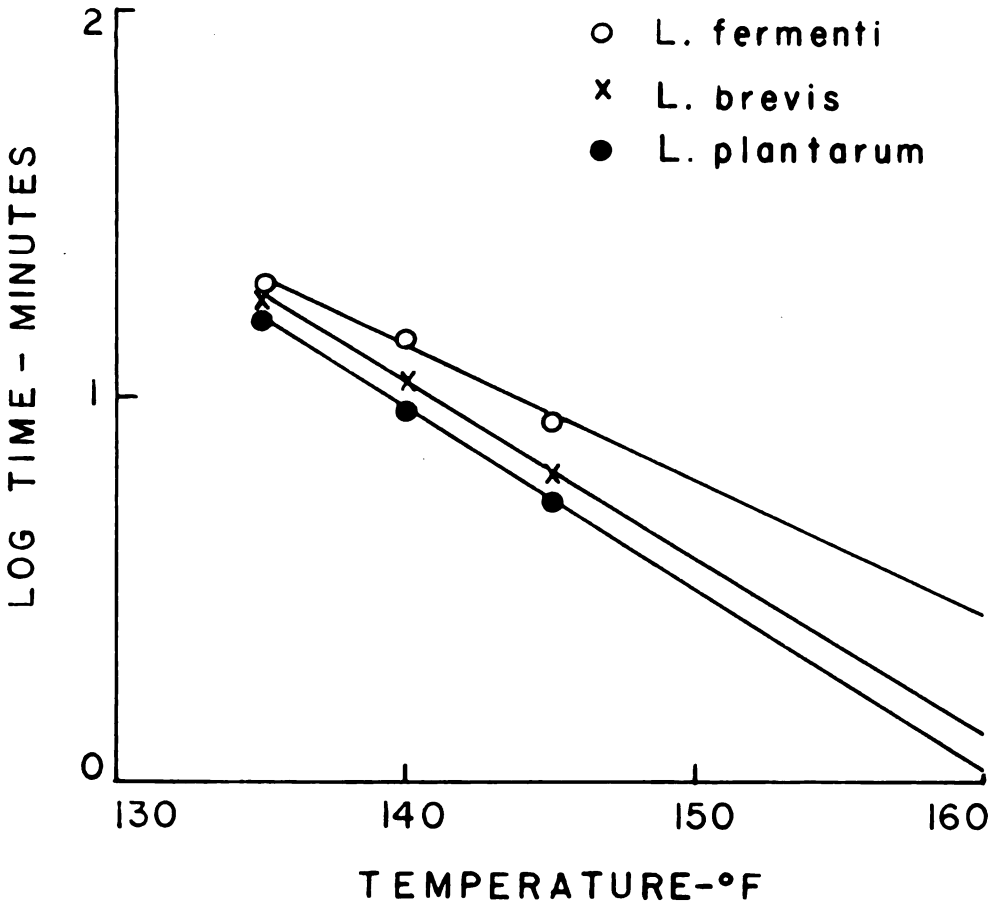

Figure 1

Thermal death times curves of L. fermenti, L. brevis and L. plantarum in phosphate buffer pH 7.0.

of 2.5 minutes as compared to 1.19 for L. plantarum and 1.08 for L. brevis. Therefore, L. fermenti was selected for all further studies.

TABLE 6

THE k, F_{160}, AND z VALUES FOR THREE SPECIES OF LACTOBACILLI HEATED IN PHOSPHATE BUFFER (pH 7.0)

Organisms	k values			F_{160}*	z
	135°F	140°F	145°F		
L. brevis	.250	.434	.666	1.19	20.5
L. fermenti	.260	.400	.444	2.5	27.5
L. plantarum	.300	.571	1.08	1.08	21.0

* Based upon 99.999 per cent destruction

The next factor to be investigated was the influence of sucrose concentrations on the heat tolerance of the lactic acid bacteria. The first step was to check the effect of various sucrose concentrations on cell suspensions without heat. Cells were suspended in 15, 25, 30, 40, and 50 per cent sucrose solutions, held at room temperature 1 hour, and survivor counts determined. The following per cent reduction in cell populations were observed: 15% sucrose, 1% reduction; 25% sucrose, 2% reduction; 30% sucrose, 80% reduction; 40% sucrose, 95% reduction; and 50% sucrose, 99% reduction. Therefore, only the 15 and 25 per cent sucrose concentrations were used for further studies.

