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Characterization of microorganisms associated with pasteurized milk

Sherry Ratledge Zimmerman

To the Graduate Council:

I am submitting herewith a thesis written by Sherry Ratledge Zimmerman entitled "Characterization of microorganisms associated with pasteurized milk." I have examined the final electronic copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Food Science and Technology.

P. Michael Davidson, Major Professor

We have read this thesis and recommend its acceptance:

Ann Draughon, John Mount, Herbert C. Holt

Accepted for the Council:
Carolyn R. Hodges

Vice Provost and Dean of the Graduate School

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Date August 1986

CHARACTERIZATION OF MICROORGANISMS ASSOCIATED WITH PASTEURIZED MILK

A Thesis

Presented for the

Master of Science

Degree

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Sherry Ratledge Zimmerman

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To my husband Bill, for all of his love, encouragement, financial support and understanding that made the preparation of this manuscript possible.

To my family and my husband's family for their love, financial support and encouragement to achieve my scholastic goals.

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ABSTRACT

Growth of psychrotrophic bacteria in refrigerated milk can be detrimental to the shelf life of the product. Normally, the presence of these bacteria are a result of post-pasteurization contamination. In some cases however, the microorganisms which affect shelf life are those that survive pasteurization and grow during refrigerated storage. The objectives of this study were to determine the influence of producer handling of raw milk on the shelf life of the pasteurized product and characterize the microorganisms which survived pasteurization.

Raw milk from producers rated as having good or poor sanitation was transported to the University of Tennessee on ice. The samples were transferred aseptically into sterile bottles and pasteurized for 30 min at 62.8^{o}C. The samples were stored at 7.2^{o}C and sampled at the raw, pasteurized and stored stages. Pasteurized samples from poor quality raw milk producers had generally higher standard plate counts than samples from good producers, but no significant difference was detected. No correlations were detected between the shelf life of the pasteurized milk and characteristics of the raw milk from good and poor producers.

Bacterial isolates were selected from raw, pasteurized and stored sample plates from one random good and poor

producer. All isolates were in two groups: gram positive sporeforming rods and gram negative non-sporeforming rods. These isolates were analyzed for their heat resistance and growth under refrigerated storage. Their heat and growth characteristics were compared to two common milk spoilage microorganisms, Pseudomonas fluorescens and Pseudomonas fragi. Some gram negative isolates were found to survive certain heat treatments better that the Pseudomonas strains. Most gram positive sporeformers survived heating treatments of 80°C to 90°C for 30 min, but they were not unusually heat resistant.

In growth testing, selected isolates were inoculated into sterile reconstituted skim milk and stored at 7.2°C for 15 days. Gram positive sporeforming isolates did not grow well and in some cases actually decreased in number over the 15 day incubation. In contrast, the gram negative isolates increased at the same rate as the P. fluorescens and P. fragi.

It was therefore concluded that it was possible for microorganisms to survive pasteurization and grow at a relatively rapid rate in the absence of post-pasteurization contamination.

TABLE OF CONTENTS

LIST OF TABLES

TABLE PAGE

CHAPTER I

INTRODUCTION

Over 100 years ago, pasteurization was developed as a heating process to destroy pathogenic microorganisms in foods. The time/temperature relationship has been modified over the years as scientists discovered more heat resistant pathogenic organisms which could survive the heat treatment process (6). In the late 1800's milk was delivered to the plant, pasteurized, cooled, bottled and delivered to household door steps well before it was 48 hours old (75) Today, we have bulk tank storage of the raw milk at the farm and processing plant. These changes in the handling of milk have initiated a trend towards longer refrigerated storage of milk before consumption and resultant predominance of a group of spoilage microorganisms known as "psychrotrophs" (44,77,99).

Gram negative bacteria of the genera Pseudomonas, Alcaligenes, Achromobacter and Flavobacterium are generally thought of as the psychrotrophic bacteria of primary importance to milk (4,6,46,74,83). However, several researchers have reported that the presence of psychrotrophic sporeformers, mainly Bacillus sp., may also create potential spoilage problems in milk (36,72).

Many of the psychrotrophic bacteria are important because they possess proteolytic and lipolytic enzymes which contribute to milk spoilage. These enzymes degrade milk protein and fat which ultimately results in off flavors and spoilage (1). Any treatment sufficient for inactivation of enzymes would severely damage the milk and render the product unacceptable to consumers.

The objective of this study was to determine the influence of bacterial types in raw milk from producers using good and poor sanitary practices on the shelf life of the pasteurized milk.

CHAPTER II

REVIEW OF THE LITERATURE

1. DEFINITION

In 1877, Forster recorded the first observation of bacteria that could grow at 0°C (43). Schmidt-Neilsen (1902) created the term "psychrophile" to describe microorganisms that grew at 0°C (43). Psychrophile is derived from the Greek word psychros meaning cold and philos meaning loving (31). A year later, Muller challenged their definition on the grounds that such bacteria cannot correctly be called psychrophilic when they have optimum temperature between 20 and 30°C (31). The basis for controversy among writers is that the name implies that the organisms prefer low temperatures when numerous studies (42,106) have shown that these bacteria grow better at higher temperatures. Instead of being cold loving, these organisms are simply cold tolerant.

Several researchers have suggested different terms to describe bacteria capable of growth at 0°C as a replacement for the term psychrophile: psychrotolerant (43), psychrobe (43), rhigophile (43), eurythermic (31) and cryophile (43).

The simplest definition of a psychrophile is a bacterium that can produce visible colonies at 0°C in 7, 10 or 14 days (4,8,43). Generally, this definition eliminates mesophiles since they usually do not grow below 5°C (8,43,73) and

bacteria which may produce colonies after incubation at 5°C for many weeks or months (8,43,73).

In 1960, Eddy suggested that organisms which grow at low temperatures but do not meet the maximum and optimum temperature requirements for psychrophiles should be called psychrotrophs (31). The root word trephein means: to increase or thrive (31), to nourish upon or to develop (31,45). Since psychrotrophs grow best at moderate temperatures they can be considered a subgroup of mesophiles (73). Eddy (31) further recommended that psychrophile be used only when a low optimum temperature is implied. Morita (67) defined psychrophiles as having an optimal temperature for growth at 15°C or lower, a maximum at about 20°C and a minimum at 0°C.

Unfortunately, many of the psychrophiles described before 1960 were not truly psychrophilic. Morita (67) cited a study done in 1908 by M. Tsiklinsky as being the only exception to this. There are few, if any true psychrophiles in foods. True psychrophiles are mainly of marine origin, including only a few genera and are of little consequence in foods (67). Most microorganisms that grow in foods at low temperatures are psychrotrophic.

In this manuscript, the term psychrotroph will be used for organisms that do not meet the definition of psychrophile, even though some of the references cited may have used the term "psychrophiles."

2. TEMPERATURE

Temperature can be the most important environmental factor affecting the growth and viability of microorganisms. Temperature may affect the duration of the lag phase, the rate of growth, the final cell numbers, the nutrient requirements and the enzymatic and chemical composition of cells (8,73). Every organism has a minimum, optimum and maximum temperature for growth.

Minimum Temperature

Minimum growth temperature is the lowest possible temperature at which the organism can grow (8). Most references report -5°C to 5°C as being the minimum growth range (8,73). However, a minimum growth temperature has been reported as low as -10°C (14,43,104). At this temperature growth is very slow and may take several months. Ingraham reported halophilic bacteria growing on bacon at -10°C (42). Below -10°C, growth is prevented, probably due to increasing salt concentrationa and dessiccation as a result of increased removal of water by freezing to achieve such a low temperature(42).

Optimum Temperature

The optimum temperature is usually based on the rate of growth but it can also be the optimum for total cell yield, rate of metabolism or rate of respiration (8). As the temperature approaches that of the optimum for total cell

yield, growth accelerates and generation time is shortest. As temperature lowers from that of the optimum, growth slows and inevitably stops (8,73,45).

Optimum temperature ranges for psychrotrophs are listed as 25-30°C in most references (8,73). Thomas and Druce (91) report optimum psychrotrophic growth range temperatures as being 20-30°C, for some as high as 30-40°C and for a few as low as 15°C or below.

Maximum Temperature

The maximum growth temperature varies widely for psychrotrophic bacteria and is perhaps higher than expected for bacteria that grow well at lower temperatures (43). The maximum is generally listed as 30°C although there are organisms that have been reported as having a maximum of 37 to 45°C (43). Banwart (8) suggests 30-40°C as an approximate maximum range. Olson and Nottingham prefer the range of 30-35°C (73).

Adaptation to Other Temperatures

There is disagreement among researchers about whether bacteria can adapt to growth at lower temperatures. Ingraham and Stokes (43) reviewed the conflicting evidence and concluded that bacteria do not adapt readily to growth at lower temperatures. In a more recent study, Zachariah and Liston (105) concluded from their results that temperature adaptation is a phenomenon shown by both mesophiles and psychrotrophs in their appropriate growth

range. They suggested that it may be necessary for biochemical changes to occur in cells transferred to a lower growth temperature to enable them to metabolize substrate normally (105).

3. TYPES OF PSYCHROTROPHIC BACTERIA

It is difficult to tabulate all of the genera that have been isolated from raw milk and dairy products. In many cases, researchers failed to identify the isolates and incorrectly labeled psychrotrophs as psychrophiles (23,43). Psychrotrophic organisms include Gram negative and Gram positive bacteria; aerobes, anaerobes and facultative anaerobes; motile and non-motile organisms; sporeformers and non-sporeformers (23,73). Psychrotrophic strains of yeasts have been reported from the genera Candida, Cryptococcus, Rhodotorula and Torulopsis (46,73). Psychrotrophic molds include those of the genera Penicillium, Cladosporium, Trichothecium and Aspergillus (73).

Psychrotrophic bacteria found in milk are mainly gram negative rod shaped bacteria in the genera Pseudomonas, Achromobacter, Alcaligenes, Enterobacter, Acinetobacter and Flavobacterium, but gram positive bacteria in the genera Bacillus and Clostridium have also been isolated (4).

Gram Negative Bacteria

Many investigators agree that organisms of the genus Pseudomonas predominate in fluid dairy products

(47,65,86,89,104). Pseudomonas can account for as much as 65% (102) of the raw milk microflora. Schultze and Olson (83) found in 586 isolates collected from pasteurized cream, milk, chocolate drink and cottage cheese that 70.6% were species of Pseudomonas, 10.8% were coliforms, 9.2% were Achromobacter, 7.9% were Alcaligenes, 0.7% were Flavobacterium and 0.8% were yeasts. Marth and Frazier (59) isolated 139 cultures of gram negative rods from raw milk. Of the representative 18 cultures they identified, 3 were Achromobacter, 3 were Aerobacter, 2 were Alcaligenes, 3 were Flavobacterium and 7 were species of Pseudomonas.

Many investigators have isolated other gram negative bacteria from raw and pasteurized milk. Studies have shown the presence of: Chromobacterium (104), Citrobacter (53,95), Escherichia (53,104), Klebsiella (53), Serratia (53,104), and Yersinia (57).

Gram Positive Bacteria

Thermoduric psychrotrophic sporeformers were first isolated by Grosskopf and Harper in 1969 (36). They attributed the loss of milk quality to the outgrowth of a psychrotrophic sporeformer identified as B. coagulans. They found outgrowth occured in 13-17 days at 2°C with a generation time of 24-30 hr. Similar lag and generation times for sporeformers were reported by others (20,64).

In 1971, Shehata and Collins (84) isolated thermoduric psychrotrophs from raw milk and identified them as belonging

to the genus Bacillus. Chung and Cannon (20) reported 83.3% of the raw milk samples they examined to contain spores.

Freshly pasteurized milk usually contains low levels of spores. Mikolajcik and Simon (66) reported spore counts immediately after heat treatments as <10 ml. After 14 and 28 days of storage, 50% and 83% of the samples developed psychrotrophic spore counts of over 100,000/ml. They concluded that growth of heat resistant psychrotrophic organisms may cause spoilage of heat treated milk after long term storage. Overcast and Atmaram (76) pinpointed B. cereus as the causative organism of sweet curdling in fluid milk. They found 28% of the commercially pasteurized milk samples obtained from various plants in Tennessee exhibited sweet curdling within 10 days of storage. Washam et al. (99) and Credit et al. (24) agreed that Bacillus occurred the most frequently in refrigerated stored pasteurized milk.

