CHANGES IN THE MICROBIAL POPULATION OF MILK DUE TO THERMAL AND ELECTRICAL TREATMENTS

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by

Richard Markham Heise

In Partial Fulfillment of the

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RICHARD MARKHAM HEISE

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I dedicate this thesis to my wife Diana, for her love and understanding I am eternally grateful.

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1. ABSTRACT

An electrical pasteurization process was selected to inactivate potential spoilage bacteria in milk. The goal of electrical pasteurization was to use a lower temperature than HTST and achieve similar results (63°C vs. 72°C). The temperature used was comparable to batch pasteurization which is also a thermal treatment.

The use of lower temperatures that pasteurize milk could generate more flavour in cheese and other fermented dairy products. The lower temperature may not heat denature or affect as many milk proteins as HTST thus producing a better product.

The quality of the treated milk was also evaluated by sensory analysis over a four week period. Batch pasteurized, electrically pasteurized and HTST treated samples were all evaluated by smell and found to be essentially similar.

Electrical pasteurization was more efficient than batch pasteurization for inactivating bacteria. The mesophilic bacteria were consistently reduced to the same low levels regardless of the initial microbial load. The thermophiles and sporeformers that were present were more difficult to

inactivate. This was true for all types of heat treatments. The destruction of these bacteria by electrical treatment was similar or superior to batch pasteurization. The psychrotrophs were the bacteria that had a very difficult time surviving electrical pasteurization. Almost all of the trials showed no survivors regardless of the initial microbial load suggesting that with further refinement the electrical method may have the potential to produce sterile milk products. The batch pasteurized samples did show some survivors at all sampling periods.

The sensory evaluation proved that the batch pasteurized samples were the least preferred samples and the HTST treated samples were the most preferred. The electrically treated samples were somewhere in between. The electrically treated samples were comparable to HTST treated samples in the beginning but as the milk aged the samples were as poor as the batch pasteurized samples.

2. INTRODUCTION

Bacteria are part of the natural microflora of milk. These microorganisms undergo a heat treatment before the milk reaches the commercial market. This heat treatment is known as pasteurization. The three types of heat treatment are batch pasteurization, High Temperature Short Time (HTST) pasteurization and ultra high temperature (UHT) sterilization. Each treatment uses a different time and temperature relationship. Batch pasteurization uses a temperature of 63 °C for 30 mins. The HTST procedure takes 16 s. at 71.6 °C. These procedures are not sterilization processes and, therefore, there are some survivors after pasteurization. Other microbes appear due to post processing contamination. The UHT pasteurization takes one to three seconds and uses a temperature of 131°C. There are no surviving bacteria in UHT treated milk but the milk is not very appealing to the oral senses due to a slight burnt taste.

After pasteurization, refrigeration is used to minimize the growth of mesophiles and sporeformers. Therefore, the main spoilage organisms in pasteurized milk are the psychrotrophs (Cousin, 1982). The elimination of these microorganisms is ultimately the main quality challenge for the dairy industry.

Electrical energy has been studied as an alternative source of pasteurization for many food products. The use of high voltage electrical current has been successful for inactivating many microorganims. This technique causes severe product deterioration by the shear force of the electrical current. A second technique of using low voltage currents has been studied and found to be very effective for reducing the bacterial load in liquids (Coichina et al., 1965).

The use of electrical current on various liquid products such as saline, fruit juices and milk has been studied (Murray and Blicq, 1992). A specially designed electrical unit has been successful for the inactivation of microorganims, even at operating temperatures as low as 29°C (Murray and Blicq, 1992). The purpose of this investigation was to compare the effectiveness of both thermal and electrical energy in the inactivation of spoilage organisms in milk.

Gelpi and Devereux (1930), used an electrical current to treat milk. They called this treatment the electropure process. They were able to inactivate 99 % of the bacteria without altering the quality of the milk, i.e. sterility was achieved.

The information gathered from these researchers proved that this topic needed further research. Therefore, the aim of this project was to illustrate the effectiveness of an alternating current on all types of microorganisms found in milk.

3. LITERATURE REVIEW

3.1 BACTERIA

3.1.1 Spoilage of milk

The pasteurization of milk has been an established procedure for many years. It has proven effective in the inactivation of pathogenic microorganisms in milk and related dairy products. Many species of bacteria make up the microflora of milk. Most of these bacteria including pathogens are eliminated from milk by normal pasteurization procedures. Significant spoilage organisms can enter milk after pasteurization (Kraft and Rey, 1979). This is known as post pasteurization contamination. Nevertheless, the milk industry has an excellent quality record for human consumption. Illness, as a result of the intake of spoiled milk, is not common. The microflora that are found in milk may be subdivided into several groups. These subdivisions are mesophiles, thermophiles, sporeformers and psychrotrophs.

3.1.2 Mesophiles

Microorganisms with mesophilic characteristics are bacteria that grow optimally at or above room temperature. The laboratory temperature and incubation conditions that are used to isolate mesophilic microorganisms are 32°C for 24-48 h. Mesophilic bacteria represent a large heterogeneous group of bacteria. Some mesophiles (especially the spore formers) are difficult to inactivate (Cousin, 1982). Also, certain mesophiles can grow sub-optimally at low temperatures and over a prolonged period they may cause product problems (Morita, 1975). There are many groups of mesophiles and it is difficult to categorize them. Microorganisms that can cause spoilage in raw milk at temperatures from 10-37°C include species from the group enterococci, lactobacilli and micrococci. Also, Streptococcus lactis and some coliform bacteria are included here (Frazier and Westhoff, 1978).

In general, mesophilic microorganisms have not been the main focus for researchers because the spoilage of milk is usually attributed to organisms that grow at refrigeration temperature. Mesophiles do not normally survive pasteurization. The microflora of raw milk largely consist of mesophilic microorganisms and some may survive. When they do, they are referred to more often as thermodurics or sporeformers.

3.1.3 Thermophiles

Microorganisms that grow effectively at 32°C and survive a heat treatment of 63°C for 30 min. were termed thermophiles. Thermophiles pose a problem with batch pasteurized milk but they are not a concern with High Temperature Short Time (HTST) pasteurized milk due to the higher temperatures. The HTST process uses a time temperature treatment of 71.6°C for 16 s (Frazier and Westhoff, 1978).

Thermophilic microorganisms are also known as thermodurics because they can grow at extreme temperatures. The term thermoduric means to survive conventional batch pasteurization. However, these organisms can grow as thermoduric psychrotrophs; that is, they are microorganisms that survive batch pasteurization and grow well at refrigeration temperatures. Thermodurics are mostly Gram positive rods or cocci. They are classified into two important groups. The first group is the non-sporeforming bacteria and the second is the sporeforming bacteria. The first group is broken down into two subclasses, the high temperature lactics such as the entrococci and certain species of Micrococcus. The second group consists mostly of bacteria from the genera Bacillus and Clostridium (Frazier and Westhoff, 1978).

Thermophiles are not usually a problem with HTST pasteurized milk. One reason is that milk is refrigerated after pasteurization. The refrigeration process slows down the growth of all microorganisms, thus providing a stressful environment for thermophiles. Also, most milk is heated to 71.6°C for 16 seconds, thereby killing the thermoduric bacteria.

Theoretically, thermophiles should not be present in pasteurized milk. However, they continue to exist. Elliker et al. (1964) noted the presence of thermodurics in milk after the plant equipment was improperly cleaned and sanitized. Therefore, the milk was contaminated.

3.1.4 Sporeformers

Sporeforming organisms are a concern to the milk industry because of the heat and time treatment necessary for pasteurization plus the enzymes these bacteria release into the milk. Sporeformers are able to survive 80°C for 10 min. This is a higher temperature than HTST pasteurization. The sporeformers consist of a variety of microorganisms in which most have been identified as Bacillus species (Cousin,

1982); (Washam et al, 1977). Mikolajcik and Simon (1978) stated that 80% of sporeformers were B. licheniformis and B. cereus. In addition, Washam et al.(1977) stated that 95% of the sporeforming bacteria were Bacillus species.