Figure 2 shows the effect of sucrose concentrations on the thermal

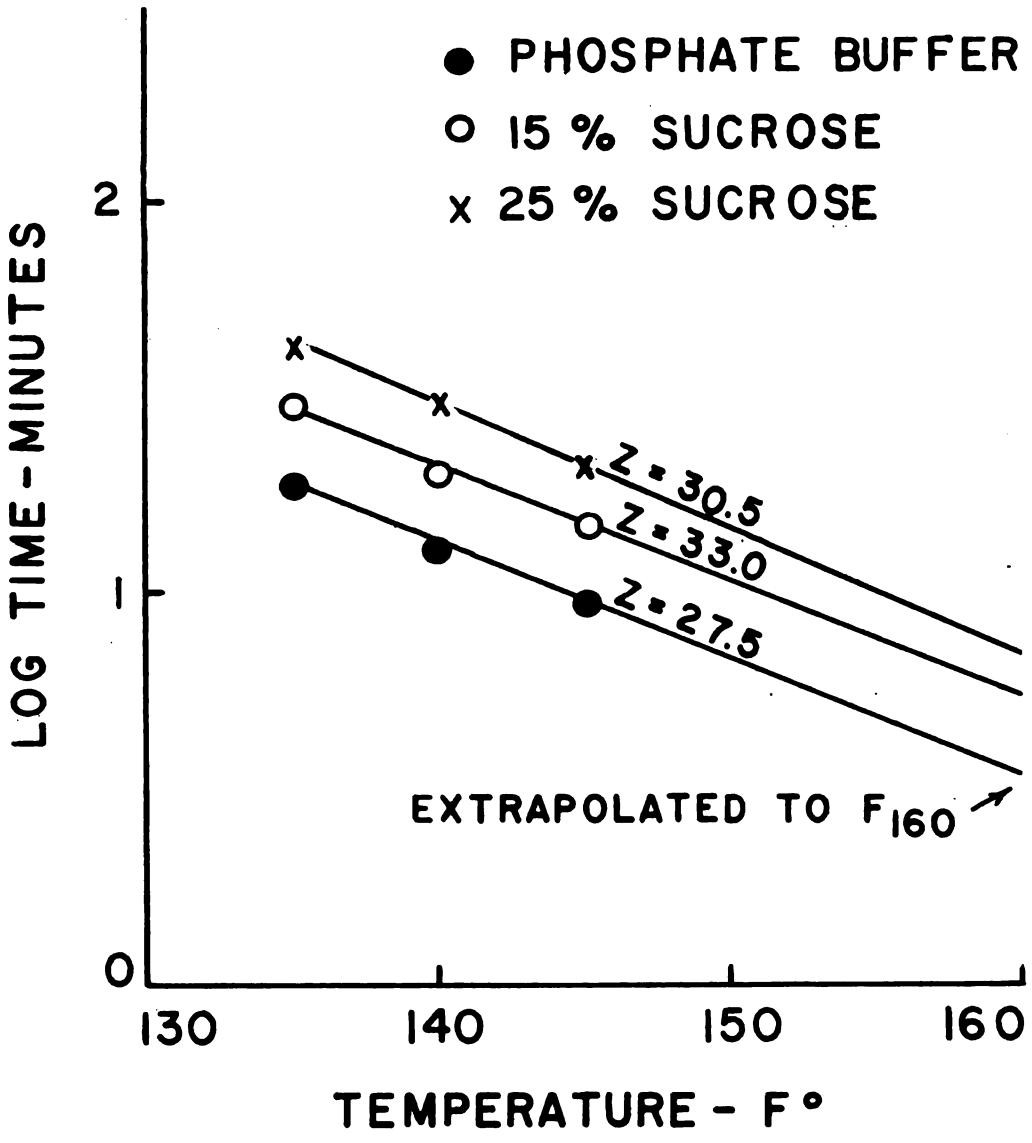

Figure 2

Effect of 15 and 25 per cent sucrose concentrations on thermal death times of L. fermenti (pH 7.0)

death time curves of L. fermenti. The resistance of the organism to heat was increased with increasing sugar levels. However, the slope of the curve (z value) was not significantly affected. The F_{160} values increased from 2.5 in phosphate buffer to 4.9 in 15 per cent sucrose and 6.6 in 25 per cent sucrose (Table 7).