Martin (60) proposed that Bacillus species account for about 95% of the total sporeforming organisms in milk and Clostridium species constituted the remaining 5%. Psychrotrophic clostridia were present in 4 of 48 raw milk samples examined by Bhadsavle et al. (15). Moreover, Johns reported isolation of other gram positive bacteria from raw milk and milk handling equipment including streptococci, micrococci, Corynebacterium and Arthrobacter (49). Gram positive psychrotrophic bacteria from the following genera have been isolated from raw and pasteurized milk: Arthrobacter (5,51,92,95), Bacillus (20,37,60,84,99),

Corynebacterium (99), *Lactobacillus* (24,51,104) *Microbacterium* (24,51,99), *Micrococcus* (5,50,96,104), *Sarcinia* (41) *Staphylococcus* (51) and *Streptococcus* (24,41,43,51,99). Most sporeformers can survive low temperature pasteurization and thus cause subsequent spoilage in the product (65).

4. METHODS TO ENUMERATE PSYCHROTROPHIC BACTERIA

The technique recommended by the American Public Health Association (APHA) (4) for determining the number of psychrotrophs in milk is incubation of pour plates for 10 days at 7±1°C. Numerous other techniques have been proposed to enumerate psychrotrophs which might reduce the time involved with the current approved method. In the industry, many of the rapid tests that are widely used, may be useful for only a single product.

Blankenagal (17) described the ideal test to detect psychrotrophic bacteria as being accurate, able to differentiate between thermoduric bacteria that survived the heat treatment and post-pasteurization contamination, provide results within a short time and be simple and economical. While there is no single enumeration method meeting those criteria, many researchers are trying to develop one.

Inhibitors or Selective Agents

Use of inhibitors or selective agents have been increasingly popular. Inhibition of gram positive bacteria has been accomplished by use of antibiotics, dyes and other chemicals (23). Freeman et al. (33) tested 58 chemicals and dyes as to their ability to inhibit gram positive bacteria. They found only five chemicals that were effective as selective agents: Sodium desoxycholate, alkyldimethyl benzyl ammonium chloride, methyl dodecyl trimethyl ammonium chloride, alpha-bromolauric acid and alpha-bromomyristic acid.

A rapid test for psychrotrophs was proposed by Hankin and Dillman and involved the oxidase test (38). Since many of the psychrotrophs involved with milk spoilage are pseudomonads, it was logical that this might be a useful differentiation tool. Standard methods agar (SMA) plates were incubated 48 hours and flooded with a reagent which turned the oxidase positive colonies blue. In the case of Pseudomonas, Alcaligenes and Aeromonas species, all oxidase tests were positive. Coliforms, Staphlyococcus and Streptococcus species and most Bacillus were oxidase negative.

Olson (70,71) developed a selective plating technique for detection of contamination subsequent to pasteurization in milk and cottage cheese. He added 1 ppm crystal violet to 100 ml SMA before autoclaving. Prior to pouring the plates, he added 50 ppm of 2,3,5 triphenyl tetrazolium

chloride. The plates were incubated at 32°C for 48 hours. Gram positive bacteria were inhibited by crystal violet and gram negatives formed distinct red colonies. He designated this count as CVT count and concluded that it appeared to be more reliable than other methods for detecting contamination.

Surface Plating

Another attempt to reduce the incubation time has been surface plating. Since most psychrotrophs are aerobic, application of sample to the agar surface would theoretically accelerate their growth (23). Punch and Olson (78) noted that surface colonies were always detected sooner and were much larger than subsurface colonies. Their method used incubation at 6°C for 5 days and they found that counts by this procedure correlated well with those of pour plates incubated at 6°C for 8 days.

Incubation at Elevated Temperatures

Incubation of plates at temperatures higher than 7oC to reduce the time necessary to get results has been proposed by several researchers. Waes (98) suggested incubation of plates at 17°C for 16 hours before incubation at 7oC for 3 days. Juffs (52) concluded that an incubation method involving preliminary incubation of plates for 24 hr at 15oC followed by incubation for 3 days at either 5oC or 7oC was a satisfactory alternative to the standard 10 days.

Oliveria and Parmelee developed a very reliable, rapid method (69). They incubated plates at 21oC for 25 hours. Many mesophiles normally present in raw and pasteurized milk appeared to be unable to form visible colonies within 25 hr and therefore did not interfere with the enumeration of psychrotrophs. Using this method, over 95% of those colonies enumerated were psychrotrophs (69).

Oehlrich and McKellar expanded on this technique and chose a temperature of 18oC and an incubation time of 45 hr to try and maintain a more uniform colony size (68). Their results indicated that the 18C/45hr test gave a reliable estimate of the psychrotroph count when compared with the 7C/10d method. Although the 18C/45h (68) method was not as rapid as the 21C/25h test (69), it produced larger more uniform colonies.

Moseley Keeping Quality

This test is designed to detect possible keeping quality problems on freshly pasteurized milk samples. After a standard plate count is done, the samples are stored at 7±1oC for 5 to 7 days and then plated again (4). Generally after 5 to 7 days a flavor evaluation is also done (101). The incubation temperature is designed to simulate conditions of commercial distribution. The disadvantage of this test is that results are not available for 7-9 days after the product has been bottled. At that time, the product has already reached the grocery store or even the consumer.

Preliminary Incubation

The preliminary incubation (PI) count technique was developed by Dr. C.K. Johns (10,48). His goal was to find a laboratory procedure that would provide a better indication of raw milk production practices (10). The test was designed to detect improperly cleaned milk contact surfaces and poor sanitation conditions that could go unnoticed due to low milk storage temperatures (82). The technique involves incubating samples of raw milk at 13oC for 18 hr and then plating samples for standard plate count (SPC) (4).

Many researchers have agreed that this technique holds considerable promise in indication of poor production practices and prediction of shelf life of the dairy products processed from raw milk (10,12,27,50,82). It has been recommended that the test be included in all quality control programs (10,102). Some have even concluded that the PI count is a better indicator of raw milk microbiological quality than standard plate count (10,50,79,82). An example of the value of the PI count was shown in a study by Johns (50). In that study, a Denver plant adopted PI count because it became obvious that something different was needed when some raw milk producers were getting low SPC's even though their production practices were very poor. Plant sanitarians were not satisfied with the SPC's ability to reflect production conditions. The sanitarians instituted several changes causing the producers to improve their sanitation practices. In addition to subjecting every

producers' sample to PI, ther sanitarians reduced the maximum allowable counts in their raw milk PI counts. Within days, 45 out of 100 producers showed high enough PI counts to exclude them from selling milk to the plant and they were subjected to weekly inspection for 2 months by fieldmen. Fieldmen had very little difficulty in detecting the cause of the high PI counts. After they began the farm visits, they noticed a sharp drop in the percent of samples with PI counts >100,000. Dr. Johns concluded that a reflection of sanitation practices of the milk producers would never have been evident without use of PI counts.

Reduction Tests

Reduction tests serve only as an index of microbial loads and are dependent upon the metabolic rates of the microorganisms. They measure the metabolic activity of bacterial populations in raw milk. Two of the types available are Methylene blue and Resazurin (28). Methylene blue is not applicable for psychrotrophs because most psychrotrophs do not reduce methylene blue (23). Therefore, resazurin is the main reduction method utilized for milk. Catchick and Gibson (19) developed a 16 hr test for detecting post pasteurization contamination based on the resazurin dye test.

5. SOURCES OF PSYCHROTROPHS

In 1892, Forster (43) showed that psychrotrophic bacteria were widely distributed in nature including fresh

and salt water, milk, meat, garden soil and street dirt. Today, most researchers agree that psychrotrophic microorganisms found in milk and dairy products usually originate from vegetation, soil and water (23,88,92,).

Soil and Vegetation

Mikolacjik (65) postulated that soil is the primary source of gram negative psychrotrophic and sporeforming bacteria. He further added that water supplies, plants, animal feed and bedding are sources of psychrotrophic bacteria that probably originate in the soil.

Water

Witter (104) stated that psychrotrophic bacteria known in older literature as water bacteria, have been found in every conceivable water source including farm water supplies, dairy plant water supplies, municipal water supplies, lakes, streams and ditches, fresh and salt water. *Pseudomonas*, *Achromobacter*, *Alcaligenes* and *Flavobacterium* dominate the psychrotrophic microflora in water (92). Witter pinpointed the primary source of psychrotrophic contamination in dairy plants as the water supply and improperly cleaned equipment and utensils as a secondary source (104). Mikolajcik (65) found the psychrotrophic bacterial counts of farm water supplies collected from the same source at three different locations ranged from <10 to 270,000/ml. Chlorinated farm water supplies have been found to have as many as 100 psychrotrophs/ml (92). Wash water

can be a major source of psychrotrophic contamination in cottage cheese and cheese manufacturing (92).

Feed

Prior to 1960, grass and hay were found to be sources of psychrotrophic bacteria with numbers exceeding 10^7/gr (92). Barley and oats had counts as high as 10^6 pychrotrophs/gr (92).

Cow and Udder

It is genarally accepted that udder microflora is made up of mostly gram positive bacteria. Usually the microflora of the milk as it leaves the udder is predominately Micrococcus, Corynebacterium and coagulase negative Staphylococcus (92). Udders of mastitic cows can contain large populations of coagulase positive staphylocci, streptococci and the coli aerogenes groups (93). Thomas et al. (92) found aseptically drawn milk usually contained no psychrotrophs. Blankenagal and Okello-Uma showed that milk collected aseptically from individual udder quarters was free of gram negative bacteria and concluded that gram negative organisms may be considered as contaminants from outside environments (18).

Swabs of washed and disinfected udders and teats have shown surprisingly high psychrotrophic (92,95) and total plate counts (35). Thomas and Thomas (95) suggested that fecal contamination, as a result of poor milking hygiene, may contribute considerable psychrotrophic contamination to

raw milk supplies. Fresh and dry cow manure have been reported to contain >10^6 psychrotrophs/g (92). Thorough preparation of the udder before milking has been effective in decreasing the bacterial numbers entering the milking system (35).

Milking Apparatus

Gottemoller (35) conducted and extensive study on dairy farm practices and their effect on preliminary incubation. He found that the gaskets, when not cleaned properly or replaced, in combination with the milker claws, take off sensors and weigh jars, added significantly to the number of bacteria present in raw milk.

Bulk Tank

Gram negative rods were the major bacterial type present on the surface of farm milk bulk tanks according to a review by Mikolajcik (65). Microflora of well cleaned tanks contained 38.6% gram negative rods while poorly cleaned tanks contained 76.7% (90). Rubber outlet plugs were common problem areas (90,93,94). Also, coli-aerogenes types were more prevalent on rubber parts of manually and automatically cleaned systems (95).

Andrey and Frazier (5) examined 220 isolates from 12 farm bulk cooling tanks in Wisconsin and found they belonged to the genera Aerobacter, Alcaligenes, Arthrobacter, Flavobacterium, Micrococcus and Pseudomonas. They noted that during the period when cows were barn fed, Arthrobacter

species predominated in the milk while Pseudomonas and Micrococcus were second and third respectively. When cows were on pasture, Flavobacterium, Arthrobacter and Alcaligenes genera were found in decreasing order of predominance (5).

Besides the farm bulk tank, road tankers are another important source of contamination (49). The reason for this is that a poorly washed or sanitized tanker will nullify the results of the best farm bulk tank cleaning program (49).

Contamination of Dairy Products

Growth of microorganisms in pasteurized milk can cause serious quality defects and reduce the shelf life of the product. Post pasteurization contamination by psychrotrophic bacteria can be the most significant in terms of reducing quality. Among the common post-process contamination sources are the high temperature short time pasteurizer (HTST), storage tanks, lines, fillers and the package (16,80).

The most common types of gram negative bacteria associated with post pasteurization contamination belong to the genera Pseudomonas, Achromobacter, Chromobacterium, Aerobacter, Alcaligenes and Escherichia (4,65). Maxcy (61) reported that post-pasteurization contamination levels are generally low in freshly pasteurized, packaged milk. However, because psychrotrophs grow well at refrigerated

temperatures, even a contamination level of one cell per container of milk can result in high counts within 5 or 7 days.

Gram positive sporeforming rods of the genus Bacillus have also been found on improperly cleaned equipment. Hileman (41) reported a source of spores as being found in "milk stone" on the equipment, as a result of improper cleaning. Milk stone is a complex film mixture of milk and water minerals that can contain entrapped protein, fat, soil and microorganisms plus sanitizing and cleaning residues. The film adheres tightly to the surface of bulk milk tanks and equipment and requires proper cleaning for complete removal (32,54).

6. CONTROL AND PREVENTION OF PSYCHROTROPHIC BACTERIA

The best way to control the number of psychrotrophic bacteria found in milk is to prevent their presence by properly cleaning and sanitizing equipment. The effectiveness of any equipment cleaning program depends upon the surface that is to be cleaned and the type of soil that is to be removed (11,54). The cow's udder, milking apparatus, lines and bulk tank are all important points to consider (11,32,54,97).