The presence of Gram positive bacteria in raw milk was believed to stimulate the formation of spores in pasteurized milk (Cousin, 1982). The presence of Gram negative microorganisms was also thought to stimulate the formation of spores (Mikolajcik and Simon, 1978). However, according to Kraft and Rey (1979), the presence of Gram negative bacteria does not stimulate the formation of spores. All researchers agree that mesophilic spores are more heat resistant than the psychrotrophic spores. Cousin (1982) estimated that 83% of raw milk samples contained sporeformers that survived pasteurization, but had a lag phase at low temperatures of 8-14 days. The survival of sporeforming bacteria was attributed to the sweet curdling of milk (Mikolajcik and Simon, 1978). The sporeforming B. cereus produce a rennin-like protease which causes this type of spoilage in milk.

3.1.5 Psychrotrophs

Psychrotrophic bacteria causing spoilage in milk originate from soil, water, air and vegetation (Cousin,

1982). The bacteria, which were called psychrotrophs, were originally termed psychrophiles, this means cold loving. This did not properly identify the bacteria which can spoil refrigerated foods, mainly milk. Many researchers have given specifications for psychrotrophs. Most agree they are bacteria that grow at 5 °C or lower, regardless of the optimum temperature. Cousin (1982) stated growth was most likely at 7 °C but the general term for psychrotrophs was bacteria that grow well at lower temperature but have a growth optimum of 10-13°C higher (Kraft and Rey, 1979).

The degradation of refrigerated milk was mainly due to the presence of Gram negative bacteria which belong to the Pseudomonas genus (Bryne et al., 1989). These bacteria spoiled the milk by breaking down proteins and lipids thereby causing a bitter flavor, rancidity and some discoloration (Kraft and Rey, 1979). Kraft and Rey (1979) also stated that many of these psychrotrophic bacteria do not survive pasteurization but cause problems in milk after pasteurization. This may be due to post-processing contamination.

Some bacteria that possess the characteristics of psychrotrophs survive to spoil milk with time. A high initial microbial load had a tremendous effect on the survival of psychrotrophs during pasteurization. The heat

treatment had the ability to inactivate only a limited number of organisms. If the initial microbial load were above this number then some bacteria survived the heat treatment. Therefore, the abuse of raw milk indicated a shorter shelf life of pasteurized milk. The incoming milk must meet specific standards and must not be abused before processing. Cousin (1982) estimated that raw milk stored at refrigeration temperature can reach up to 29 million C.F.U./ml within 72 h. The survival of psychrotrophs in milk also depends on the temperature and time of exposure to heat. Weckbach and Langlois (1977) found that a lower temperature and longer treatment time, as compared to normal pasteurization procedures, were more effective at inactivating psychrotrophs than HTST. They believed that the higher temperature caused cell destruction or cell injury. This created better conditions for faster recovery of microorganisms compared to lower temperature (Weckbach and Langlois, 1977).

The surviving psychrotrophs have been identified as Pseudomonas species. Pseudomonas fluorescens was the dominant bacteria which caused proteolysis and lipolysis (Cousin, 1982). Washam et al. (1977) stated that B. cereus was the predominant psychrotroph that survived 72°C for 16 s. He found that 135 out of 700 culture samples of B. cereus survived these temperatures.

3.1.6 General Psychrotrophs

The presence of bacteria in milk contributes to the spoilage and degradation of milk components. Cox and MacRae, (1988) stated that the spoilage of refrigerated milk must reach a minimum level of 10 million microorganisms per mL for milk to exhibit spoilage. Generally, the number of Gram negative bacteria was larger than the amount of Gram positive bacteria (Cousin, 1982). The minimum temperature for survival of psychrotrophs is - 12°C (Kraft and Rey, 1979). There is no possibility of growth in frozen conditions; therefore, psychrotrophs must have the ability to survive a frozen state. Once reheated above 0°C, they continue to grow.

The amount of bacteria present in milk also depends on the type of milk. In general, chocolate milk (2%) had the highest bacterial counts/mL which was then followed by skim milk. Milk with 2% butter fat was next followed by whole milk. These variations could be due to different protective effects on the various microbes by lipid levels in the different products. Also, the addition of a plant extract (cocoa) to milk could increase the overall microbial load.

Pseudomonas species were the main spoilage organisms

that had the ability to survive pasteurization. Cox and MacRae, (1988) stated that P. fragi was more competitive than P. fluorescens. However, Washam et al., (1977) found that Clostridium could also survive pasteurization. Bacteria that survive pasteurization were sensitive to further treatments or harsh conditions. Cousin (1982) stated the surviving bacterial cells were most fastidious in their requirements for nutrients. Bacteria also became more sensitive to pH alteration and have a difficult time recovering at lower temperatures. These were all stress factors. Upon cell injury due to heat, the cells become more sensitive and were easily destroyed by the thermal treatment. The recovery on optimum medium at optimum temperatures may also overestimate the survivors of pasteurization. Cousin (1982) stated that milk has harsher conditions than the standard laboratory growth conditions. The survivors of the heat treatment had a difficult time recovering in milk.

In many cases, the spoilage of pasteurized milk has been attributed to post processing contamination. Great care must be taken to keep the initial bacterial load low. Cousin (1982) stated that the problems of spoilage may be hidden to the human eye. Rubber parts of the improperly cleaned milking equipment contained 10-117 times more bacteria than the metal

parts. Cousin (1982) confirmed the statements of Thomas and Thomas (1973) in that the milking equipment was the most common microbial contamination after processing.

3.1.7 Cell Membrane

The cell membrane plays a very important role in the ability of the cell to survive. The permeability of the membrane and the ability of the membrane to take up substrates have been linked to the survival of microorganisms at low temperatures (Cousin, 1982). The transport of solute into Vibrio sps. grown at low temperature was affected by the degree of unsaturation of fatty acid side chains in membrane lipids. Cousin (1982) proposed that psychrotrophs have higher levels of unsaturated fatty acids than mesophiles. This is the reason why psychrotrophs could survive. E. coli proved that at temperatures of 12°C and 37°C, the unsaturated fatty acid levels were similar. Gill and Suisted, (1978) as stated in Cousin (1982), found similar patterns with P. fluorescens. It was found that an increase in the saturation level of lipids and an increase in growth temperature could be linked to the inability of the cell to control the mechanism responsible for alteration caused by environmental changes. Cousin (1982) followed this by noting that moderate temperature changes can alter physiological or

permeability functions of microorganisms grown at low temperature. Therefore, if the membrane were damaged, cell contents would leak out and cause cell lysis. The psychrotrophic bacteria (Cousin, 1982) showed an increase in amounts of respiratory enzymes which enabled the cells to continue to survive. The production of these enzymes did have one side effect in that the cells were not able to reproduce quickly at sub-optimum temperature.

Washam et al. (1977) studied the metabolism of bacterial cells at different temperatures and found that the cells failed to oxidize acetate through the tri-carboxylic acid cycle (TCA) at 32°C. At lower temperatures the cells were able to actively oxidize acetate through the TCA cycle.

3.1.8 Future and Control of Microbes

The food industry is always open to inventions to increase the shelf-life of a product. Europe has developed a process called thermization. This process involves special temperature treatments for milk refrigerated before pasteurization to prevent psychrotrophs from growing (Cousin, 1982). This process used a temperature of 63-66°C for 15 s.

Researchers at the University of Saskatchewan in the Food Science department have been working on this theory. Humbert

et al. (1985) called the process thermization. They recommended a treatment of 65°C for 20 s. This process increased the storage of raw milk an extra four days. They suggested that thermization be used in the dairy industry where raw milk is transported or stored for extended periods of time.

Ohmic heating (Skudder and Bliss, 1987) is another food processing technique that is introduced to extend the shelf life of food products. Ohmic heating uses alternating electrical current to generate heat internally in the food product. The heat produced is used to pasteurize or sterilize various food products. The products must contain 30 % or more water and must have moderate amounts of ionic salts. The procedure uses a continuous flow method with aseptic packaging. These two methods, similar to pasteurization, take advantage of various thermal time/temperature effects on living microbes.

The development of resistance by microbes, especially to antibiotics and heat, is an increasingly common occurrence. Food pasteurization or sterilization techniques that uses mechanisms other than thermal inactivation are of increasing interest. Such mechanisms include gaseous effects, radiation treatment and electrical effects. Although, Murray et al.

(1986) demonstrated that relatively low electrical treatment (400 volts) could kill microbes when sample temperatures were maintained at 29°C, the electrical lethal effect was not dependent on a thermal input.