TABLE 7

THE k, F_{160}, AND z VALUES FOR L. FERMENTI HEATED IN 15 AND 25 PER CENT SUCROSE SOLUTIONS

Per Cent Sucrose	k values				F_{160}*	z
	135°F	140°F	145°F	150°F		
15	.198	.235	.740	--	4.9	33.0
25	--	.266	.53	.684	6.6	30.5

* Based upon 99.999 per cent destruction

Table 8 and Figure 3 give the effect of the addition of acetic acid on the thermal death times of L. fermenti in 25 per cent sucrose concentrations. The results demonstrate that the presence of acetic acid greatly reduces the thermal tolerance of this organism. However, there were no significant differences demonstrated between the 0.5 and 1.0 per cent levels of acid at the temperatures tested. This apparent similarity of results at the two levels of acid may have been the result of allowing a time lag before heating the samples containing 0.5 per cent acid; therefore, permitting the combined action of acid and sugar to produce a detrimental effect upon the organism.

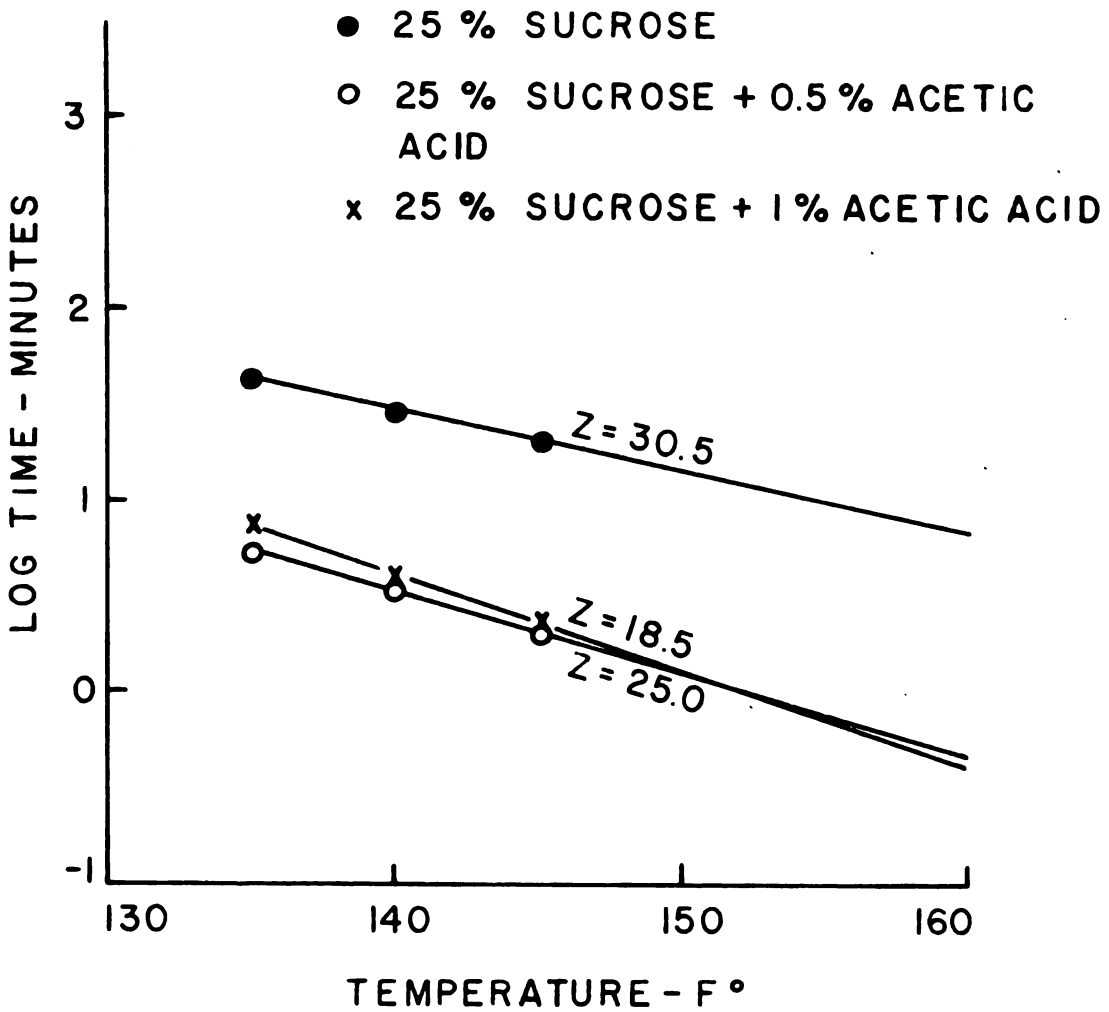

Figure 3

Effect of the addition of acetic acid on the thermal death times of L. fermenti in 25 per cent sucrose

TABLE 8

THE k, F_{160} AND z VALUES FOR L. FERMENTI HEATED IN 25 PER CENT SUCROSE SOLUTION PLUS 0.5 AND 1.0 PER CENT ACETIC ACID

Per cent acetic acid	k Values 135°F	140°F	145°F	F_{160}*	z
0.5	.707	1.50	2.00	.55	25.0
1.0	.638	1.33	2.50	.365	18.5

* Based upon 99.999 per cent destruction

The effect of sodium chloride on the thermal death time curve was determined using a combination of 3.0 per cent NaCl, 1.0 per cent acetic acid, and 25 per cent sucrose. The same cell suspensions were used to obtain the curves represented in Figure 4. The addition of sodium chloride to the heating menstrum had little effect upon thermal tolerance. For all practical purposes, both curves were identical.

Survivor counts in all menstra may be found in the Appendix.