Cow's Udder

A good udder washing program consists of washing the quarters and teats using individual towels and an iodine

washing solution, drying the udder with individual towels before placing the milkers on the udder and then follow up with teat dip after milking (11,54,97). Use of teat dip can be a very important factor because it has been shown to effectively reduce the number of new mastitis infections (11,100). Following milking, teat canals remain dilated for a short period of time. Teat dip contains iodine and seals the teat ends, helping prevent entrance of bacteria into the teat canal before the teat contracts to normal size after milking (11). If teat dip is not used, then as the cow leaves the parlor, if the udder contacts mud or manure, bacteria can enter the teats, invariably inviting future mastitis problems.

Bulk Tank and Milk Transport Lines

An effective procedure for cleaning the bulk tank includes rinsing with hot water to remove milk residue, scrubbing with alkaline or acid cleaner, rinsing , sanitizing, and rinsing again (11,32,54).

Today, most milk transport lines are installed so that they can be easily cleaned and sanitized without disassembling (11). This system is termed "CIP" or cleaned-in-place. With this type system, after milking, the equipment is rinsed with warm water and then a hot detergent solution is continously pumped through the lines for no less than 30 minutes (11). The length of time and temperature ensures removal of all traces of residue. Finally, the

equipment is sanitized immediately before use (11,54,97).

Rubber parts within the milking system are particularly hard to clean because milk solids and fat accumulate in the pores of the rubber parts (35,54). It is essential that all surfaces contacting the milk be thoroughly cleaned and sanitized (11,54).

7. EFFECT OF PASTEURIZATION

Pasteurization was developed as a heat treatment process to destroy pathogenic microorganisms in foods. Pasteurization is achieved by heating at 145°F for 30 minutes (low temperature holding method) or at 161oF for 15 seconds (high temperature short time). These temperature and time relationships are sufficient to destroy the two most heat resistant non-sporeforming pathogenic organisms: Mycobacterium tuberculosis and Coxiella burnettii (45).

With the development and use of pasteurization, it was assumed that all of the organsisms that survived the process would be sporeformers. In 1901, Russell and Hastings as cited by Hileman (41), discovered a micrococcus which survived 168.8oF. He also reported several research studies on strains of cocci surviving pasteurization. Among the most frequently occurring were Micrococcus epidermidis, M. candidus, M. varians, M. luteus, Streptococcus faecium, S. liquifaciens and S. thermophilus.

Gram Positive Bacteria

As stated earlier, some gram positive thermoduric bacteria may survive pasteurization and causie spoilage of the product. Maxcy (61) observed 40% non-sporeforming rods, 32% micrococci, 22% bacilli , 5% streptococci and 1% coliforms from 246 isolates obtained from freshly pasteurized, packaged milk. Credit et al. (24) reported 84% of the bacteria isolated from commercially pasteurized milk as belonged to the genus Bacillus while Micrococcus, Microbacterium, Achromobacter and Alcaligenes species were also detected at lower levels.

Mikolajcik conducted a study on the heat destruction of spores in skim milk (63). He found that no one Bacillus strain was consistently the most heat resistant at all three temperatures studied. D-values at 100°C ranged from 0.875 minutes for B. pumilus to 4.10 minutes for B. licheniformis.

B. cereus has been implicated in foodborne illness. However, growth of psychrotrophic sporeformers rarely results in foodborne illness because most species can not grow below 10°C. Stewart (87) found that few B. cereus strains could grow below 10°C. In contrast, Coghill and Juffs (22) observed growth of some strains of B. cereus at 7°C. In view of these findings, Mikolajcik (64) suggested that continued refrigerated storage has promoted adaptation of these sporeformers to growth at lower temperatures. Should new B. cereus strains be found which grow in the psychrotrophic growth temperature range, then the presence

of this microorganism in raw milk will take on a new significance.

Gram Negative Bacteria

Gram negative bacteria, once assumed to be destroyed by proper pasteurization are emerging as potential problems to the dairy industry. In 1963, Macaulay et al. (58) evaluated the effect of pasteurization on survival of certain psychrotrophic bacteria. They inoculated Pseudomonas fluorescens and Alcaligenes tolerans in a series of cell densities ranging from 10 to 10^9 cells per ml into 5 ml of trypticase soy broth. Pasteurization was at 73°C for 16 seconds. They found when cell density was greater than 10^5, viable cells were detected immediately after pasteurization. When the cell count was between 10^4 and 10^5, only after 10 days at 5°C were survivors detected. When cell numbers were less than 10^4, no survivors were detected. This confirmed the importance of the relationship of initial numbers to the success of the pasteurization heat treatment of milk.

It was generally believed that few, if any, gram negative bacteria survive the pasteurization process. Witter reported that few had been found to survive pasteurization, yet after 7-10 days of storage, high counts were found (104). These findings suggests that bacteria assumed killed by heating may recover their ability to grow after extended storage. Dabbah et al. (25) studied a Pseudomonas species which appeared killed by heat recovered

and grew normally after incubation for long periods. They listed 3 factors that controlled the cell's ability to recover: 1) physiological state of the bacterial cell, 2) type of heating medium and 3) type of recovery medium.

Among the gram negative bacteria present in raw milk, Yersinia enterocolitica, a pathogen has been implicated in food borne illnesses from consumption of contaminated chocolate milk (57). If this gram negative rod could indeed survive pasteurization, re-evaluation of our pasteurization process would be needed. Hanna (39) tested 5 strains for heat resistance and reported no survival at 60oC for several minutes and concluded that if these organisms are present, post pasteurization contamination is the likely source vehicle. Another foodborne pathogen of current interest, Listeria monocytogenes, has been reported to be heat resistant under certain circumstances.

Enzymes

Bulk cooling of raw milk has increased the length of time between the dairy farm and the processing plant. Thus, there is considerable opportunity for psychrotrophic bacteria to grow and produce extracellular enzymes, namely proteinases and lipases. Although pasteurization will inactivate natural milk lipase (21), many of the other proteinases and lipases will not be inactivated (1,21,26). These enzymes can affect the quality and shelf life by acting on the constituents of the heat treated product.

Proteinases. Some thermoduric bacteria as well as psychrotrophic thermoduric sporeformers are well known for their ability to produce extracellular proteinases but no evidence linking them to these defects in milk has been reported (13,55). Proteinase-producing bacterial types include: Pseudomonas, Acinetobacter, Achromobacter, Aeromonas, Flavobacterium, Xanthomonas, Proteus, Enterobacter liquefaciens, Escherichia freundii (55). Bentgsson et al. (13) concluded that some Pseudomonas strains that survive pasteurization and attack casein during storage. Mayerhofer et al. (62) reported on an enzyme from P. fluorescens P26 that required up to 9 min at 121°C for a 90% loss of activity. Adams et al. (1) compared inactivation of another protease from P. fluorescens MC60 at 149°C with that of bacterial spores used to establish UHT sterilization parameters. The protease was found to be 400 times more resistant than spores of Clostridium sporogenes PA 3679 and 4000 times more resistant than B. stearothermophilus. The D^{149C} and Z values were 1.5 min and 32.5 C, respectively.

To overcome the problem protease, Aggarwal (3) proposed a UHT treatment of 146°C for 2 seconds, but even this destroyed less than 10% of MC60 proteinase. In fact, any UHT treatment capable of inactivating a significant amount of proteinase would render the product unacceptable. Therefore, Barach et al. (9) suggested that the destruction of heat resistant proteinases using sub-sterilization

temperatures may prove feasible. Using data collected at higher temperatures, they calculated an expected 30% loss of proteinase activity, at 55°C for 60 min. However, inactivation actually occurred more rapidly and to a greater extent than was expected. They concluded that low temperature inactivation could be useful if the mechanism was understood.

Lipases. Chemical breakdown of milk fat by the enzyme lipase results in free fatty acids (FFA). Some FFA produce unpleasant odors and bitter flavors. Agitation of raw milk can cause disruption of the fat globule membrane and lipase can gain access to the fat.

Dreissen and Stadhouders (29) found two heat resistant lipases produced by P. fluorescens 22F that exhibited different inactivation rates. The different rates were exhibited only at heat treatments above 55°C. They proposed that this may be due to two distinct enzymes or one enzyme with two active forms, one of which is modified to a heat stable form when heated above 55°C. Cogan (21) theorized the enzyme exhibiting the faster rate of inactivation was an extracellular lipase that was released when the bacterial cell died.

Organoleptic Changes/Keeping Quality.

Proteinases may attack casein (55) causing development of bitter flavor (1,2), coagulation or clearing (9,13,55) of the milk and development of bitter flavor in cottage cheese

(103). Gelation is the primary problem with UHT milk because it limits the shelf life (9,55,56,103). This defect becomes evident after extended periods of storage. Flavor defects by sensory testing can be detected as early as 4-5 days even with small concentrations of the enzyme present (103).

Gebre-Egziabher et al. (34) observed the activity of proteinase from six different pseudomonads and found their optimum temperatures to be 40°C. Above 40°C, the activity declined quickly. However, below 40°C even at room and refrigerated temperatures (25°C), the proteinases had good activity. These findings can be particularly important in UHT processing since the product is not refrigerated.

8. SHELF LIFE STUDIES

Patel and Blankenagel (77) conducted a shelf life study on 216 samples of milk. The raw milk was laboratory pasteurized, stored at 7oC and evaluated for flavor after 1 and 2 weeks. Raw milk samples exhibiting bacteria counts of >1,000,000/ml before pasteurization inevitably developed off flavors after pasteurization and storage. The researchers noticed a bitter flavor within 2 weeks as a common defect in nearly all samples that contained >10,000,000/ml as a raw milk sample. The off flavor developed in the absence of post pasteurization contamination in spite of low numbers in the pasteurized product.

Washam et al. (99) isolated more than 700 bacterial cultures from 227 different pasteurized samples. Of those 700 isolates, 135 were resistant to heating at 72oC for 16 seconds and could re-establish growth at 7.2oC. Thirty five isolates, representing 15 different types were subjected to detailed examination in refrigerated milk, growth temperatures, heat resistance at different temperatures and their identities.

The significance of sporeforming and non sporeforming thermoduric psychrotrophs depends on several factors including identity of the bacterium, number of cells present, other microflora competing, storage temperature and expected shelf life of the product. These bacterial types may become the most important areas of further research especially with increased usage of lower refrigeration temperatures and the trend towards longer shelf life.

CHAPTER III

MATERIALS AND METHODS

1. DESIGNATION OF PRODUCERS

Prior to collection, three producers were identified as having good quality sanitation and three as having poor quality sanitation. These producers were classified by two experts who were very familiar with the individual producers and their dairy operations. One expert was associated with the milk processor which received the producers milk and the other was an agent for a company which marketed cleaners and sanitizers. Criteria used for classification were: historical microbiological data, farm sanitation practices in and out of the milking parlor, adequate facilities to produce a quality product and general health of the dairy herd.

2. SAMPLE COLLECTION AND PREPARATION

One gallon plastic jugs of raw milk were gathered from producers of good and poor quality milk and transported to the University of Tennessee on ice. Samples were randomly assigned alphabetical letters A-H to designate producers. Upon arrival at the laboratory, 100 ml subsamples of the raw milk were aseptically transferred to twelve sterile 125 ml screw-capped bottles.

3. PASTEURIZATION OF SAMPLES

Two-100 ml bottles of each sample were set aside for raw milk testing. The remaining milk subsamples were lab pasteurized at 62.8°C for 30 min in a water bath. The samples were randomly distributed within the heating vat. Following pasteurization, the samples were cooled on ice and stored at 7.2°C.

4. METHODS FOR MICROBIOLOGICAL ANALYSES

All microbiological analyses were done using a Spiral Plater (Spiral Systems, Inc., Cincinnati, OH). Samples were evaluated as raw and pasteurized samples. All stored samples were examined at 0, 7, 10, 14, 17, 21 days as to their microbiological profiles. Replicate 1 was carried thru 35 days and replicate 2 was carried to 21 days. In the event that a sample was determined to be spoiled before the storage study was completed the sample was evaluated by (a) standard plate count, (b) coliform count, (c) psychrotrophic count, (d) pH, (e) titratable acidity, and (f) acid degree value.

Standard Plate Count (SPC)

Raw milk samples were plated on standard methods agar (SMA; BBL, Cockeysville, MD) using the spiral plater method outlined by the American Public Health Association

(APHA) (4). Plates were incubated at 32°C and counted at 48 ±2 hours.

Coliform Count

Raw and pasteurized samples were plated on violet red bile agar (BBL) using the method outlined in APHA (4). Preliminary testing indicated that overlaying the plates disturbed the known volumes dispensed in each plate segment of the spiral plated samples and therefore, they were not overlayed.

Psychrotrophic Count

Raw and pasteurized samples were plated on Crystal Violet Tetrazolium Agar (CVT). This medium was SMA with 1 ppm crystal violet (Fisher Scientific Co., Fair Lawn, NJ) added before sterilization. Prior to pouring the plates, a 50 ppm solution 2,3,5 triphenyl tetrazolium chloride (Difco, Detroit, MI) dissolved in distilled water was added. Since crystal violet inhibits gram positives, this technique is designed to detect gram negative bacteria which gives an estimation of psychrotrophic bacteria (4,85).