3.2 ELECTRICAL EFFECTS ON MICROBES

3.2.1 Cellular Effects

Exposure of microorganisms to electricity results in various effects. The first effect is death due to the heat which is produced by the electrical current. This can be termed as an ohmic heating effect, an established food process for many liquid and semi-liquid food products. The voltage (up to 75 kV) can produce large amounts of heat and some researchers do attribute the destruction of microorganisms to the ohmic heating effect. Gilliand and Speck (1967a) stated that the bactericidal action produced by these treatments was attributed for the most part to heat produced by the flow of current through the liquid.

The second effect is the death of bacterial cells by the alternating electrical current. The possibility of inactivating microorganisms with alternating current has been proposed for approximately 70 yrs. However, no processes have been designed to sterilize foods with electrical energy that can be used commercially.

Allan and Stoike (1966) used high voltages (25 kV) to shock bacterial cells and sterilize water. Bacteria such as Bacillus subtilis and E. coli were inactivated in less than one minute. The input energy used to inactivate E. coli was 7.5 kJ/1.2L and B. subtilis was destroyed with 10 kJ/1.2L. The initial microbial loads of these samples were between 1 and 100 million viable microorganisms per mL. The mechanism by which death occurred was unknown to these researchers. Their best conclusion was that the shockwave pressure caused damage to the internal contents of the cell which resulted in death. Researchers Gilliand and Speck (1967b) also used similar high voltage discharges and explained the mechanism as a electro-hydraulic shock. Although considerable work has been done on the effects of high voltage (25-75 kV) on bacteria, only a few workers have investigated the use of low voltage.

Coichina et al. (1965) reported that E. coli was removed (presumably inactivated) by only 14 v with a current density of 0.15 A/cm . The bacteria were exposed to the electrical current for only 5-30 mins. This was low voltage for a longer period of time as compared to the high voltage treatment. The possibility of low voltage kill rates was established but was never pursued until later. The high voltage shock treatment was a preferred procedure because

large numbers of microorganisms could be inactivated in seconds. However, most of the time other organic material in the sample is destroyed. Therefore, the use of high voltage shock treatment in the food industry would cause severe product deterioration in most food products. Coichina et al. (1965), sugessted that low voltage treatments worked just as effectively as the high voltage treatments.

A low voltage electrical effect was also noted by Ockerman and Szczawinski (1984). They reported, in five studies investigating electrical tenderizing of meat, that the microbial population was significantly reduced on electrically stimulated meat. The heat produced by the electrical system raised the temperature to 60 °C. The microbial load was reduced on this meat sample only and caused a pH reduction. They stated that pH had little effect on the inactivation of microbes. The reseachers did not speculate on the combined effects of electricity and heat. They did, however, suggest there was a synergistic effect between these two parameters and that electrical current did have a detrimental effect on microbes.

It is well known that microorganisms carry a net negative charge (Harper et al, 1964). It is also known that electronic counting devices (such as the Coulter Counter) make use of these cellular charges and each time a living cell passes through an electrical counting orifice, it registers on a counting mechanism. Interestingly, a dead cell goes to a ground state and carries no net charge. Such cells

register differently on a Coulter Counter oscilloscope. Therefore, a key question becomes - is it possible to manipulate/ neutralize the charge on a living cell and thereby kill it? This question forms the underlying working hypothesis for work on the effect of electricity on microbes (Murray et al., 1986).

Researchers at the University of Manitoba studied the effect of electrical current on food. They proved that alternating current in liquid systems (saline, fruit juices) that did not go above 29 °C had a lethal effect on microorganisms (Murray and Blicq, 1992). This method was fast and used relatively low amounts of energy. A unique glass apparatus (as shown in the Materials and Methods section, Figure 1) was designed in the Food Science Department and used a flow through technique to handle liquid food products. This method sent alternating current through two graphite end pieces. The graphite pieces were designed to not produce any free radicals. The food product comes in contact with the graphite plate and transmits the current through the food product. A low voltage system was used (2-5 W/mL). Under these conditions it often took 10-20 mins. for the liquid product to pass through the system. The electrical current produced heat as it passed through the conductive sample. One modification to keep the temperature low was to use a cooling

jacket around the treatment tube. By doing this, it was possible to obtain sterile samples with electrical treatments that did not cause sample temperatures to rise above 29 °C. (Murray and Blicq, 1992). Clearly, the electrical lethal effect on microbes was different than the thermal effects.

Modifications of the orginal design have been produced. The different models all have few moving parts and are easy to operate. The newest designs can be scaled to the pilot plant level without difficulty.

3.2.2 Pulse Length and Current Density

Electrical current can be broken down into pulse length and current density. The pulse length is to electricity what the wave length is to light. Researchers believe that the length of the pulse along with the frequency of pulses attribute to the effective kill rate of microorganisms. Also, a minimal rise in temperature does not result in any significant change in the kill rate (Gilliand and Speck, 1967b; Hamilton and Sale 1967). The current density is similar to the frequency of pulses and is expressed in W mL^{-1} min^{-1}. Hamilton and Sale (1967) claimed that other researchers found the power density to be a major factor in the reduction of microorganisms. They concluded

that the destruction of microorganisms was non-thermal and was not due to heating of the suspension as a whole (Sale and Hamilton, 1967). The damage caused by electrical current was irreversible and was dependent upon field strength and total time of treatment. (Sale and Hamilton, 1967).

Electricity has also been used to electrify microorganisms. This was done by sending shock waves through the liquid. When Gilliand and Speck (1967b) used electricity in this respect, they did not raise the temperature of the treated milk; however, significant amounts of electricity were used. They reported an 85% destruction of bacteria when using high voltage (25 kV). They termed this procedure as electrohydraulic shock and found the most efficient destruction of bacteria was at lower voltage levels for each level of capacitance (Gilliand and Speck, 1967a).

3.2.3 Membrane Damage

Electrical current has the ability to affect the membrane in a way that normal thermal treatment cannot. A membrane damaged by electrical current can show the leakage of intracellular contents and the inability to plasmolzye in a hypertonic medium (Hamilton and Sale, 1967). It was proposed that the electric field or electricity caused an irreversible loss of membrane function as a semi-permeable

barrier between the cell and its environment. (Sale and Hamilton, 1967). This occurred with vegetative bacteria and some sporeformers and this was the main mechanism of the destruction of cells. The research in this area has been inconclusive. Reseachers do admit, however, that the direct current (d.c.) pulse treatments may only affect certain parts of the membrane (Hamilton and Sale, 1967).

3.2.4 Spore Resistance

Certain microorganisms form spores to enable them to survive harsh conditions. It is well known that sporeformers are very difficult to inactivate by normal thermal pasteurization procedures. Electrical current is also limited with respect to the inactivation of sporeformers. Bacillus cereus spores are resistant to electricity; their resistance comes from the spore coat and cortex layers (Hamilton and Sale, 1967).

Heat and electrical current cause the cells to undergo stress. The plasma membrane of the vegetative cell becomes the core membrane which is surrounded by the spore coat and cortex layers. The electrical pulse can penetrate these layers but requires longer exposure or a high frequency of pulses to destroy the cells. Once the stressful situation is

eliminated, the cortex layer disappears. The cell gradually expands and the coat layers dissolve within the cell (Hamilton and Sale, 1967). This pattern is continuous throughout germination; similar patterns were noted in B. subtilis when exposed to broth (Hamilton and Sale, 1967). The potential use of electrical current to destroy or inactivate sporeformers was established by Sale and Hamilton (1967), who illustrated the sensitivity of the sporeformers to the direct current pulse treatment. They noted that the spore coat splits, opens and the vegetative cell emerges. The membrane is left unprotected causing the possibility of a lethal effect to the once sturdy sporeforming microorganism.

3.2.5 Structural Damage

The membrane of the cell is one of the most important structural features of the cell. Electrical current causes the membrane to weaken and split (Hamilton and Sale, 1967). Therefore, cell death occurs. Direct current pulses also affect other parts of the cell. The most noticeable effect is the loss of cell motility and the synthesis of enzymes. The loss of motility does not allow the cell to move to less stressful environments. The cells are, in a sense, trapped and easily destroyed. The loss of ability to synthesize enzymes only occurs with the induced enzyme B-galactosidase in vegetative bacteria (Hamilton and Sale, 1967). The destruction of this enzyme does not allow the breakdown of essential sugars. Therefore, potential for growth is reduced.

Without the ability to synthesize food, the microorganisms that are not destroyed will starve and cease to exist. The researchers concluded that the individual enzymes in the cells are not affected by the electrical currents (Hamilton and Sale, 1967).