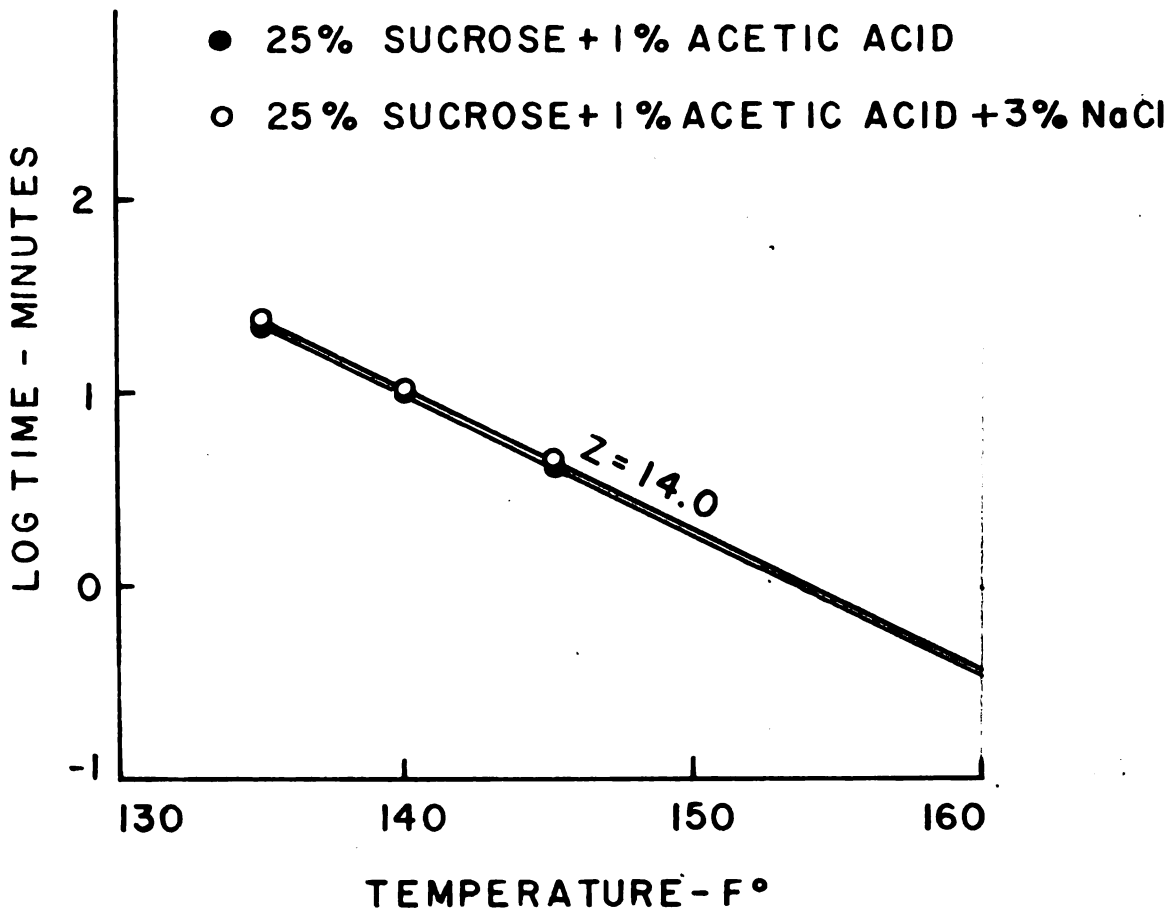

Figure 4

Effect of the addition of 3 per cent sodium chloride on the thermal death time in 25 per cent sucrose

DISCUSSION

The isolation and identification of three species of lactobacilli causing spoilage in fresh sweet pickles was accomplished in this study. The organisms were placed in the genus Lactobacillus according to their morphological and physiological characteristics as described by Pederson (20, 21) and Bergey's Manual of Determinative Bacteriology (5). As indicated by the data, a slight variation from the standard description of the genus was encountered. Sixteen strains of Lactobacillus fermenti were found capable of reducing nitrates to nitrites when grown on indole-nitrate medium (BBL). Costilow and Humphreys (6) demonstrated that certain strains of Lactobacillus plantarum reduced nitrate when grown on this medium. Therefore, not all members of the genus Lactobacillus are nitrate negative.

Etchells and Goresline (11) and Etchells and Ohmer (12) have demonstrated that two main groups of organisms are responsible for the spoilage of pasteurized pickle products; viz., acid-forming bacteria and yeasts. Anderson et al. (2) also isolated acid-forming bacteria and yeasts from jars of spoiled Kosher style pickles. No identification of these organisms was offered. In part, the data acquired in this investigation agreed with the above authors. However, the results of the present work indicated that the lactic acid bacteria would survive higher pasteurization levels than the yeasts.