Preliminary Incubation Count

Raw milk (10 ml) was aseptically transferred to sterile screw-capped tubes and incubated at 13°C for 18 hours. After the preliminary incubation period, a SPC was conducted on the raw milk samples. Plates were incubated at 32°C and counted at 48 ±2 hours.

Moseley Keeping Quality

Raw milk samples were vat-pasteurized and stored for 7 days at 7°C. The milk samples were then subjected to a standard plate count (SPC).

Proteolytic Thermoduric count

Raw milk samples (10 ml) were heated in sterile screw-capped tubes at 80°C for 15 minutes. The samples were packed on ice and then plated on Skim Milk agar (85). Plates were incubated at 21°C for 48-72 hr. Following incubation, the addition of 1% HCl or 10% acetic acid to plates distinguished true "proteolytic" zones from clear zones produced by weak acid producing organisms. False-positive zones became turbid after flooding with acid, true proteolytic zones remained clear (4).

Somatic Cell Counts

The direct microscopic method for somatic cell counts were used to evaluate samples (4).

5. METHODS FOR CHEMICAL ANALYSES

pH

The pH of the samples were measured using a Fisher Accumet pH meter model 600 (Fisher Scientific, Pittsburgh, PA). The instrument was standardized using buffer solutions at pH 4.0 and 7.0. Accuracy was within $\pm$ 0.1. The pH was measured at the raw and pasteurized stages and on the last day of storage.

Titratable Acidity (TA)

Eighteen milliliters of milk was titrated with 0.1N NaOH to a phenolpthalein endpoint. Titratable acidity of the sample was expressed as % lactic acid (4).

Acid Degree Value

Acid degree values of the samples were determined using the method outlined in APHA (4).

6. CHARACTERIZATION OF BACTERIAL ISOLATES

Isolates were selected using Harrison's disc (40) and transferred to nutrient agar (BBL) slants and stored at 4°C. They were transferred routinely to maintain viability.

Isolates were gram stained and grouped by morphological characteristics into two groups: sporeformers and non-sporeformers.

7. HEAT RESISTANCE OF SPOREFORMING BACTERIA

Preparation of Spore Suspensions

Bacteria were grown at 32°C for 48 hours in nutrient broth (BBL). The tubes were vortexed to mix contents and then 0.1 ml was spread plated on nutrient agar supplemented with 10 mg/ml $MnSO_4$ (Fisher Scientific). The plates were incubated at 32°C for 48 hours.

Wet mounts were examined under phase microscopy for sporulation. At a sporulation rate of >70%, spores were

washed off the plate with three 10 ml portions of distilled water. An ethanol-flame sterilized glass rod was used to loosen the spores and the mixture was pipetted into a sterile plastic centrifuge tube. The tubes were centrifuged at 7600 X G for 15 min at 4°C. The supernatant was decanted and discarded. The spores were washed with 30 ml sterile distilled water and centrifuged again at 1900 X G for 15 min. The supernatant was decanted and discarded. Finally, the spores were centrifuged at 500 X G for 15 min. The supernatant was again discarded and spores were suspended in phosphate buffer and stored at 4°C until use.

Determination of Spore Population

Tubes containing the spore suspension were heat shocked at 80°C for 15 min. After cooling on ice the suspension was spiral plated on Dextrose Tryptone Agar (85) and incubated at 32°C for 24 hr.

Heat Treatments

The spore suspension (1 ml) was diluted into 99 ml of M/15 phosphate buffer (pH 7.2). Five milliliters of this suspension was dispensed into sterile screw-capped tubes, in duplicate. Tubes were packed on ice prior to heat treatments. The tubes were heated at 80°C, 90°C and 100°C for 0, 10, 20, 30 minutes. After heating, the spore suspensions were cooled on ice. Double-strength Trypticase Soy Broth (BBL, Cockeysville, MD) was added (5 ml) to the suspensions and tubes were incubated at 32°C for 48 hours.

8. HEAT RESISTANCE OF NON-SPOREFORMING BACTERIA

Preparation of Bacterial Suspension

Selected isolates were grown in Brain Heart Infusion (BHI) broth (BBL) at 32°C for 48 hr. After incubation, tubes were vortexed and 0.1 ml was spread plated on BHI agar (BBL). The plates were incubated at 32°C for 24 hours. The bacteria were harvested by flooding the plate 3 times with M/15 phosphate buffer (pH 7.2) and gently lifting the bacteria off the surface of the agar with a sterile glass rod. The mixture was centrifuged at 7600 X G for 15 min at 4°C. The cells were washed twice with 30 ml of M/15 phosphate buffer (pH 7.2) and centrifuged at 1900 X G for 15 min. Finally, the cells were suspended in 100 ml phosphate buffer and stored at 4°C.

Heat Treatments

The cell suspension (1.0 ml) was diluted in 99 ml of phosphate buffer and dispensed into tubes. Cells were heated at 55°C and 65°C for 10, 20, 30 minutes. Sample tubes were done in duplicate. After heating, tubes were cooled on ice and 5 ml of double strength BHI broth was added before incubation at 32°C. After 48 hr, tubes were examined for growth. Cell suspensions at zero time were used in order to determine initial population.

9. GROWTH STUDIES OF SELECTED ISOLATES

Selected isolates were inoculated into sterile skim milk and incubated at 7.2°C to monitor their growth characteristics. Two control organisms, Pseudomonas fluorescens and Pseudomonas fragi were used as indices of growth. Litmus milk was rehydrated with distilled water, dispensed into test tubes and sterilized at 114°C for 20 min. Skim milk was rehydrated by dissolving 100 grams of nonfat dry milk into 1 liter of distilled water (10%). The solution was heated to near 100°C, dispensed into dilution bottles and autoclaved at 114°C for 20 minutes. Isolates were first inoculated into a tube of litmus milk and incubated at 30°C for 24 hr. Then 1 ml of litmus milk was inoculated into 99 ml of sterile skim milk and incubated at 7.2°C. Standard plate counts were done on day 0, 3, 6, 9, 12, and 15. The plates were incubated at 30°C and counted within 48 ±hr. On day 15, the titratable acidity was also determined.

10. STATISTICAL ANALYSIS

All comparative data was analyzed using Analysis of Variance (ANOVA). If significant differences ($p<0.05$) were detected by ANOVA, the means were separated using Duncan's mean separation.

CHAPTER IV

RESULTS AND DISCUSSION

1. DESIGNATION OF GOOD AND POOR PRODUCERS

Producers were categorized as having good and poor quality sanitation by two experts based upon several criteria. Good quality producers (1,2 and 3) had historically lower standard plate, coliform and somatic cell counts. They also had good udder washing programs including individual towels and use of teat dip immediately after milking. In addition, the producers had adequate hot water for cleaning equipment, a good equipment and maintenance program and a good housing program. Overall, these three herds were very well managed.

In contrast, producers 4,5 and 6 were designated as being poor sanitation producers primarily because of a history of high microbiological counts. Possible factors contributing to those high counts included inadequate hot water supplies for proper bulk tank and equipment cleaning, poor housing maintenance programs and several free standing muddy areas. Moreover, all three producers had poor udder washing programs with little or no teat dip used, poor culling programs (keeping older cattle with chronic mastitis problems and broken down udders). In general, these three producers had poorly managed herds and insufficient facilities and/or resources to produce a quality product.

2. LAB PASTEURIZATION AND STORAGE OF MILK SAMPLES FROM GOOD AND POOR PRODUCERS

Raw milk from the six producers was sampled at the raw, freshly pasteurized and stored stages. The samples were evaluated by standard plate, coliform, and psychrotrophic counts, titratable acidity and pH. On the last day of the storage test, if the sample had not spoiled, it was subjected to those same tests and termed "end" sample. It should be noted that trial 1 extended through 35 day of storage and trial 2 extended through 21 days.

Microbiological Analysis of Raw Milk

There were no significant differences ($P>0.05$) among the raw milk samples for SPC, coliform, crystal violet tetrazolium (CVT) (gram negative bacteria), preliminary incubation, or proteolytic thermoduric counts due to individual producers or quality groups. However, there was a significant difference ($P<0.05$) in the counts from trial 1 and 2. This could have been due to biological variations including herd health and seasonal variation influencing the numbers and types of bacteria present in the milk from each trial. The standard plate counts (SPC) for raw milk samples of good and poor producers ranged from log 4.05 to 5.50 CFU/ml (Tables 1 and 2). The mean SPC for each quality group was log 5.01 for the good producers and log 5.19 CFU/ml for poor producers (Table 3).

Table 1. Microbiological analyses of milk samples collected from producers rated as having good sanitation practices.[a]

ANALYSES	PRODUCER 1	PRODUCER 2	PRODUCER 3[b]
	--------$\log_{10}$ CFU/ml --------		
Standard Plate Count			
Fresh (Raw)	4.05	5.49	5.50
Pasteurized	1.85	4.40	3.72
7 day	2.94	4.45	3.72
10 day	1.89	4.59	3.78
14 day	1.99	4.24	3.55
17 day	1.87	4.66	3.61
21 day	1.93	5.30	5.07
Coliform Count			
Fresh (Raw)	2.22	2.28	4.89
Pasteurized	<1.43	<1.43	<1.43
Final	<1.43	<1.43	<1.43
Psychrotrophic Count			
Fresh (Raw)	2.84	4.64	5.10
Pasteurized	<1.43	2.43	<1.43
Final	3.36	3.90	1.43
Preliminary Incubation	4.13	5.93	6.52
Proteolytic Thermoduric	1.67	1.43	1.43

[a]Milk samples were vat pasteurized and stored at 7.2°C.

[b]Milk samples from Producer 3 spoiled at 28 days in trial 1 but not by 21 days in trial 2.

Table 2. Microbiological analyses of milk samples collected from producers rated as having poor sanitation practices.[a]

ANALYSES	PRODUCER 4[b]	PRODUCER 5[c]	PRODUCER 6[d]
	--------$\log_{10}$ CFU/ml----------		
Standard Plate Count			
Fresh (Raw)	5.10	5.26	5.22
Pasteurized	2.03	2.30	2.18
7 day	2.74	1.97	3.84
10 day	2.28	3.21	5.26
14 day	4.83	3.60	4.98
17 day	5.54	4.19	4.46
21 day	6.37	5.56	3.80
Coliform Count			
Fresh (Raw)	2.47	4.15	3.36
Pasteurized	<1.43	<1.43	<1.43
Final	<1.43	<1.43	<1.43
Psychrotrophic Count			
Fresh (Raw)	4.18	4.77	4.16
Pasteurized	<1.43	<1.43	<1.43
Final	3.16	3.54	1.43
Preliminary Incubation	5.05	5.72	5.72
Proteolytic Thermoduric	1.82	1.43	2.03

[a]Milk samples were vat pasteurized and stored at 7.2°C.

[b]Milk from Producer 4 spoiled at 21 days in trial 1, but not by 21 days in trial 2.

[c]Milk from Producer 5 spoiled at 28 days in trial 1, but not by 21 days in trial 2.

[d]Milk from Producer 6 spoiled at 28 days in trial 1, but not by 21 days in trial 2.

Table 3. A comparison of microbiological analyses of raw milk samples from different quality milk producers.

Analyses	Qual #1[a]	Qual. #2[b]
	---------$\log_{10}$ CFU/ml----------	
SPC	5.01	5.19
Coliform	3.13	3.33
CVT	4.19	4.29
PI	5.52	5.50
PT	1.51	1.76

[a]Good sanitation producers

[b]Poor sanitation producers

The mean coliform count on raw milk for all producers was log 3.23 CFU/ml (Table 1 and 2). Counts were highest for producers 3 and 5. In general, good producers had slightly lower coliform counts than poor producers. One reason for high coliform counts could be that producers have poor udder sanitation programs and are not adequately removing these microorganisms from the udder.

The mean CVT count was log 4.24 CFU/ml for all producers. Psychrotrophic bacteria were in greatest number in the raw milk of producer 3. Mean log counts for Preliminary Incubation (PI) and Proteolytic Thermoduric (PT) were 5.51 and 1.64 respectively (Table 3). Preliminary Incubation counts appeared to have little use in prediction of the shelflife of the raw milk. The PT counts were slightly lower in the good producer group than the poor producers.

Other Analyses of Raw Milk

There was little variation in pH among the individual producers with the means being 6.53 and 6.55 for good and poor quality groups, respectively (Table 4). These values were within the pH range for normal raw milk of 6.5 to 6.6 (30,81).

All milk samples had titratable acidity (TA) values within the normal range of 0.1 - 0.26% lactic acid (30,81) for raw milk (Table 4). There was no significant difference ($P<0.05$) in TA among individual producers but there was a

Table 4. A comparison of other analyses from different quality milk producers.