3.2.6 Formation of Radicals

The use of higher voltage(25 kV) causes the formation of radicals. This formation can cause a secondary effect with electrical currents. Radicals themselves have the ability to inactivate microorganisms. A combination of radicals and electricity could be devastating to the viability of the cell. The charge on the radical could induce a more lethal effect. Gilliand and Speck (1967b) illustrated the bactericidal action produced by electrohydraulic shock. They suggested that lower voltages can also form radicals. They believed that hydrogen atoms which are present in milk could form hydroxyl radicals. Gilliand and Speck (1967b) stated that the indirect effects of radiation resulted in bacterial death. Death was due to chemical reactions mediated by free radicals produced by intracellular water. Therefore, the death of the cell was not by the inactivation of one component but by several cell components.

3.3 ELECTRICITY ON MILK

The electropure process was introduced in the 1920's and is very similar to a electrical pasteurization unit at the University of Manitoba. The electropure process used milk that flowed through a continuous system. The liquid passed through a narrow rectangular center piece with side walls consisting of carbon electrodes (Gelpi and Devereux, 1930). The researchers were able to inactivate 99% of the microorganisms without altering the quality of the milk. The only surviving microorganisms were of sporeforming nature (Gelpi and Devereux, 1930).

Gelpi and Devereux (1930) used skim milk to prevent the interference of fat in the test tubes. The milk was placed in test tubes that were pasteurized by the batch procedure (62.8°C for 30 min) and then cooled to 10°C. The milk from the same raw source was also passed through their electropure process for 10-14 s at 71°C. These researchers failed to mention the power levels used during their treatment. The time of exposure was similar to the research done at the University of Manitoba. Therefore, one can assume this was not a high voltage shock treatment. Gelpi and Devereux (1930) concluded that for spore destruction, the electropure process was superior to batch pasteurization.

4. MATERIALS AND METHODS

4.0.0 ELECTRICAL EQUIPMENT

4.0.1 Sample Storage

Milk was obtained from the University of Manitoba dairy in 20 L lots and was stored in an 8 L glass pyrex Erlenmeyer flask. The pyrex container was first autoclaved. The milk was then transferred from the plastic bag obtained from the dairy to the Erlenmeyer flask in a laminar flow hood. The glass Erlenmeyer flask was used until 10 L Nalgene plastic carboys were obtained. Two carboys stored all of the 20 litres of milk. The carboys had a screwtop lid which allowed for minimal bacterial contamination during storage. The raw milk was stored at 4°C.

4.0.2 Sample Delivery

The Erlenmeyer flasks and plastic carboys were removed from the cold room where control samples were taken. Conductivity and pH measurements were also performed on all samples. The samples were then transferred from the storage

vessel to the electrical unit by a peristaltic (Cole-Palmer #WZ1R057) pump. The pump was fitted with one cm diameter plastic tubing (Cole-Palmer #6419-45) which was required to remove the milk from the flask or carboy. The milk then travelled through the pump into the glassware of the electrical system. The milk flowed through the tubing to a 10/19 Quickfit end piece which had the dimensions of 1cm x 12cm.

4.0.3 Glassware

The glassware for electrical treatment can be divided into two major categories. These are the center tube and end pieces (Figure 1). The end pieces and center tube were constructed at the glass blowing shop Chemistry Department at the University of Manitoba. These were the two main pieces of the electrical unit. Once the milk flowed through the 10/19 "Quickfit" section (1 cm) , it then entered one of the end pieces. There were two end pieces which were constructed exactly the same except that one had a female end and the other had a male end. The end pieces were made up of five different components (Figure 2) that were held together with spring tension clamps obtained from Canadian Tire. The first component was the outer portion of the end piece which was a

Figure 1. Skematic Diagram of Female End Piece of Electrical Treatment Assembly

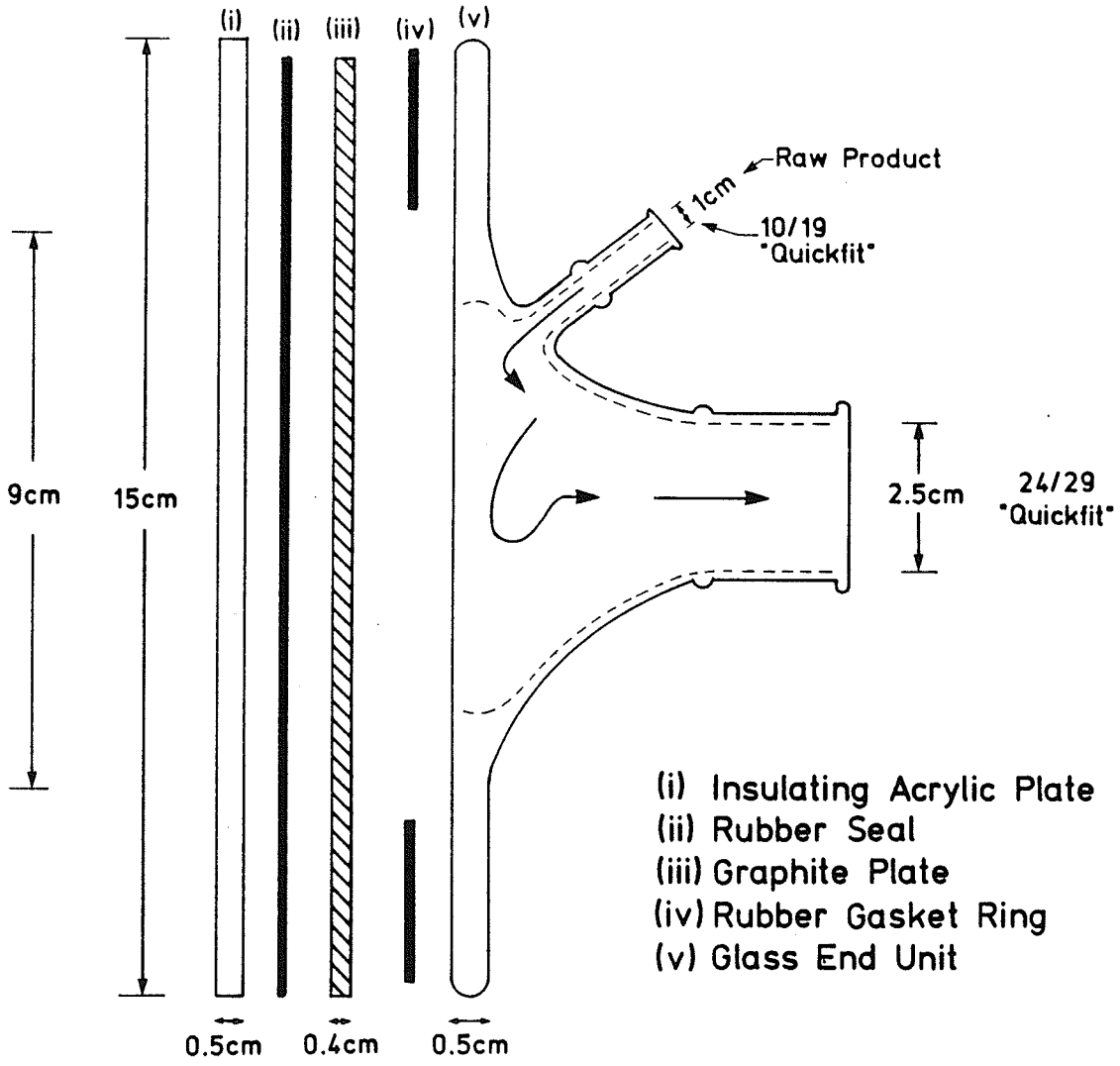
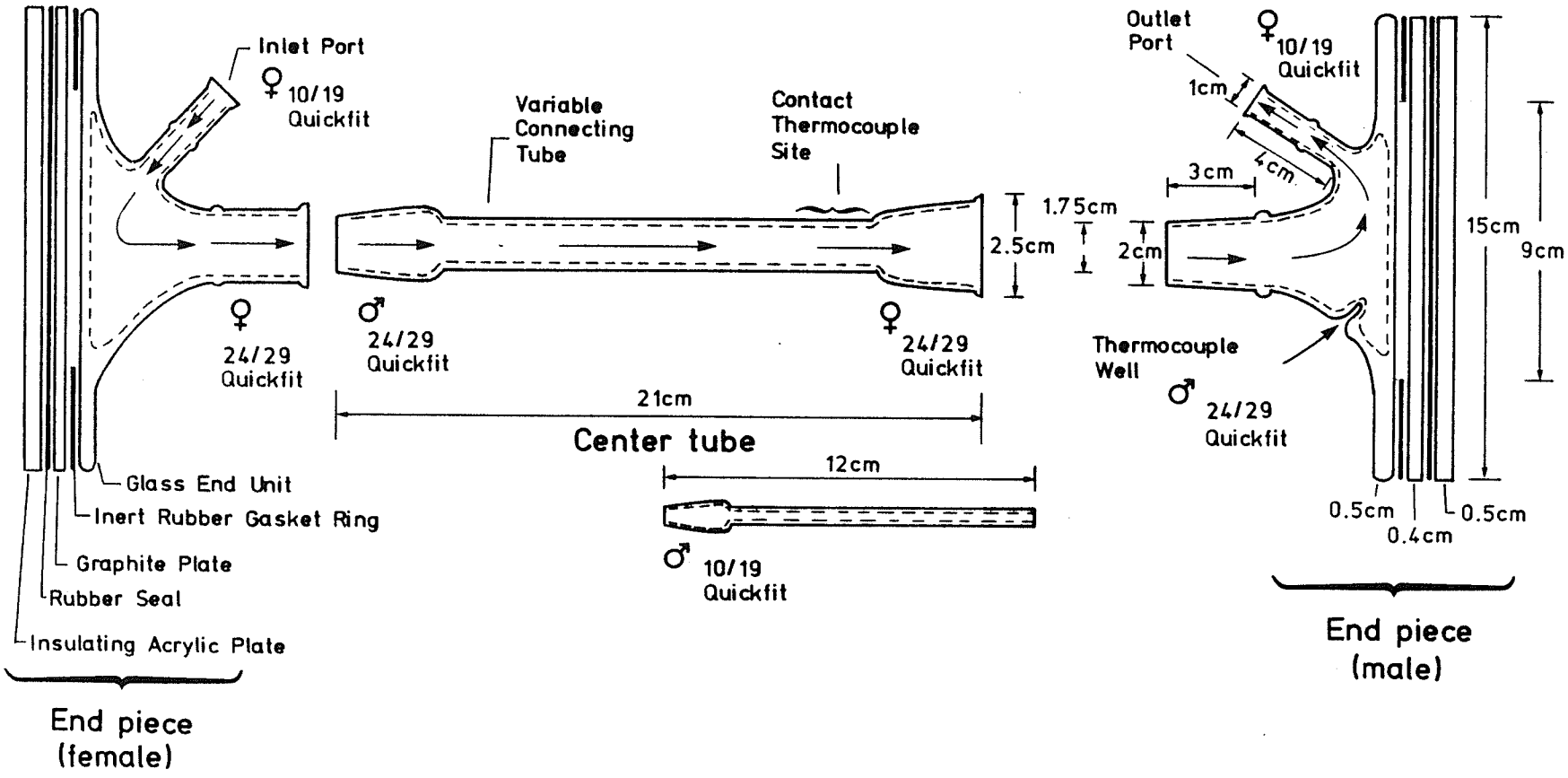