Anderson et al. (2) indicated that the thermal resistance of the lactobacilli isolated from spoiled Kosher style pickles was less than

the test organism—a yeast. Heat tolerance was determined in brine containing 0.5 per cent acetic acid and 5.5 per cent salt; pH of the brine was 2.9. Thermal death times indicated that the lactobacilli would not tolerate 1 minute at 130°F in this menstruum. The yeast had an F_{160} value of only 0.5 in such a brine. This is about the same resistance noted here for L. fermenti in 0.5 per cent acetic acid and 25 per cent sucrose.

L. fermenti was found to be the most heat tolerant of the three species of lactobacilli tested. The F_{160} of this species was about two times that of the other two organisms and its z value was much greater. Therefore, one might expect it to be more frequently encountered in the spoilage of such products than L. brevis or L. plantarum, but this is not evident in the literature.

Various combinations of sugar, acid, and salt were used to determine their effects upon the thermal death time of L. fermenti. As indicated by a number of workers (2,14,16,17), acetic acid produces a marked effect upon the heat tolerance and growth of microorganisms. It was somewhat surprising that as the per cent acid was increased from 0.5 to 1.0 per cent, the thermal tolerance of L. fermenti did not decrease accordingly at low temperatures. However, this was demonstrated in the presence of 25 per cent sucrose which might affect the results. As the temperature was increased to 145°F, the effect of the increased acid level became apparent.