Analyses	Qual. #1[a]	Qual. #2[b]
pH	6.53	6.55
TA	.19[c]	.17[d]
ADV	1.05	1.28
SCC	5.84	6.05
Babcock	4.17[c]	3.40[d]

[a]Good sanitation producers

[b]Poor sanitation producers

[c,d]Significant difference ($P<0.05$) between quality group #1 and #2.

significant difference between the good and poor quality groups (Table 4). This could be related to the solids not fat (SNF) concentration which was higher for producers 1 and 2.

Acid degree value (ADV) is a measure of the lipolysis of milk fat (4). Normal raw milk should have an ADV in the range of 0.25 to 0.40 (4). Using these values as a guideline, producers 3 and 5 (Table 5 and 6) had the most acceptable values of 0.85 and 0.64. Remaining producers had values ranging from 1.10 through 1.68. Raw milk having an ADV of 1.2-1.5 is slightly lipolyzed and ADV >1.5 is unsatisfactory (4). One possible cause for the high values could have been the mishandling of the raw milk resulting in activation of the enzyme lipase.

For somatic cell count, there was no significant difference ($P<0.05$) among individual producers, trials or quality groups. The mean log somatic cell counts were 5.84 for good quality and 6.05 for poor quality producers (Table 4).

There was a significant difference ($P<0.05$) in raw milk fat concentration among the producers as measured by the Babcock test (Table 4). Using Duncan's mean separation test, it was found that producers 1 and 2 had significantly higher percent butterfat than the other producers. This can be explained by the fact that these producers had herds of Jersey cattle which generally produce higher butterfat than

Table 5. Other analyses of milk from producers rated as having good sanitation practices.[a]

ANALYSES	PRODUCER 1	PRODUCER 2	PRODUCER 3[b]
pH			
Fresh (Raw)	6.5	6.6	6.6
Pasteurized	6.5	6.6	6.6
Final	6.6	6.6	6.5
Titratable Acidity (TA)			
Fresh (Raw)	.19	.19	.18
Pasteurized	.19	.19	.19
Final	.21	.21	.20
Acid Degree Value			
Raw	1.10	1.20	.85
Final	.81	.98	.88
Somatic Cell Count	6.02	5.74	5.75
Babcock Fat Test	5.40	5.07	4.78

[a]Milk samples were vat pasteurized and stored at 7.2°C.

[b]Milk samples from producer spoiled at 28 days in trial 1 but not by 21 days in trial 2.

Table 6. Other analyses of milk from producers rated as having poor sanitation practices.[a]

ANALYSES	PRODUCER 4[b]	PRODUCER 5[c]	PRODUCER 6[d]
pH			
Fresh (Raw)	6.6	6.6	6.6
Pasteurized	6.5	6.5	6.6
Final	6.4	6.3	6.6
Titratable Acidity (TA)			
Raw	.16	.18	.17
Pasteurized	.17	.18	.18
Final	.19	.21	.20
Acid Degree Value			
Raw	1.52	.64	1.68
Final	1.30	.61	1.31
Somatic Cell Counts	6.20	5.58	6.40
Babcock Fat Test	3.30	3.30	3.55

[a]Milk samples were vat pasteurized and stored at 7.2°C.

[b]Milk from Producer 4 spoiled at 21 days in trial 1, but not by 21 days in trial 2.

[c]Milk from Producer 5 spoiled at 28 days in trial 1, but not by 21 days in trial 2.

[d]Milk from Producer 6 spoiled at 28 days in trial 1, but not by 21 days in trial 2.

other breeds (30,81). In contrast, Holsteins rank lowest in average butterfat among the other dairy breeds with a reported average of 3.40% (30,81). Producers 4 and 5 had herds of Holsteins. Producer 6 had a predominantly Guernsey herd which is intermediate to Jerseys and Holsteins in percentage butterfat (30,81). Producer 3 had a mixed herd of Jersey and Holsteins.

Microbiological Analyses of Pasteurized Milk

Following initial day (0) testing, all samples were stored at 7°C and evaluated at 7, 10, 14, 17 and 21 days. On day 0, the mean standard plate count for quality groups 1 and 2 were log 3.32 and 2.17 CFU/ml respectively (Table 7). Quality group 1 (good producers) had an initally higher mean than quality group 2 (poor producers). There was a significant difference ($P<0.05$) between individual producers with producer 1 having significantly lower counts than the other 5. Quality group 1 had a higher mean than did group 2. These differences could be due to a higher number of gram positive sporeformers present in milk from producers 1, 2 and 3.

There were no coliforms detected in the pasteurized samples for any of the six producers (Table 1 and 2). This result was expected since coliforms are not normally resistant to pasteurization temperatures.

Table 7. Mean standard plate count for 0, 7, 10, 14, 17 and 21 days storage among quality groups #1 and #2.

Group	Day 0	Day 7	Day 10	Day 14	Day 17	Day 21
	----------$\log_{10}$ CFU/ml----------					
Qual. #1[a]	3.32	3.69	3.42	3.26	3.38	4.10
Qual. #2[b]	2.17	2.85	3.58	4.47	4.73	5.24

[a]Good sanitation producers

[b]Poor sanitation producers

Psychrotrophic bacteria were not detectable in pasteurized milk samples from producers 1, 2, 4, 5, and 6 (Table 1 and 2). However, a small number of gram negatives was observed in pasteurized milk samples from producer 2.

It should be noted that all samples had at least one sampling time that had a SPC lower than that of the previous time. A possible reason for this could be that a new bottle of each producers pasteurized milk was taken for analysis each time, thus creating some variation in counts. While, the exact reason for this is not fully understood, it would indicate that milk is not a completely homogenous system.

On day 7, quality group 1 still had a higher mean SPC than quality 2. However, mean SPC counts from group 2 were higher than group 1 for days 10, 14, 17 and 21 (Table 7). Overall, there was a large increase in the counts with the poor quality group increasing more over the 21 day storage time than the good quality group.

Other Analysis of Pasteurized Milk

Sample pH either remained the same or dropped slightly among producers during storage. Titratable acidity changes were similar to those found with pH. TA increased slightly after pasteurization.

Microbiological Analysis of Final Stored Samples

As reported earlier, trial 1 extended through 35 days and trial 2 only through 21 days. Therefore, comparisons between trials can only be through 21 days storage. One of

the justifications for terminating replicate 2 at 21 days was that code dates for most fluid milk products would never exceed 21 days at 7°C. Therefore, information beyond that point would not be applicable to the current industry situations.

Milk from producers 1 and 2 did not spoil through 35 days of storage at 7°C. Producer 5 milk spoiled at 21 days in trial 1. Producers 3, 4 and 6 spoiled at 28 days storage. However, during trial 2, no spoilage was found in any of the samples stored 21 days.

Other Analysis of Final Stored Samples

The pH remained the same or dropped slightly as in the case of the freshly pasteurized samples. All decreases in pH were most likely due to the growth of microorganisms in the milk and their production of acid as a metabolic waste product. Titratable acidity was also greater than that found in the freshly pasteurized product again indicating the growth of microorganisms. Acid degree value decreased in all milk samples except that from producer 3.

3. CHARACTERIZATION OF SELECTED ISOLATES

Ten bacterial isolates each were randomly selected from raw, pasteurized and final sample plates using a Harrison's disc (40). Because of the large number of isolates that would be obtained from all six producers, producers 1 and 5

were randomly chosen as representative producers of the good and poor quality groups.

The predominant bacterial types in raw milk samples of the good producer were gram positive rods and cocci (Table 8). Some gram negative rods and coccobacilli were also present.

Gram positive sporeforming rods predominated in pasteurized milk samples from producer 1 (Table 9). The survival of gram positive sporeforming rods during low temperature, long time (LTLT) pasteurization has been well documented (24,41,71). Isolate 21 was the only pasteurized isolate obtained from trial 1 (Table 9). Isolates 22-31 were selected from trial 2. As reported earlier, good sanitation producers had higher pasteurized plate counts than did poor producers. These higher counts may have been due to a high percentage of gram positive sporeformers such as that found with producer 1.

Isolates 32-41 were obtained during trial 1 and 42-47 during trial 2 (Table 10). The milk sample from this producer (No. 1) had not spoiled after 35 days storage at 7^{o}C in trial 1. Isolates present on the final day of storage were gram negative and gram positive rods. Again in trial 2, the milk had not spoiled by day 21. The isolates present were predominately gram positive rods.

Isolates 51-71 were taken from the raw milk plates of the poor producer (No. 5) (Table 11). Gram positive rods

Table 8. Characteristics of selected bacteria isolated from raw milk of good producers.

ISOLATE CODE	GRAM STAIN/MORPHOLOGY
#1	G+ cocci
#2	G+ cocci
#3	G+ rods
#4	G+ rods
#5	G+ rods
#6	G+ cocci
#7	G- rods
#8	G+ rods
#9	G+ rods
#10	G+ rods
#11	G+ rods
#12	G- rods
#13	G- rods
#14	G+ cocci
#15	G+ rods
#16	G+ coccobacilli
#17	G+ coccobacilli
#18	G+ coccobacilli
#19	G+ cocci
#20	G+ cocci

Table 9. Characteristics of selected bacteria isolated from pasteurized milk of good producers.

ISOLATE CODE	GRAM STAIN/MORPHOLOGY
#21	G+ rods, spores
#22	G+ rods, spores
#23	G+ rods, spores
#24	G+ rods, spores
#25	G+ rods, spores
#26	G+ rods, spores
#27	G+ rods, spores
#28	G+ rods, spores
#29	G+ rods, spores
#30	G+ rods, spores
#31	G+ rods, spores

Table 10. Characteristics of selected bacteria isolated from stored milk of good producers.

ISOLATE CODE	ORIGIN	GRAM STAIN/MORPHOLOGY
#32	35 day plate	G- rods
#33	35 day plate	G+ rods, spores
#34	35 day plate	G- rods
#35	35 day plate	G- rods
#36	35 day plate	G- rods
#37	35 day plate	G+ cocci
#39	35 day plate	G- rods
#40	35 day plate	G- rods
#41	35 day plate	G- rods
#42	21 day plate	G+ rods
#44	21 day plate	G+ rods
#45	21 day plate	G+ rods
#46	21 day plate	G+ rods
#47	21 day plate	G+ rods

Table 11. Characteristics of selected bacteria isolated from raw milk of poor producers.

ISOLATE	GRAM STAIN/MORPHOLOGY
#51	G- Rods
#52	G+ rods
#53	G- rods
#54	G+ rods
#55	G+ rods
#56	G- rods
#57	G+ rods
#58	G+ rods
#59	G- rods
#60	G- rods
#61	G+ rods
#62	G+ rods
#63	G+ rods
#64	G- rods
#67	G+ rods
#68	G- rods
#69	G- rods
#70	G+ coccobacilli
#71	G+ coccobacilli

and gram negative rods were common types found.

In trial 1, only two isolates were obtained from pasteurized milk samples of the poor producer, 72-73 (Table 12). One isolate was a gram positive sporeforming rod and the other a gram negative rod. There were no isolates to select from in trial 2 because on day 0 of sampling no colonies were present on the plates.

Isolates 74-83 were obtained from 28 day spoiled samples in trial 1 (Table 13). Two thirds of the isolates were gram positive rods and the other one third were gram negative rods. In trial 2, isolates 84-93 were obtained from plated samples that had not spoiled by 21 days.

4. DETERMINATION OF HEAT RESISTANCE

Heating studies were conducted on isolates selected from pasteurized and final samples (spoiled or end) of producers No. 1 and 5. Heating parameters were 80, 90 and 100^{o}C for 0, 10, 20 and 30 minutes for gram positive sporeformers. Gram negative non-sporeforming rods were heated at 55 and 65^{o}C for 0, 10, 20 and 30 min.

Gram Positive Sporeforming Rods

With the exception of isolate 28, all isolates produced countable colonies on Dextrose Tryptone Agar (DTA) after heating at 80^{o}C for 30 min (Table 14). Changes in number of viable spores after heating at 80^{o}C, from initial counts of the good producer averaged log 1.10 CFU/ml (Table 15). This

Table 12. Characteristics of selected bacteria isolated from pasteurized milk of poor producers.

ISOLATE CODE	GRAM STAIN/MORPHOLOGY
#72	G- rods
#73	G+ rods, spores

Table 13. Characteristics of selected bacteria isolated from stored milk of poor producers.