Figure 2 Skematic Diagram of the Electrical Treatment Assembly for Fluid Milk



1.5 cm thick acrylic plate , 15 cm in diameter. This piece was the only component of the end piece which was not autoclaved because it did not come in contact with the food product. The next portion of the end piece was insulating rubber which was 2 cm thick and 15 cm in diameter. This piece was described as the rubber mat. There were two insulating rubber pieces but one was on the other side of the graphite electrode plate and had an inside diameter of 12 cm. This was the gasket of the end piece. The graphite plate was 2 cm thick and 15 cm in diameter. The graphite plate was the only material that conducted electricity and, therefore, came in contact with the liquid food product. Graphite was used to minimize any free radicals which may have been produced. The last component and most inner portion of the end unit was the glass end piece which had a 15 cm outside diameter. It had a 2.5 cm inner diameter which was tapered to a 24/29 "Quickfit" connection. The end pieces were assembled in the laminar flow hood and held there until the whole unit was ready to be assembled.

The center tube was fitted between the two end pieces; therefore, it had 24/29 "Quickfit" adaptators which interlocked with each end pieces. The center tube was composed of autoclavable glass which was 15 cm in length and

had an inside diameter of 1.5 cm. The length and width of the tube could be varied to achieve different electrical treatments.

4.0.4 Power Supply

The power supply consisted of a 1500 V. transformer (Hammond 2000 V. Variac) with an operating range of 120-1200 V. The electrical current flowed through a rheostat (Variac - Fisher Brand #9-521-130B2) which was then stepped up with a stepper motor (transformer controlled). The current was transferred to the electrical treatment unit through pin point connectors. The connectors were inserted between the graphite plates and the rubber insultating mats (Figure 2).

4.0.5 Monitoring/Control

The power supply was controlled and monitored by a voltmeter and ammeter. The voltmeter was a digital multimeter (Fluke #8000) and the ammeter was a Avometer (model 15/16) enclosed in a plastic box. The temperature was monitored by an Omega computer monitoring system. This system used contact thermocouples (K type) which were attached at the liquid entry and exit ports. A third thermocouple was placed in the exit well of the electrical system. The thermocouples were

encased in glycerol and stoppered with a cork. The temperature inside the male end piece could then be monitored. The last and most important thermocouple was placed on the center tube which was the point on the unit where the maximum temperature was achieved. If the computer was occupied, a digital thermometer (Tegam 871 A) was used and was attached on the centre tube.

4.0.6 Sample Collection

The milk samples were collected in sterile 20 mL test tubes. The tubes were opened and placed under the exit hose of the electrical system. The test tubes were opened for a minimal amount of time to prevent contamination. The sample was collected carefully with a liquid overflow. Great caution was exerted at this point because the electrical current could flow through the liquid.

4.1.0 UNIT ASSEMBLY

4.1.1 System Sterilization

The electrical unit was sterilized before assembly. The glassware was a Pyrex material and was easily autoclaved after it was wrapped in aluminium foil. After the pieces of

the unit were autoclaved they were placed in a laminar flow hood until they had cooled. The pieces were then assembled using an aseptic technique in the flow hood.

The tubing and connectors were not autoclaved because they could not survive the temperatures used for sterilization. However, this equipment was washed thoroughly and sanitized with 500 ppm chlorine.

4.1.2 Wiring Connections

The electrical system was attached to the graphite plate of the end pieces. Pin point connectors were used to connect the power supply to the electrical apparatus. The electrical power first travelled through the junction box which connected the voltmeter and ammeter.

4.1.3 Temperature and Thermocouple

The temperature was monitored by an Omega computer system. This system monitored the change in temperature every two seconds and recorded each temperature on a computer disk. It was stored on the Omega Computer Data Acquisition System.

The thermocouples were attached to this system and were also attached to various parts of the electrical unit. The

thermocouples used were K type surface units. They were attached to the surface inlet and exit ports to monitor incoming and exiting liquid sample temperatures. Another thermocouple was inserted in the sampling well of the exit end piece. This was used to monitor the temperature of the liquid at this point near the graphite plate. The final thermocouple was placed on the centre tube where the maximum operating temperature was achievable.

4.2.0 UNIT OPERATION

4.2.1 Flow Rate

The flow rate of the electrical unit was the first parameter established. The flow rate was variable due to the nature of the pump and could be set at a variety of speeds. Once the flow rate of the system was established, it remained constant throughout the entire run.

4.2.2 Temperature

Although earlier work had established that the electrical treatment alone was sufficient to sterilize

various liquids, normal operation of the electrical apparatus caused the product to warm as it passed through the system. Consequently, the sample was allowed to warm to a temperature of 63°C. This temperature was selected because it was the same as that used with batch pasteurization. The temperature remained constant throughout the run but could change during the treatment. This slight change was due to the raw milk becoming warmer over time. The slight increase (< 5°C) did not have any effect on the final product. Therefore, less electricity was needed to pasteurize the raw milk. The temperature could be controlled by a slight increase or decrease in electrical power. The temperature equilibrium of the system was established before any alteration to the power was made.

4.2.3 Unit Preparation

The electrical unit was pre-filled with the raw product before the current was applied. The pump transferred the liquid into the electrical appartus; however it did not completely fill the tubing and end pieces. Trapped air would have created a problem if left in the end pieces. Therefore, the air was removed by running the pump backwards, then forwards. The thermocouples were attached to the glassware while the pump filled the unit.