Several concentrations of sucrose were used to determine their effect

upon the growth and thermal death times of L. fermenti. Two concentrations (15 and 25 per cent) were found to afford protection during heating without inhibiting the organism to any great extent. However, concentrations of 30, 40, and 50 per cent sucrose produced an inhibitory effect. These data were in agreement with Tarkow et al. (27) who reported that high concentrations of sucrose inhibited microorganisms. The inhibitory action is believed to be due to osmotic pressure and affinity of sucrose for water. Braun et al. (4) demonstrated a protective effect of high concentrations of sucrose upon food spoilage organisms during processing, but this was with bacterial spores which are extremely tolerant to osmotic influences.

The addition of 3 per cent sodium chloride to a 25 per cent sucrose solution containing 1.0 per cent acetic acid did not greatly affect the thermal death time curves of L. fermenti. Levine et al. (17) and Anderson et al. (2) determined the effect of salt concentrations in solutions containing acetic acid and sucrose upon microorganisms. These data indicated that salt concentrations of 5 and 15 per cent produced little or no effect upon the organisms studied. Lower concentrations of salt were not evaluated.

The data presented in this study indicates that the controlled pasteurization procedures recommended by Etchells (10), Etchells and Jones (13), or Esselen et al. (8) would be sufficient to eliminate any spoilage which might result due to the three species of lactic acid bacteria isolated and tested. However, variations in the nature of the

pack may produce conditions in which organisms might survive. In many experimental packs, it has been demonstrated that organisms may be protected due to pickles pressing tightly together or against the sides of the jar. In such cases, heat penetration is retarded and the lethal effect of the acid is lost. Also, there is always the possibility that the cucumber tissue may exert some protective effect aiding the bacteria. This is certainly a possibility which deserves investigation.

SUMMARY

Twenty-one cultures of acid-forming bacteria were isolated from spoiled experimental packs of fresh sweet pickles. These spoiled jars of pickles had been pasteurized in a 180°F water bath for 20 minutes. On identification, 16 cultures were found to be Lactobacillus fermenti; 3, Lactobacillus plantarum; and 2, Lactobacillus brevis.

All cultures isolated were screened to determine their approximate heat resistance; and the one strain of each species found to be most resistant selected for further studies. Thermal death time studies in phosphate buffer at pH 7.0 showed that L. fermenti (F_{160} = 2.5) was the most heat resistant of the three species. L. brevis (F_{160} = 1.19) and L. plantarum (F_{160} = 1.08) were not greatly different in heat tolerance. Therefore, L. fermenti was used for all further studies.

Sucrose solutions of 30 to 50 per cent were found to have a lethal effect upon the organism, even at room temperature. However, solutions of 15 and 25 per cent had no significant effect at room temperature and exerted a great protective action at pasteurizing temperatures. The F_{160} values for L. fermenti in 15 and 25 per cent sucrose solutions were 4.9 and 6.6 respectively.

The addition of acetic acid to the substrate in which the cells were heated reduced the thermal death time of the organism greatly. With 0.5 per cent acid and 25 per cent sucrose, the F_{160} was only 0.55; and with 1.0 per cent acid in this solution, the F_{160} was further re-

duced to 0.365.

The inclusion of 3 per cent salt (NaCl) in the solution containing 25 per cent sucrose and 1.0 per cent acid had no measurable effect on the thermal death time of L. fermenti.

From these data, it is evident that any of the processes recommended in the literature today (8, 10, 13) should be sufficient to protect sweet pickles from microbiological spoilage. In fact, they should provide adequate safety factors. However, since such spoilage still occurs in these products, further studies of the effects of other factors on the heat tolerance of spoilage organisms are indicated.

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APPENDIX

APPENDIX I

SURVIVOR COUNTS FROM HEATING IN PHOSPHATE BUFFER (pH 7.0)

Organism	Temp. °F	No./ml surviving after various heating times (Heating times—minutes)					
		0	2	3	5	8	10
L. plantarum (10-1)	135	6.6×10^{6}	-	1.1×10^{6}	2.5×10^{5}	3.0×10^{4}	8.0×10^{3}
	140	6.8×10^{6}	-	1.0×10^{5}	1.0×10^{4}	2.0×10^{2}	20
	145	8.0×10^{6}	-	1.2×10^{4}	1.5×10^{2}	----	----
L. fermenti (15-2)	135	5.0×10^{7}	-	----	2.1×10^{6}	3.4×10^{5}	1.0×10^{5}
	140	1.0×10^{7}	-	----	1.0×10^{5}	7.0×10^{3}	1.0×10^{3}
	145	1.0×10^{7}	-	----	3.5×10^{4}	1.6×10^{3}	----
L. brevis (15-3)	135	2.5×10^{7}	-	----	----	1.9×10^{5}	6.0×10^{4}
	140	9.0×10^{6}	-	----	----	2.5×10^{4}	300
	145	8.0×10^{6}	7.0×10^{4}	----	100	----	----