ISOLATE CODE	ORIGIN	GRAM STAIN/MORPHOLOGY
#74[a]	28 day plate	G+ rods
#75[a]	28 day plate	G+ rods
#76[a]	28 day plate	G+ rods
#78[a]	28 day plate	G+ rods
#79[a]	28 day plate	G- rods
#80[a]	28 day plate	G- rods
#81[a]	28 day plate	G- rods
#82[a]	28 day plate	G+ rods
#83[a]	28 day plate	G+ rods
#84	21 day stored	G+ rods, spores
#85	21 day stored	G+ rods, spores
#86	21 day stored	G+ rods, spores
#87	21 day stored	G+ rods, spores
#88	21 day stored	G+ rods, spores
#89	21 day stored	G+ rods, spores
#90	21 day stored	G+ rods, spores
#91	21 day stored	G+ rods, spores
#92	21 day stored	G+ rods, spores
#93	21 day stored	G+ rods, spores

[a]Sample spoiled at 28 days storage at 45°F.

Table 14. Initial and final log CFU/ml of gram positive sporeforming organisms isolated from pasteurized milk stored for 21 days at 7.2°C. Spore suspensions were heated at 80°C, 90°C and 100°C for 30 minutes.

Isolate	Initial	80°C-30 min.	90°C-30 min.	100°C-30 min.
		$\log_{10}$ CFU/ml		
#22	8.51	8.23	4.77	<1.43
#24	9.38	9.01	4.95	<1.43
#26	9.26	8.05	4.33	<1.43
#27	3.82	3.83	<3.43	<3.43
#28	4.27	<3.43	<3.43	<3.43
#29	5.50	4.70	<3.43	<3.43
#30	4.80	4.17	<3.43	<3.43
#33	9.37	9.14	5.95	<3.43
#42	6.16	5.20	<3.43	<3.43
#44	7.08	6.30	3.43	<3.43
#45	7.09	6.76	3.73	<3.43
#46	9.45	9.04	5.48	<1.43
#47	6.47	5.69	4.71	<3.43
#84	7.37	6.35	<1.43	<1.43
#88	7.03	5.39	3.43	<1.43
#92	6.92	5.05	3.43	<1.43
#93	6.88	3.67	<1.43	<1.43

Table 15. Decrease in log number of viable spores after heating at 80°C, 90°C and 100°C for 30 minutes.

Isolate	80°C	90°C	100°C
	-----$\log_{10}$ CFU/ml-----		
#22	0.28	3.74	>8.51
#24	0.37	4.43	>9.38
#26	1.21	4.93	>9.26
#27	NC[a]	>3.82	>3.82
#28	4.27	>4.27	>4.27
#29	0.80	>5.50	>5.50
#30	0.63	>4.80	>4.80
#33	0.23	3.42	>9.37
#42	0.96	>6.16	>6.16
#44	0.78	3.65	>7.08
#45	0.33	3.36	>7.09
#46	0.41	3.97	>9.45
#47	0.78	1.76	>6.47
#84	1.02	>7.37	>7.37
#88	1.64	3.60	>7.03
#92	1.87	3.49	>6.92
#93	3.21	>6.88	>6.88
Mean[b]	1.10	>4.42	>7.02

[a] No change

[b] Mean for 17 isolates

value includes a value of log 4.27 and 3.21 CFU/ml for isolates 28 and 93. If these values were excluded the mean would be log 0.66 CFU/ml.

Of the isolates heated at 90°C for 30 minutes, only 8 of the 13 isolates (22, 24, 26, 33, 44, 47, 88, 93) produced countable colonies on DTA after 48 hr incubation at 32°C (Table 14). Mean change in log count (including using values listed as ">") was log >4.42 CFU/ml (Table 15).

At 100°C for 30 min, there were no countable colonies obtained after incubation for any of the gram positive isolates (Table 14). Therefore, it could be concluded that these isolates were not particularly heat resistant but could survive pasteurization if present in the raw milk sample.

Recovery from duplicate tubes was determined by checking for turbidity of the broth suspension, which would be indicative of growth (Table 16). In general, growth in tubes was found to substantiate results from final plate counts. In some cases however, differences occurred. At 90°C, isolate 27 displayed growth at 0 time (control) but not after 30 min (Table 14). This agreed with duplicate tube results at 90°C for 30 minutes (Table 16). Isolates 29, 30 exhibited no growth in the tubes heated 10 or 20 min and growth in one tube at 30 min (Table 16). On final plate counts for these isolates, no colonies were observed (Table 14). Isolates 28, 42, 84, 93 all displayed growth in tubes at various times but no colonies on final DTA plates were

Table 16. Recovery of gram positive sporeforming organisms heated in screw-capped tubes at 80°C, 90°C and 100°C for 0, 10, 20 and 30 minutes.

Isolate Code	80°C				90°C				100°C			
	0	10	20	30	0	10	20	30	0	10	20	30
#22	+	+	+	+	+	+	+	+	+	-	-	-
#24	+	+	+	+	+	+	+	+	+	-	-	-
#26	+	+	+	+	+	+	+	+	+	-	-	-
#27	+	+	+	+	+	-	-	-	+	-	-	-
#28	+	+	+	+	+	+	+	+	+	-	-	-
#29	+	+	+	+	+	-	-	±	+	-	-	-
#30	+	+	+	+	+	-	-	±	+	-	-	-
#33	+	+	+	+	+	+	+	±	+	-	±	-
#42	+	+	+	+	+	+	+	+	+	-	-	-
#44	+	+	+	+	+	+	+	+	+	-	-	-
#45	+	+	+	+	+	+	+	+	+	-	-	-
#46	+	+	+	+	+	+	+	+	+	±	+	-
#47	+	+	+	+	+	+	+	+	+	-	-	-
#84	+	+	+	+	+	+	+	+	+	-	-	-
#88	+	+	+	+	+	+	+	+	+	+	-	-
#92	+	+	+	+	+	+	+	+	+	±	-	-
#93	+	+	+	+	+	+	+	+	+	±	-	-

\+ growth in both tubes

\- no growth in either tube

± one tube positive, one negative

found. At 100°C, growth was observed in heating tubes at 10 min for isolates 46, 88, 92 and 93. Growth was evident in duplicate tubes for 33 and 46 at 20 min. Any small discrepencies could be explained by the fact that the lower limit of detection of the spiral plater was 28 CFU/ml while only one spore could produce a positive reaction in the culture tubes.

Gram Negative Non-Sporeforming Rods

Initial populations of gram negative non-sporeforming rods ranged from log 9.29 to 10.34 CFU/ml (Table 17). After heating at 55°C for 30 min, the mean decrease in viable cells for the six isolates was log 3.29 CFU/ml (Table 18) . All six cultures had survivors after 30 min of heating. Moreover, growth was observed in duplicate recovery tubes (Table 19) confirming this result.

At 65°C growth was again evident for the six isolates on Brain Heart Infusion (BHI) plates with an average decrease in viable cells of log 3.30 CFU/ml (Table 18). Although a greater kill was expected at 65°C than at 55°C, essentially there was no difference. One reason for this could be clumping of bacteria at 55°C and subsequent breakup of those clumps at 65°C, giving higher apparent numbers at 65°C.

Pseudomonas fragi, which was used as an index microorganism, showed growth in duplicate tubes and on plates after heating at 65°C for 30 min. Interestingly, 72

Table 17. Initial and final log CFU/ml of gram negative non-sporeforming organisms isolated from milk heated at 55°C and 65°C for 30 minutes.

Initial Count	55°C-30 min.	65°C-30 min.
	log10 CFU/ml	
#32	9.55	5.28	5.94
#35	9.38	5.26	6.11
#36	9.34	5.52	6.00
#39	9.39	7.53	7.60
#40	9.38	7.51	6.33
#41	9.29	7.45	7.33
P. fragi	10.34	4.60	2.84
P. fluor.	9.28	<3.43	<1.43

Table 18. Decrease in log number of viable cells after heating at 55°C and 65°C for 30 minutes.

Isolate	55°C	65°C
	--$\log_{10}$ CFU/ml--	
#1	3.52	3.28
#4	3.74	3.74
#5	4.19	3.27
#8	3.10	3.02
#9	2.42	3.50
#10	2.74	2.98
Mean[a]	3.29	3.30
P. fragi	4.77	7.14
P. flour.	>6.91	>8.75
Mean[b]	>5.84	>7.95

[a]Mean for six isolates

[b]Mean for indices

Table 19. Recovery of gram negative non-sporeforming organisms heated in screw capped tubes at 55°C and 65°C for 0, 10, 20 and 30 minutes.

Isolate Code	55°C				65°C			
	0	10	20	30	0	10	20	30
#32	+	+	+	+	+	+	+	+
#35	+	+	+	+	+	+	+	+
#36	+	+	+	+	+	+	+	+
#39	+	+	+	+	+	+	+	+
#40	+	+	+	+	+	+	+	+
#41	+	+	+	+	+	+	+	+
P. fragi	+	+	+	+	+	+	+	+
P. fluor.	+	+	+	+	+	±	-	±

\+ both were positive for growth

\- both were negative for growth

± one positive, one negative tube

hr were required before P. fragi colonies became countable on BHI Agar when heated at 65oC for 30 min indicating possible heat injury. Despite the fact that there were survivors of P. fragi after heating for 30 min at 65°C, it was relatively heat sensitive. Under normal conditions, if initial populations were as high as those used in the heat study, the raw milk would be unacceptable before pasteurization. Therefore, for all practical purposes, P. fragi would not survive pasteurization.

Pseudomonas fluorescens exhibited growth in one tube heated at 65°C for 10 min and one 30 min (Table 19) but did not form countable colonies on 30 min BHI plates (Table 17). Two possible reasons for this discrepency could have been: 1) one cell in the tube could have recovered or been protected by other cells and began to grow, and 2) if no more than 28 cells recovered from the heating, they would not have been detected by the spiral plate procedure.

The indices had a mean decrease of greater than or equal to log 5.84 CFU/ml viable cells at 55°C (Table 18). For 65°C, the mean decrease in viable cells was greater than or equal to log 7.95 CFU/ml. The most important conclusion to be drawn from the heat study was that these six isolates appeared to be more heat resistant than P. fragi or P. fluorescens and could possible survive pasteurization depending upon the initial number present.

5. GROWTH STUDIES OF SELECTED ISOLATES

Growth studies of selected isolates indicated large differences in behavior of gram positive sporeforming rods and gram negative non-sporeforming rods (Table 20 and 21). In general, gram negative isolates from milk multiplied relatively rapidly over time (Table 21) and equalled ($P>0.05$) the growth of the indices, P. fluorescens and P. fragi. The mean log count of the gram negative isolates over the 15 day incubation period was 7.17 log CFU/ml. The indices P. fragi and P. fluorescens had means of log 7.64 and 7.63 CFU/ml, respectively over the same period. Conversely, the gram positive sporeformers grew much slower than the gram negative isolates and indices ($P<0.05$) and in two cases (No. 28 and 42) actually decreased in numbers over time (Table 20). This group had an mean of log 5.54 CFU/ml over the storage time of 15 days. Individual gram positive sporeforming isolates 74, 75, 76, 78, 82, 83, 84, 85, 86 and 92 increased in numbers slowly ranging from an log 0.98 to 2.07 CFU/ml over 15 days storage at 7.2°C.

There were few reactions observed on litmus milk. Isolates 74, 75, 76, 78, 82 and 83 caused a reduction reaction in the medium. These were the only isolates causing reactions. Effect on the TA by the isolates was varied (Appendix). The greatest changes occurred with isolates 74, 75, 76, 78, 82, 83, 84 and 85. These isolates caused a mean increase in TA of .25 % lactic acid.

Table 20. Growth of gram positive sporeforming rods in sterile reconstituted skim milk stored at 7°C.