4.2.4 Voltage Delivery

The voltage delivery was applied after the pin point connectors were attached . The pin point connectors were attached during the filling of the unit. Once the unit preparation was complete, a consistent flow rate was established. The flow rate was set and not adjusted until the electrical treatment was complete. Simultaneously, the voltage or power was applied. The voltage could be applied at near precision as the operator gained more knowledge of the equipment. However, for the first several runs, it was desirable to apply a minimal voltage, then increase the voltage after periods of equilibrium.

4.2.5 Equilibrium

Once the system was running, it would set itself into equilibrium. The first equilibrium established was the temperature of 63°C. The next was the flow rate and, finally, the voltage and the amperage. All equilibrium points intertwined with each other and were affected by the adjustment of each. Once the system was operating, only one of these equilibrium points could be altered to keep the other conditions constant. The voltage was the one parameter that was adjusted throughout the run to keep the temperature at 63°C.

4.2.6 Lag Time

The system did not reach equilibrium immediately. Therefore, there was a lag time. The first lag time occurred when the system had reached 63°C. The second lag time was imposed to allow the system to pass two volumes (equivalent to system volume) after reaching equilibrium temperature. This was to ensure that the sample taken had been exposed to the desired operating conditions.

4.2.7 Sampling

Triplicate samples were taken from each trial. Each sample was stored in ice water which was approximately 4°C. Plate counts were then completed. The control sample was also taken at this time and then stored at 4°C until it was ready to be plated. However, the control sample could have been taken before system operation.

4.2.8 Plating

The samples were removed from the ice water along with the control samples . All samples were plated on Standard Plate Count Agar (S.P.C. Agar) and incubated at 32°C for 24-48 hrs. or 7°C for seven days.

4.3.0 THERMAL BATCH TREATMENTS

4.3.1 Sample Procedure

The raw milk obtained from the University of Manitoba dairy was stored in a 10 L Nalgene plastic carboy at 4°C. The carboys were removed and tranferred to a laminar flow hood; test samples were taken and the carboys were returned to the refrigerator until the next treatment run.

The raw milk was transferred from the carboys to 20 mL sterile test tubes. The transfer took place in a laminar flow unit to reduce the chance of microbial contamination. The test tubes were marked to indicate the treatment temperature. The test tubes were stored at refrigeration (4°C) temperature until the electrically treated tubes were ready for the second heat treatment.

4.3.2 Monitoring and Pasteurization System

A glass thermometer (Fisher-Scientific 14-985C) was placed in a test tube containing raw milk. This tube was used to monitor the temperature for batch pasteurization. The thermometer was placed in the control tube with continous agitation until it reached 63°C or 80°C depending upon the treatment. The time of pasteurization was monitored by a box

timer (Gralab Universal : Model 171). The pasteurization was performed by a constant temperature water bath (Magni Whirl : Model MW-1120A-1). The temperature of 63°C was held constant for 30 mins.

4.4.0 HEAT DELIVERY

The delivery of heat to the milk in the test tubes was performed by the water. The heat to the water bath was controlled thermostatically to ensure a proper consistent temperature. The milk temperature was monitored by the thermometer in the control test tubes.

4.4.1 Equilibrium

The control tube temperature began at 4°C and increased to 63°C. Therefore, each tube was agitated until the control tube reached the desired temperature of 63°C. Once the tube had reached this temperature the timer was set for 30 mins. During the 30 mins. of holding time, the tubes were agitated every two minutes.

4.4.2 Sampling and Plating

The test tubes were removed from the water bath and placed in an ice water bath to cool the milk to 4°C. The medium used was a Standard Plate Count (S.P.C.) agar with a pour plate technique. The mesophiles and psychrotrophs were the only samples that were plated immediately. The other tubes needed to be reheated to the specific temperatures.

4.5.0 ISOLATION OF MICROORGANISMS

4.5.1 Mesophiles

The mesophilic organisms from the raw milk sample and the treated milk samples were plated onto S.P.C. agar. The plates were incubated at 32°C for 24-48 hrs.

4.5.2 Thermophiles

The thermophile isolation experiment used a heat treatment of 63°C for 30 mins. The tubes were heated, agitated and then placed again in the ice water bath. The samples were plated with the same pour plate method with S.P.C. agar. The plates were then incubated at 32°C for 24-48 hrs.

4.5.3 Sporeformers

The isolation of sporeforming microorganisms required that the samples be heated to 80°C for 10 mins. The milk samples were then placed in an ice water bath, cooled and finally plated on S.P.C. agar (pour plate method). The plates were incubated along with the mesophiles and thermophiles at 32 °C for 24-48 hrs.

4.5.4 Psychrotrophs

The samples of psychrotrophs were plated on S.P.C. agar (pour plate method) and incubated at 7°C for 7-10 days.

4.6.0 SENSORY EVALUATION

4.6.1 System Preparation

The sensory evaluation began the week of February 12, 1990. The milk used in this experiment was received from the University of Manitoba dairy. Both raw and pasteurized milk were obtained from the dairy. The pasteurized milk was processed at the U of M dairy which used the standard High Temperature Short Time (HTST) technique. The raw sample was thermally treated (63°C) by batch pasteurization at the Food Science pilot plant. The electrically treated milk was processed under laboratory conditions and also used a temperature of 63°C. The treated milk samples were placed in sterile plastic carboys and stored at refrigeration temperature (4-7 °C). The HTST sample was also stored in a sterile carboy at refrigeration temperature.

4.6.2 System Operation

The sensory evaluation was performed weekly. The panelists were Food Science students and were not experienced panelists. They were asked to give their preference on a 9 point hedonic scale. The value of 9 meant the sample was most preferred and a value of 1 meant, least preferred. The samples were placed in clean paper cups and allowed to aerate

for 10-20 minutes where upon the panelist was asked to smell the samples and designate a preference for each sample of milk. The samples were not taken orally due to a possible health risk factor. Samples of milk that sat in the refrigerator for four weeks could contain high microbial contents. Therefore, smelling the samples was sufficient for the experiment.

4.6.3 System Analysis

The weekly samples were analyzed on S.P.C. agar for microbial growth. The plates were stored at 32 °C to isolate the mesophilic microoragnisms and at 7 °C for psychrotrophic microorganisms. The sensory analysis was tabulated and stored onto computer. The computer system was able to analyze the results by analysis of variance (ANOVA) and the Duncan's Range test. These tests illustrated a difference between the weeks of storage and type of treatment.

5. RESULTS

5.1.0 MICROORGANISMS

5.1.1 Classification of Microorganisms in Raw Milk

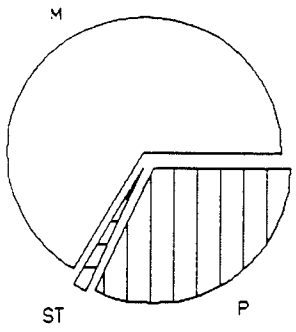
Mesophilic bacteria were the most prevalent microorganisms found in raw milk according to the standard plate count method. By definition, a mesophile is an organism which has an optimum growth at 32°C. Raw milk, which was stored at 4°C for one day, had a mesophilic population of approximately 66.5 % (Figure 3) of the whole population. The increase in storage time resulted in an increase in all microorganisms. However, by the ninth day of storage at 4°C, the mesophiles had increased to 76.2 % of the total population.

The thermoduric organisms or thermophiles were the next most frequent organisms that were isolated from the milk samples. These organisms grew above room temperature and presented problems in milk that was pasteurized by the batch method. To isolate these organisms a temperature of 63°C was used to heat the samples. Therefore, the control samples were raw milk that was heated to 63°C and cooled. The treated samples were exposed to 63°C (for the process of pasteurization) and were cooled in ice water (0°C). In order

Figure 3: Microbial Population Distributions of Raw Milk

(Average of 15 Runs)

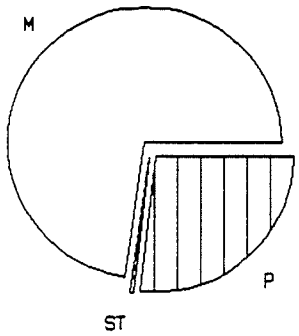
STORAGE 1 DAY (4°C)



SURVIVAL RATE (%)

M: 66.5 %
P: 31.8 %
ST: 1.7 %

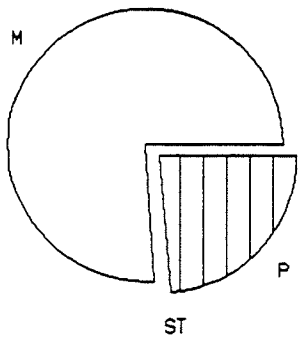
STORAGE 3 DAYS (4°C)



SURVIVAL RATE (%)

M: 72.9 %
P: 26.8 %
ST: 0.3 %

STORAGE 9 DAYS (4°C)



SURVIVAL RATE (%)

M: 76.2 %
P: 23.8 %
ST: 0.01 %

to isolate the thermoduric organisms the samples were then again exposed to 63°C for 30 mins. Following this, the samples were then cooled in an ice water bath and plated on S.P.C. agar for 48 hours at 32°C. This two step heating procedure essentially involved a thermal kill step (for the non-thermoduric bacteria) and then a thermal isolation step for assay purposes.