APPENDIX II

SURVIVOR COUNTS OF L. FERMENTI AFTER HEATING IN SUCROSE SOLUTIONS (pH 7.0)

% Sucrose	Heating time	Temperature of heating (°F)			
		135	140	145	150
	min	No./ml	No./ml	No./ml	No./ml
15	0	5.0×10^6	7.0×10^6	1.0×10^7	----
	5	9.0×10^5	5.0×10^5	9.0×10^4	----
	8	2.0×10^5	5.0×10^4	1.2×10^4	----
	10	8.0×10^4	1.5×10^4	3.0×10^3	----
	20	1.0×10^3	400	60	----
25	0	----	8.0×10^6	6.0×10^6	4.2×10^6
	1	----	----	----	1.0×10^6
	5	----	----	4.0×15^5	3.0×10^3
	10	----	2.0×10^5	2.0×10^4	----
	20	----	6.0×10^3	60	----

APPENDIX III

SURVIVOR COUNTS AFTER HEATING IN 25 PER CENT SUCROSE SOLUTIONS WITH 0.5 PER CENT ACETIC ACID

Heating time	Trial	Temperature of heating (°F) 135	140	145
min.		No./ml	No./ml	No./ml
0	A*	5.0×10^6	1.0×10^7	3.0×10^6
	B	5.0×10^6	4.0×10^6	1.0×10^7
1	A	1.0×10^6	6.0×10^6	1.0×10^4
	B	1.0×10^6	----	1.0×10^5
2	A	----	2.0×10^4	1.0×10^3
	B	----	7.0×10^4	3.0×10^4
3	A	1.5×10^4	2.0×10^3	20
	B	----	3.0×10^3	20
5	A	7.0×10^2	6	----
	B	5.0×10^3	20	----
8	A	----	----	----
	B	100	----	----
10	A	----	----	----
	B	60	----	----

* Data obtained from repeating experiment

APPENDIX IV

SURVIVOR COUNTS AFTER HEATING IN 25 PER CENT SUCROSE SOLUTIONS WITH 1.0 PER CENT ACETIC ACID

Heating time	Trial	Temperature of heating (°F)		
		135	140	145
min.		No./ml	No./ml	No./ml
0	A*	1.0×10^7	6.0×10^6	4.5×10^6
	B	8.0×10^6	6.0×10^6	3.0×10^6
1	A	1.0×10^6	1.0×10^5	2.0×10^4
	B	----	----	2.0×10^5
2	A	1.0×10^5	4.0×10^5	20
	B	----	8.0×10^5	7.0×10^4
3	A	1.5×10^4	1.0×10^2	----
	B	----	3.0×10^5	4.0×10^3
5	A	20	----	----
	B	1.0×10^6	5.0×10^4	50
8	A	----	----	----
	B	2.0×10^5	2.5×10^3	----
10	A	----	----	----
	B	1.0×10^5	450	
20	A	----	----	----
	B	1.0×10^3	----	----

* Data obtained from repeating experiment

APPENDIX V

SURVIVOR COUNTS AFTER HEATING IN 25 PER CENT SUCROSE SOLUTION PLUS 1 PER CENT ACETIC ACID AND 3 PER CENT NaCl

Heating time	Temperature of heating (°F)		
	135	140	145
min.	No./ml	No./ml	No./ml
0	8.0×10^6	6.0×10^6	3.0×10^6
1	----	----	2.0×10^5
2	----	8.0×10^5	7.0×10^4
3	----	3.0×10^5	4.0×10^3
5	1.0×10^6	5.0×10^4	50
8	2.0×10^5	2.5×10^3	----
10	1.0×10^5	450	----
20	1.0×10^3	----	----

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