Iso.#	0 day	3 day	6 day	9 day	12 day	15 day	Δt[a]
	----------	----------	----------	$\log_{10}$ CFU/ml	----------	----------	
#21	5.47	5.39	5.42	5.44	5.54	5.38	-.09
#22	5.80	5.45	5.34	5.39	5.36	5.33	-.47
#23	5.61	4.87	4.86	4.78	4.77	4.65	-.96
#24	5.64	5.39	5.43	5.48	5.40	5.46	-.18
#25	5.74	5.42	5.39	5.40	5.37	5.40	-.34
#26	5.50	5.22	5.15	5.13	5.08	5.21	-.29
#27	5.42	5.10	5.00	5.04	4.98	5.02	-.40
#28	5.83	5.13	5.19	4.84	4.66	4.69	-1.14
#29	4.48	5.2o	5.21	5.27	5.31	5.26	+.78
#30	5.51	5.43	5.34	5.41	5.39	5.36	-.15
#31	6.10	5.86	5.62	5.24	5.28	5.42	-.68
#33	5.44	5.48	5.51	5.54	5.55	5.53	+.09
#42	5.44	5.02	4.34	4.46	4.63	4.22	-1.22
#44	4.81	4.92	4.62	4.57	4.58	4.67	-.14
#45	5.81	5.67	5.63	5.57	5.76	5.61	-.20
#46	5.88	5.57	5.51	5.49	5.46	5.46	-.42
#47	5.48	5.25	5.39	5.32	5.28	5.26	-.22
#73	5.43	4.66	4.69	4.62	4.35	4.52	-.91
#74	5.74	6.46	7.29	7.57	7.59	7.56	+1.82
#75	5.72	6.39	7.24	7.52	7.42	7.36	+1.64
#76	5.22	6.22	7.16	7.34	7.29	7.29	+2.07
#78	5.93	6.22	7.09	7.32	7.44	7.71	+1.78
#82	5.78	6.02	6.99	7.51	7.75	7.63	+1.85
#83	5.84	5.79	7.20	7.55	7.61	7.60	+1.76
#84	4.84	4.88	4.96	5.64	5.86	6.38	+1.54
#85	5.10	5.10	5.10	5.20	5.75	6.08	+.98
#86	4.94	4.88	4.94	4.99	5.46	6.00	+1.06
#87	4.84	5.02	5.06	5.00	5.24	5.38	+.54
#88	5.04	5.19	5.26	5.23	5.36	5.66	+.62
#89	4.98	4.96	5.02	5.02	5.34	5.40	+.42
#90	5.05	4.99	4.99	5.06	5.12	5.62	+.57
#91	4.86	4.87	4.92	4.96	5.37	5.40	+.54
#92	5.03	5.07	5.09	5.36	5.76	6.34	+1.31
#93	5.78	5.33	5.31	5.34	5.38	5.76	-.02
Mean	5.64	5.45	5.60	5.72	5.86	5.96	+.46

[a] Δt Change in log CFU/ml from day 0 to day 15

Table 21. Growth of gram negative non-sporeforming rods in sterile reconstituted skim milk stored at 7°c.

Isolate	0 day	3 day	6 day	9 day	12 day	15 day	Δt[a]
	----------$\log_{10}$ CFU/ml----------						
#32	5.72	5.93	7.92	8.16	8.43	8.61	+2.89
#35	6.03	7.10	7.68	8.06	8.39	8.42	+2.39
#36	5.55	6.89	7.56	7.72	8.10	8.17	+2.62
#39	5.73	6.22	6.82	7.75	8.02	8.10	+2.37
#40	6.18	5.67	7.10	7.60	7.98	7.94	+1.76
#41	5.70	6.78	7.49	7.75	8.26	8.98	+3.28
Mean	6.20	6.79	7.37	7.62	8.17	8.08	+2.55
P. fragi	6.10	7.19	7.60	8.12	8.32	8.51	+2.41
P. fluor.	5.98	7.17	7.48	8.06	8.50	8.60	+2.62
Index Mean	6.37	7.52	7.76	8.09	8.67	8.89	+2.52

[a] Δt Change in log CFU/ml from day 0 to day 15.

In summary, the gram negative isolates seemed to follow the same growth patterns as those of the indices (Table 22). These six isolates grew very well increasing an average log 2.55 CFU/ml over the 15 days. The indices had a mean increase of log 2.52 CFU/ml for the same period.

Table 22. Summary of behavior of bacterial types during refrigerated storage at 7°C.

Type	Day 0	Day 3	Day 6	Day 9	Day 12	Day15
	----------$\log_{10}$ CFU/ml----------					
G+ rods	5.64	5.45	5.61	5.72	5.86	5.96
G- rods	6.20	6.79	7.37	7.62	8.17	8.08
Indices	6.37	7.52	7.76	8.09	8.67	8.89

CHAPTER V

CONCLUSIONS

The first objective of this study was to determine if pasteurized milk received from producers with poor sanitation practices would spoil sooner during refrigerated storage than milk from producers with good sanitation. Results showed that there was no significant differences between individual producers in each group or between the groups collectively as far as the microbiological counts were concerned. It might be concluded that if more attention to the finer points of udder and equipment sanitation had been used for original classification, the results may have been more conclusive. Since there were no definite correlations, it must be asked "what is good sanitation?" It is important to note that several factors can contribute to bacterial loads on the raw milk product. For example, if one of the good producers was running short of time the morning his milk was sampled for this evaluation, he may not have devoted enough time to proper sanitation before he began. This could have lead to high counts on that particular day and an error in the classification scheme used.

It must be realized that in order to completely understand the conditions in which the milk was produced,

more than just a general characterization of the producers sanitation and equipment cleaning, udder washing, herd health and housing conditions must be made. Special attention must be given to specific points such as the producer's equipment cleaning process (i.e. how efficient was it in properly avoiding build-up of bacteria?), udder washing (is one technique more sanitary than the other?), and the realization that a "good" sanitation producer can have a "bad" day and vice versa. In order to accurately assess the sanitation practices, evaluations of the producers sanitation procedures must be done at the time of sampling, not the night before or the morning of sampling. Otherwise the effect of deviations from the normal procedure each producer uses will never be known.

The second objective of this study was to characterize the bacterial types linked to spoilage of the stored pasteurized product. The predominate types found were gram positive sporeformers and gram negative non-sporeforming rods. The heat resistance of these isolates was then determined. The gram positive sporeforming bacteria survived the heating treatments at 80 and 90^{o}C. While they were not highly heat resistant, they were able to survive pasteurization. The gram negative bacteria were resistant enough to survive pasteurization if the initial numbers were high enough. The gram positive sporeforming bacteria did not grow well at refrigeration temperatures indicating that

they generally would not decrease the shelf life of the milk. In contrast, the gram negative bacteria proliferated well under refrigerated storage conditions indicating that they could cause potential shelf life problems.

LITERATURE CITED

LITERATURE CITED

1. Adams, D.M., J.T. Barach, and M.L. Speck. 1975. Heat resistant proteases produced in milk by psychrotrophic bacteria of dairy origin. J. Dairy Sci. 58:828-834.

2. Adams, D.M., J.T. Barach, and M.L. Speck. 1976. Effect of psychrotrophic bacteria from raw milk on milk proteins and stability of milk proteins to ultrahigh temperature treatment. J. Dairy Sci. 59:823-827.

3. Aggarwal, M.L. 1974. Commercial sterilization and aseptic packaging of milk products. J. Milk Food Technol. 37:250-254.

4. American Public Health Association. 1985. Standard Methods for the Examination of Dairy Products. 15th ed. American Public Health Association, Washington, D.C.

5. Andrey, J. and W.C. Frazier. 1959. Psychrophiles in milk held two days in farm bulk cooling tanks. J. Dairy Sci. 42:1781-1784.

6. Ayres, J.C., J.O. Mundt and W.E. Sandine. 1980. Microbiology of Foods. W.H. Freeman and Company, San Francisco, CA.

7. Baker, S.K. 1983. The keeping quality of refrigerated pasteurized milk. Aust. J. Dairy Tech. 38(9):124-127.

8. Banwart, G.J. 1981. Basic Food Microbiology. Chapter 4. Abridged Textbook Edition. AVI Publishing. Westport, CT.

9. Barach, J.T., D.M. Adams and M.L. Speck. 1976. Low temperature inactivation in milk of heat-resistant proteases from psychrotrophic bacteria. J. Dairy Sci. 59:391-395.

10. Barnard, S.E. 1981. Getting good preliminary incubation counts. Dairy Food San. 1:248-249.

11. Bath, D.L., F.N. Dickinson, H.A. Tucher and R.D. Appleman. 1978. Dairy Cattle: Principles, Practices, Problems and Profits. 2nd ed. Lea & Febiger, Philadelphia.

12. Baumann, D.P. and G.W. Reinbold. 1963. The enumeration of psychrophilic microorganisms in dairy products. J. Milk Food Technol. 26:351-356.

13. Bengtsson, K., L. Gardhage, and B. Isaksson. 1973. Gelation in UHT treated milk, whey and casein solution . The effect of heat resistant proteases. Milchwissenschaft. 28:495-499.

14. Berry, J.A. and C.A. Magoon. 1934. Growth of microorganisms at and below 0oC. Phytopathology. 24:780-796.

15. Bhadsavle, C.H., T.E. Shehata and E.B. Collins. 1972. Isolation and identification of psychrophilic species of Clostridium from milk. Appl. Microbiol. 24(5):699-702.

16. Bigalke, D.L. 1984. Sources of psychrotrophic organisms found in raw milk. Dairy Food San. 4(4):152-153.

17. Blankenagal, G. 1976. An examination of methods to assess post- pasteurization contamination. J. Milk Food Technol. 39(4): 301-304.

18. Blankenagal, G. and I. Okello-Uma. 1969. Gram-negative bacteria in raw milk. Can. Inst. Food Tech. J. 2(2):69-71.

19. Catchick, A. and D.L. Gibson. 1967. Sixteen-hour method for detecting post-pasteurization contamination of market milk. Can. Dairy Ice Cream J. 46(6):25-27.

20. Chung, B.H. and R.Y Cannon. 1971. Psychrotrophic sporeforming bacteria in raw milk samples. J. Dairy Sci. 54:448. (Abstract)

21. Cogan, T.M. 1977. A review of heat resistant lipases and proteinases and the quality of dairy products. Ir. J. Fd. Sci. Technol. 1:95-105.

22. Coghill, D. and H.S. Juffs. 1979. Incidence of psychrotrophic sporeforming bacteria in pasteurized milk and cream products and effect of temperature on their growth. Aust. J. Dairy Technol. 3:150-153.

23. Cousin, M.A. 1982. Presence and activity of psychrotrophic microorganisms in milk and dairy products: a review. J. Food Prot. 45:172-207.

24. Credit, C., R. Hedeman, P. Heywood, and D. Westoff. 1972. Identification of bacteria isolated from pasteurized milk following refrigerated storage. J. Milk Food Technol. 35(12):708-709.

25. Dabbah, R., W.A. Moats and J.F. Mattick. 1969. Factors affecting resistance to heat and recovery of heat injured bacteria. J. Dairy Sci. 52:608-613.

26. Deeth, H.C. and C.H. Fitzgerald. 1976. Lipolysis in dairy products: a review. Aust. J. of Dairy Technol. 31(6):53-64.

27. Desai, M.N. and T.J. Claydon. 1964. Preliminary incubation of raw milk samples as an aid in evaluating bacteriological quality. J. Milk Food Technol. 27:333-338.

28. DiLiello, L.R. 1982. Methods in food and dairy microbiology. AVI Publishing Company, Inc. Westport, CT.

29. Dreissman, F.M. and J. Standouders. 1974. Thermal activation and inactivation of exocellular lipases of some gram negative bacteria found in milk. Neth. Milk Dairy J. 28:10.

30. Eckles, C.H., Combs, W.B. and H. Macy. 1943. Milk and Milk Products. 3rd ed. McGraw-Hill Book Company, New York.

31. Eddy, B.P. 1960. The use and meaning of the term "psychrophilic". J. Appl. Bacteriol. 23(2):189-190.

32. Ensminger, M.E. 1971. Dairy Cattle Science. 1st ed. The Interstate Printers and Publ. Inc. Danville, Ill.

33. Freeman, T.R., N.V. Nanavati and W.E. Glenn. 1964. Faster enumeration of psychrophilic bacteria in pasteurized milk--a preliminary report. J. Milk Food Technol. 27:304-307.

34. Gebre-Egziabher, A., A.K. Cheong, G. Blankenagel, and E.S. Humbert. 1985. Extended storage of raw milk. I. Effect on microbiological quality. Can. Inst. Food Sci. Technol. J. 18(3):247-250.

35. Gottemoller, C.A. 1983. Dairy farm practices and their effect on preliminary incubation counts. Dairy Food San. 3(10):376-385.

36. Grosskopf, J.C. and W.J. Harper. 1969. Role of psychrophilic sporeformers in long life milk. J. Dairy Sci. 52:897. (abstract)

37. Grosskopf, J.C. and W.J. Harper. 1974. Isolation and identification of psychrotrophic sporeformers in milk. Milchwissenschaft. 29:467-470.

38. Hankin, L. and W.F. Dillman. 1968. A rapid test to find "potentially" psychrophilic organisms in pasteurized dairy products. J. Milk Food Technol. 31:141-145.

39. Hanna, M.O., J.C. Stewart, Z.L. Carpernter and C. Vanderzant. 1977. Heat resistance of Yersinia entercolitica in skim milk. J. Food Sci. 42:1134,1136.

40. Harrigan, W.F. and M.E. McCance. 1976. Laboratory methods in food and dairy microbiology. Academic Press, New York.

41. Hileman, J.L. 1940. Thermoduric bacteria in pasteurized milk. A review of literature. J. Dairy Sci. 46:1143-1159.

42. Ingraham, J.L. 1958. Growth of psychrophilic bacteria. J. Bacteriol. 76:75-80.

43. Ingraham, J.L. and J. L. Stokes. 1959. Psychrophilic bacteria. Bacteriol. Rev. 23:97-108.

44. Janzen, J.J., J.R. Bishop, A.B. Bodine and C.A. Caldwell. 1982. Shelf-life of pasteurized fluid milk as affected by age of raw milk. J. Dairy Sci. 65:2233-2236.