The sporeformers survived temperatures above 80°C for 10 mins. The control samples for the sporeformers were raw milk samples which had been exposed to 80°C for 10 mins. The treated samples were subjected to 63°C, cooled and then exposed to 80°C for 10 mins and finally cooled in a water bath. The samples were then plated on S.P.C. agar for 48 hrs at 32°C.

The thermodurics and sporeforming microorganisms occurred in low numbers in raw milk. Results from the first day of storage showed that the sporeformers and thermophiles consisted of less than 2 percent of the total raw milk population. As the milk aged the relative numbers of thermophiles and sporeformers decreased proportionately because the mesophilic and psychrotrophic microorganisms grew at a faster rate. The total number of thermophiles and sporeformers remained roughly the same overall but the population percentage decreased to 0.01 % of the total raw milk population after storage at 4°C for nine days. Since

thermophiles and sporeformers make up roughly 90 to 100 % of the survivors in the thermal and electrically treated milk, they were clearly very difficult to kill using these methods.

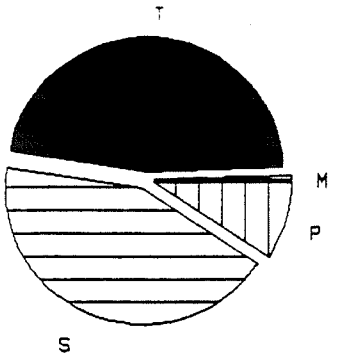
The organisms of greatest concern were the psychrotrophs as these organisms are responsible for primary spoilage of pasteurized milk when it is held at refrigeration temperatures. The raw milk counts indicated that the psychrotrophs were the second most prevalent group of microorganisms. The psychrotrophs were isolated through the use of selective incubation temperatures: the treated samples were exposed to 63°C and both the samples and controls were incubated at 7°C for 7-10 days.

5.2.0 THERMAL PASTEURIZATION

Conventional batch pasteurization affected each group of microorganisms differently. In general, the mesophiles were most affected whereas the sporeformers and thermophiles were least affected by the thermal treatment. The results shown in Figure 4, give the percentage of survivors over an average of fifteen different experimental trials. These fifteen trials were for a specific day of storage, with a total of forty five trials represented in this figure. Plotting percentage of survivors against the respective controls gave a direct comparison which can be made with the results for the survivors of electrical pasteurization.

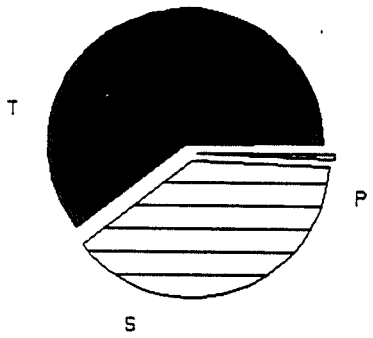
Figure 4: Microbial Population Distributions of Thermally Treated Milk (Average of 15 Runs)

STORAGE 1 DAY (4°C)



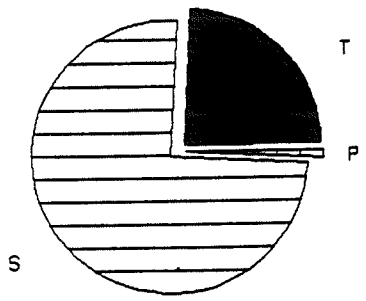
SURVIVAL RATE (%)
M: 0.6 %
P: 9.1 %
T: 46.9 %
S: 43.4 %

STORAGE 3 DAYS (4°C)



SURVIVAL RATE (%)
M: 0.2 %
P: 0.5 %
T: 60.5 %
S: 38.8 %

STORAGE 9 DAYS (4°C)



SURVIVAL RATE (%)
M: 0 %
P: 0.7 %
T: 24.3 %
S: 75.6 %

5.2.1 Mesophiles

The bacteria isolated with the mesophilic characteristics were the most prevalent microorganisms in the raw milk. Conventional batch pasteurization consistently inactivated most of these bacteria (Figure 4). The highest survival rate of the mesophiles was 0.6%, after the milk was stored for one day. The increase in storage time illustrated that the heat treatment was able to reduce the survival of the mesophilic bacteria to 0.2 and 0% of the total surviving population. This was the best kill rate for any of the microorganisms studied. Storage at 4°C meant that the mesophilic bacteria under went logarithmic growth over an extended time frame. The heat treatment was still able to reduce the mesophiles to 0% of the total population. A typical run on milk stored for nine days (Table 1) revealed the effectiveness of the heat treatment; it was able to reduce the mesophilic microorganisms from 50,000,000 as obtained in the control milk to 1,500 C.F.U./mL (Table 1).

Table 1. A Random Sampling of Milk Treatments Using Thermal Pasteurization

MICROORGANISM	STORAGE PERIOD (d)	ELECTRICAL SURVIVORS (C.F.U./mL)	RAW MILK CONTROL (C.F.U./mL)
MESOPHILE	1	90	9,000
PSYCHROTROPH	1	5	5,000
THERMOPHILE	1	320	380
SPOREFORMER	1	290	150
MESOPHILE	3	180	60,000
PSYCHROTROPH	3	5	29,000
THERMOPHILE	3	250	190
SPOREFORMER	3	170	240
MESOPHILE	9	1,500	50,000,000
PSYCHROTROPH	9	1,200	3,000,000
THERMOPHILE	9	800	1,100
SPOREFORMER	9	920	140

5.2.2 Thermophiles

The microorganisms that were isolated with thermophilic characteristics were most resistant to thermal pasteurization. The survival rate of the total population was the highest for the thermophiles when the milk was stored for one and three days. The survivors consisted of 46.9% and 60.5% of the total population, respectively (Figure 4). This strong survival rate was attributed to the fact these bacteria had been exposed to 63°C twice. The bacteria that survived the first exposure to heat usually survived a second treatment. The lowest percentage of survivors occurred when the milk reached nine days of storage. This was attributed to the tremendous increase in sporeformers as the milk became older. Therefore, the thermophilic bacteria decreased in percentages only. A typical run of milk that was stored for three days showed an increase of thermophiles. The survivors outnumber the control sample by 60 C.F.U./mL (Table 1).

5.2.3 Sporeformers

The sporeforming microorganisms were the most difficult bacteria to inactivate. They were subjected to consecutive temperatures of 63°C and 80°C. They became more prevalent

as the storage time increased. The percentage of survivors increased to 75.6% of the total survivors from 43.4% and 38.8% from day one and day three, respectively (Figure 4). The heating of the milk promoted the growth of sporeformers to the extent that the survival rate for a typical run of milk stored for nine days was six to seven fold (Table 1). Most runs illustrated that the thermally treated samples were similar or higher than the control sample.

5.2.4 Psychrotrophs

The psychrotrophic microorganisms were easily inactivated by the thermal treatment. The survival rate was the highest for milk which was stored for one day at refrigeration temperature (4°C) . The psychrotrophs consisted of 9.1% of the total surviving population. For the other days of storage the percentage of survivors was below 1% which gave an indication that the heat treatment may be effective for psychrotrophs during stressful conditions. The typical heat treatment of raw milk illustrated that the psychrotrophs were inconsistent and ranged from 1 to 1,000 C.F.U./mL. The milk that was stored for nine days showed a survival of 1,200 microorganisms/mL. The bacterial count of the control sample was 3 million C.F.U./mL which is sufficient for thermally treated milk.