45. Jay, J.M. 1978. Chapter 12. Modern Food Microbiology. 2nd ed. Van Nostrand Reinbold Company, New York.

46. Jay, J.M. 1978. Chapter 20. Modern Food Microbiology. 2nd ed. Van Nostrand Reinbold Company, New York.

47. Jezeski, J.J. and H. Macy. 1946. Cryophilic organisms in water and butter. J. Dairy Sci. 29:439.

48. Johns, C.K. 1960. Preliminary incubation for raw milk samples. J. Milk Food Technol. 23:137-141.

49. Johns, C.K. 1967. Are your tankers clean and sanitary? Can. Dairy Ice Cream J. 46(7):29-30.

50. Johns, C.K. 1975. Use of counts after preliminary incubation to improve raw milk quality for a Denver plant. J. Milk Food Technol. 38(8):481-482.

51. Johns, C.K. and G.B. Landerkin. 1969. Effects of preliminary incubation on the bacterial flora of bulk-tank milks produced in three different Canadian milk sheds. J. Dairy Sci. 52:1935-1940.

52. Juffs, H.S. 1970. A four-day count for psychrotrophs. Aust. J. Dairy Technol. 25:30-32.

53. Juven, B.J., S. Gordin, I. Rosenthal and A. Laufer. 1981. Changes in refrigerated milk caused by Enterobacteriaceae. J. Dairy Sci. 64:1781-1784.

54. Lampert, L.M. 1975. Modern Dairy Products. 3rd ed. Chemical Publishing Company, Inc. N.Y.

55. Law, B.A. 1979. Reviews of the progress of dairy science: enzymes of psychrotrophic bacteria and their effects on milk and milk products. J. Dairy Res. 46:573-588.

56. Law, B.A., A.T. Andrews and M.E. Sharpe. 1977. Gelation of ultra-high-temperature sterilized milk by proteases from a strain of Pseudomonas fluorescens isolated from raw milk. J. Dairy Res. 44:145-148.

57. Lee, W.H. 1977. An assessment of Yersinia enterocolitica and its presence in foods. J. Food Prot. 40:486-489.

58. Macaulay, D.M., R.Z. Hawirko and N. James. 1963. Effect of pasteurization on survival of certain psychrophilic bacteria. Appl. Microbiol. 11:90-92.

59. Marth, E.H. and W.C. Frazier. 1957. Bacteriology of milk held at farm bulk cooling tank temperatures. III. Psychrophiles found and their growth. J. Milk Food Technol. 20:93-99.

60. Martin, J.H. 1974. Significance of bacterial spores in milk. J. Milk Food Technol. 37:94-98.

61. Maxcy, R.B. 1967. Nature and growth response of the microflora of pasteurized packaged milk. J. Milk Food Technol. 30:213-218.

62. Mayerhofer, H.J., R.T. Marshall, C.H. White and M.Lu. 1973. Characterization of heat stable protease of Pseudomonas fluorescens P26. Appl Microbiol. 25(1):44-48.

63. Mikolajcik, E.M. 1970. Thermodestruction of Bacillus spores in milk. J. Milk Food Technol. 33:61-63.

64. Mikolajcik, E.M. 1978. Psychrotrophic sporeformers. A possible keeping quality problem in market milk. Am. Dairy Rev. 40(4):34A,34D.

65. Mikolajcik, E.M. 1979. Psychrotrophic bacteria and dairy product quality. I. Major organisms involved and defects produced. Cult. Dairy Pro. J. 14(4):6-10.

66. Mikolajcik, E.M. and N.T. Simon. 1978. Heat resistant psychrotrophic bacteria in raw milk and their growth at 7C. J. Food Prot. 41:93-95.

67. Morita, R.Y. 1975. Psychrophilic bacteria. Bacteriol. Rev. 39(2):144-167.

68. Oehlrich, H.K. and R.C. McKellar. 1983. Evaluation of an 18oC/45 hour plate count technique for the enumeration of psychrotrophic bacteria in raw and pasteurized milk. J. Food Prot. 46(6):528-529.

69. Oliveria, J.S. and C.E. Parmelee. 1976. Rapid enumeration of psychrotrophic bacteria in raw and pasteurized milk. J. Milk Food Technol. 39(4):269-272.

70. Olson, H.C. 1961. Selective media for detecting spoilage organisms in dairy products. J. Dairy Sci. 44:970-971. (Abstract)

71. Olson, H.C. 1963. Selective plating technique for detecting contamination in pasteurized milk. J. Dairy Sci. 46:362. (Abstract).

72. Olson, H.C. 1963. Spoilage of milk by thermoduric psychrophiles. J. Dairy Sci. 46:362. (abstract).

73. Olson, J.C. and P.M. Nottingham. 1980. Temperature. In Microbial Ecology of Foods, V.I. International Commission on Microbiological Specifications for Foods. (ed.) Academic Press, New York.

74. Olson, J.C. Jr., R.B. Parker and W.S. Mueller. 1955. The nature, significance and control of psychrophilic bacteria in dairy products. J. Milk Food Technol. 18:200-203.

75. Overcast, W.W. 1968. Psychrophilic microorganisms and keeping quality of milk and its products. J. Dairy Sci. 51(8):1336-1338.

76. Overcast, W.W. and K. Atmaram. 1974. The role of Bacillus cereus in sweet curdling of fluid milk. J. Milk Food Technol. 37:233-236.

77. Patel, G.B. and G. Blankenagel. 1972. Bacterial counts of raw milk and flavor of the milk after pasteurization and storage. J. Milk Food Technol. 35:203-206.

78. Punch, J.D. and J.C. Olson, Jr. 1964. Comparison between standard methods procedure and surface plate method for estimating psychrophilic bacteria in milk. J. Milk Food Technol. 27:43-47.

79. Reinbold, G.W., C.K. Johns and W.S. Clark, Jr. 1969. Modification of the preliminary incubation treatment for raw milk samples. J. Milk Food Technol. 32:42-43.

80. Richter, R. 1979. Psychrotrophic bacteria and shelf-life. Am. Dairy Rev. 41(7):50J.

81. Roadhouse, C.L. and J.L. Henderson. 1950. The Market Milk Industry. 2nd ed. McGraw Hill Book Company, Inc. New York.

82. Ryan, J.J., R.H. Gough and C.H. White. 1984. Preliminary incubation count as an index of raw milk microbiological quality during storage. J. Food Prot. 47(3):206-208.

83. Schultze, W.D. and J.C. Olson, Jr. 1960. Studies on psychrophilic bacteria. I. Distribution in stored commercial dairy products. J. Dairy Sci. 43:346-350.

84. Shehata, T.E. and E.B. Collins. 1971. Isolation and identification of psychrophilic species of Bacillus from milk. Appl. Microbiol. 21:466-469.

85. Speck, M.L. (ed). 1984. Compendium of Methods for the Microbiological Examination of Food. 2nd ed. American Public Health Association, Washington, D.C.

86. Stadhouders, J. 1975. Microbes in milk and dairy products. A ecological approach. Neth. Milk Dairy J. 29:104-126.

87. Stewart, D.B. 1975. Factors influencing the incidence of B. cereus spores in milk. J. Soc. Dairy Technol. 28(2):80-85.

88. Stokes, J.L. and M.L. Redmond. 1966. Quantitative ecology of psychrophilic microorganisms. Appl. Microbiol. 14(1):74-78.

89. Thomas, S.B. 1970. Psychrotrophic microorganisms in market cream. A review, Part 1. Dairy Ind. Int. 35(2):79-84.

90. Thomas, S.B. 1974. The microflora of bulk colected milk-part 1. Dairy Ind. 39(7)237-240.

91. Thomas, S.B. and R.G. Druce. 1969. Psychrotrophic bacteria in refrigerated pasteurized milk: a review. Part 1. Dairy ind. 34(6):351-355.

92. Thomas, S.B., R.G. Druce and A. Davies. 1966. The significance of psychrotrophic bacteria in raw milk. Dairy Ind. 31(1):27-32.

93. Thomas, S.B., R.G. Druce and M. Jones. 1971. Influence of production conditions on the bacteriological quality of refrigerated farm bulk tank milk - a review. J. Appl. Bacteriol. 34(3):659-677.

94. Thomas, S.B., R.G. Druce and K.P. King. 1966. The microflora of poorly cleansed farm dairy equipment. J. Appl Bacteriol. 29(2):409-422.

95. Thomas, S.B. and B.F. Thomas. 1973. Psychroptrophic bacteria in refrigerated bulk-collected raw milk - part 2. Dairy Ind. 38(2):61-66.

96. Thomas, S.B. and B.F. Thomas. 1976. The bacterial content of farm bulk milk tanks. Dairy Ind. Int. 41(5):162-164. (P.1)

97. Vanstone, E. and B.M. Dougall. 1960. Principles of Dairy Science. Cleaver-Hume Press Ltd. London.

98. Waes, G. 1966. Some observations on the enumeration oF psychrotrophic bacteria in milk. Milchwissenschaft. 21:282-285.

99. Washam, C.J., H.C. Olson and E.R. Vedamuthu. 1977. Heat resistant psychrotrophic bacteria isolated from pasteurized milk. J. Food Prot. 40(2):101-108.

100. Wesen, D.P. and L.H. Schultze. 1970. Effectiveness of a post-milking teat dip for preventing new udder infections. J. Dairy Sci. 53:1391.

101. White, C.H. 1981. Microbiological tests for evaluation of dairy products-today and tomorrow. Dairy Food San. 1:238-244.

102. White, C.H., W.T. Gillis, D.L. Simmler, M.K. Galal, J.R. Walsh and J.T. Adams. 1978. Evaluation of raw milk quality tests. J. Food Prot. 41:356-360.

103. White, C.H. and R.T. Marshall. 1973. Reduction of shelflife of dairy products by a heat stable protease from Pseudomonas fluorescens P26. J. Dairy Sci. 56:849-853.

104. Witter, L.D. 1961. Psychrophilic bacteria - a review. J. Dairy Sci. 44:983-1015.

105. Zachariah, P. and J. Liston. 1973. Temperature adaptability of psychrotrophic Pseudomonas. Appl. Microbiol. 26(3):437-438.

106. Zobell, C.E. and J.E. Conn. 1940. Studies on the thermal sensitivity of marine bacteria. J. Bacteriol. 40:223-238.

APPENDIX

Table 23. Titratable acidity of gram positive sporeforming rods inoculated into reconstituted sterile skim milk and stored at 7°C.

Isolate	Initial	15 day	ΔTA
#21	.40	.44	+.04
#22	.40	.40	.00
#23	.40	.41	+.01
#24	.40	.42	+.02
#25	.40	.42	+.02
#26	.40	.39	-.01
#27	.40	.40	.00
#28	.40	.39	-.01
#29	.40	.40	.00
#30	.40	.41	.01
#31	.40	.41	+.01
#33	.40	.42	+.02
#42	.40	.41	+.01
#44	.40	.42	+.02
#45	.40	.40	.00
#46	.40	.40	.00
#47	.40	.40	.00
#73	.40	.42	+.02
#74	.40	.67	+.27
#75	.40	.65	+.25
#76	.40	.61	+.21
#78	.40	.62	+.22
#82	.40	.65	+.25
#83	.40	.63	+.23
#84	.40	.46	+.26
#85	.40	.47	+.27
#86	.40	.44	+.04
#87	.40	.44	+.04
#88	.40	.42	+.02
#89	.40	.42	+.02
#90	.40	.44	+.04
#91	.40	.44	+.04
#92	.40	.45	+.05
#93	.40	.46	+.06

Table 24. Titratable acidity of gram negative non-sporeforming rods inoculated into reconstituted sterile skim milk and stored at 7°C.

Isolate	Initial	15 day	ΔTA
#32	.40	.45	+.05
#35	.40	.46	+.06
#36	.40	.44	+.04
#39	.40	.44	+.04
#40	.40	.45	+.05
#41	.40	.43	+.03
P. fragi	.40	.47	+.07
P. fluor.	.40	.62	+.22

VITA

Sherry Lynne Ratledge was born on April 15, 1961 to Charles A. and Louise Ratledge of Maryville, Tennessee. She graduated from Friendsville High School in June 1979 and entered the University of Tennessee-Knoxville the following September. In June 1983, she received a Bachelor of Science degree in Animal Science. She married William H. Zimmerman on August 13, 1983 and began work on a Master of Science degree in Food Technology and Science the following September. Later she received a research assistantship and the degree was awarded August 1986.

The author is a member of the Institute of Food Technologists, Gamma Sigma Delta, Alpha Zeta, Delta Zeta sorority, Block & Bridle club and FarmHouse fraternity.

Sherry Zimmerman has accepted a position as Quality Assurance Inspector with Stouffer Foods Corporation in Gaffney, South Carolina.