5.3.0 ELECTRICAL PASTEURIZATION

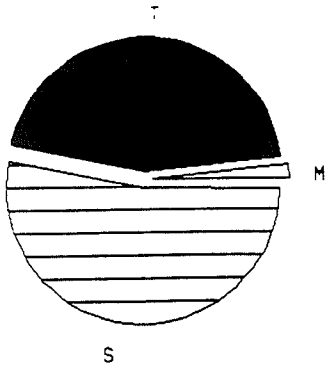
The populations of the mesophilic, thermophilic, sporeforming and psychrotrophic microorganisms after electrical pasteurization process are shown in Figure 5. These charts illustrate the percentage of survivors for an average of fifteen trials. The batch pasteurized samples were from identical raw material as the electrical treated samples to allow for direct comparison of the two treatments.

5.3.1 Mesophiles

The most prevalent microorganisms in raw milk were easily killed by the electrical current. The largest survival rate occurred when the milk was stored for three days. It reached 6.1 % of the total surviving population. The other two storage periods had lower survival rates where neither climbed above 2%. A typical run of milk showed the electrical current was inconsistent as far as killing microorganisms was concerned (Table 2). The electrical current was able to reduce the mesophilic bacteria into a range of 70 to 650 C.F.U./mL for each storage period. The reduction was always the same even though the control counts varied extremely. Therefore, the age of the milk did not have an affect on the ability of the electrical current to inactivate the mesophilic microorganisms.

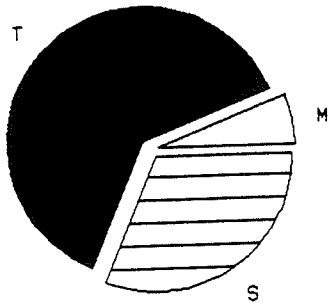
Figure 5: Microbial Populations Distributions of Electrically Treated Milk (Average of 15 Runs)

STORAGE 1 DAY (4°C)



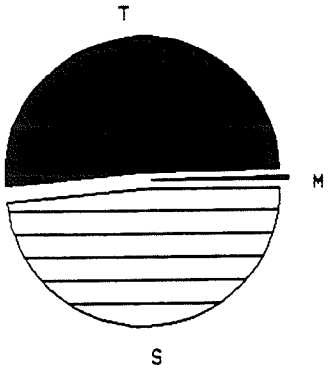
SURVIVAL RATE (%)
M: 1.8 %
P: 0.1 %
T: 44.9 %
S: 53.2 %

STORAGE 3 DAYS (4°C)



SURVIVAL RATE (%)
M: 6.1 %
P: 0.1 %
T: 62.6 %
S: 31.2 %

STORAGE 9 DAYS (4°C)



SURVIVAL RATE (%)
M: 0.4 %
P: 0 %
T: 51.2 %
S: 48.4 %

Table 2. A Random Sampling of Milk Treatments Using Electricity

MICROORGANISM	STORAGE PERIOD (d)	ELECTRICAL SURVIVORS (C.F.U./mL)	RAW MILK CONTROL (C.F.U./mL)
MESOPHILE	1	260	5,000
PSYCHROTROPH	1	0	14,000
THERMOPHILE	1	60	400
SPOREFORMER	1	80	120
MESOPHILE	3	650	4,600
PSYCHROTROPH	3	2	30,000
THERMOPHILE	3	130	65
SPOREFORMER	3	80	240
MESOPHILE	9	70	26,000,000
PSYCHROTROPH	9	2	23,000,000
THERMOPHILE	9	120	1,100
SPOREFORMER	9	390	2,700

5.3.2 Thermophiles

The microorganisms that were isolated as thermophiles were the most difficult group of bacteria to kill by the electrical treatment. The percentage of thermophilic survivors was 44.9% for fresh milk, 62.6% and 51.2% of the total surviving population as the storage time increased (Figure 5). A typical run of milk illustrated that the treated samples were very similar to the control samples (Table 2). The milk stored for three days showed a larger number of treated survivors than untreated survivors. The treated sample showed an increase of 65 microorganisms/mL which may be promoted by the heat. The other storage days showed a decrease of two to three log cycles from the control sample to the treated sample.

5.3.3 Sporeformers

The next most prominent group of survivors were the sporeforming bacteria. The percentage of survivors was very similar to the thermophiles and when the milk was stored for one day, the sporeformers had the greatest survival rate. It was 53.2 % of the total surviving population and as storage time increased the survival rate dropped to 31.2% and finally

to 48.4% (Figure 5). The highest survival rate for a typical run was 80 sporeformers from a control sample of 120 C.F.U./mL (Table 2). The electrical current was able to reduce the microorganisms. The bacteria treated by the electrical current however, did show signs of growth during heating.

5.3.4 Psychrotrophs

The psychrotrophic bacteria are the most predominant group of bacteria that spoil refrigerated milk. The electrical current had a great effect on the psychrotrophic bacteria. The survival rate was normally 0% of the total surviving population. A typical run of milk, (Table 2) illustrated no growth for freshly stored milk and only two survivors for milk stored for nine days. This was significant considering the control for this storage period was 23 million C.F.U./mL.

5.4.0 EMPHASIS ON ELECTRICAL PASTEURIZATION

The flow rates were consistent throughout the run but were different between runs. Therefore, the effective

treatment power (E.T.P.) was the factor that was used to compare each run with another. Overall the kill rate for the mesophilic and psychrotrophic bacteria was consistent. The electrical current generated tremendous kill rates against the psychrotrophs.

5.4.1 Mesophiles

5.4.1.1 Day One of Stored Raw Milk

The microorganisms isolated as mesophiles illustrated different effects for each day of storage. The increase in storage time increased the control bacterial counts but the electrical current had the ability to inactivate the number of survivors consistently and keep them low. Electrically treated milk had 140-600 C.F.U./mL mesophilic bacteria compared with 5000-400,000 C.F.U./mL (Table 3) for raw milk after one day of storage.

5.4.1.2 Day Three of Stored Raw Milk

The increase in storage time did increase the control population and therefore, an increase in mesophilic survivors. The mesophilic bacteria illustrated the inability to survive the electrical current and were reduced to similar

Table 3. Comparison of Mesophilic and Psychrotrophic Organisms in Electrically Treated Raw Milk (1 day storage)

		MICROORGANISMS (C.F.U./mL)			
FLOW RATE mL/min	POWER (W mL^{-1} min^{-1})	ELECTRICAL SURVIVORS MESO	PSYCHRO	RAW MILK CONTROL MESO	PSYCHRO
39	4.40	220	0	200,000	600,000
	4.50	150	0		
	4.70	220	0		
55	3.57	270	0	5,000	400
	3.59	210	0		
	3.59	260	0		
40	3.27	330	0	400,000	6,000
	3.27	600	15		
	3.29	490	0		
42	3.94	400	0	300,000	4,200
	3.92	190	0		
	3.93	140	0		

Table 4. Comparison of Mesophilic and Psychrotrophic Organisms in Electrically Treated Raw Milk (3 days storage)

		MICROORGANISMS (C.F.U./mL)			
FLOW RATE (mL/min)	POWER (W mL^{-1} min^{-1})	ELECTRICAL SURVIVORS MESO	PSYCHRO	RAW MILK CONTROL MESO	PSYCHRO
46	3.95	260	8	9,000,000	5,600,000
	3.95	260	1		
	3.95	200	0		
48	3.90	270	0	8,800,000	4,000,000
	3.89	160	0		
	3.94	150	0		
48	3.35	220	0	10,000	11,000
	3.50	190	1.5		
	3.51	310	1.5		
42	3.93	110	0	17,000	10,000
	3.93	100	0		
	3.96	80	1		
55	3.40	10,000	6	5,000	4,000
	3.38	3,000	0		
	3.47	3,800	3		
35	3.77	1,200	0	4,600	3,500
	3.94	650	1		
	3.95	600	0		

Table 5. Comparison of Mesophilic and Psychrotrophic Organisms in Electrically Treated Raw Milk (9 days storage)

		MICROORGANISMS (C.F.U./mL)			
FLOW RATE (mL/min)	POWER (W mL^{-1} min^{-1})	ELECTRICAL SURVIVORS MESO	PSYCHRO	RAW MILK CONTROL MESO	PSYCHRO
38	3.66	13	0	140,000,000	24,000,000
	4.08	6	0		
	4.33	11	0		
38	3.92	1	500	350,000	8,500,000
	4.10	15	19		
	4.15	20	40		
38	4.21	70	2	26,000,000	21,000,000
	4.22	50	0		
	4.22	80	0		

colony forming units, as observed in milk stored for one day. The treated sample was double that of the control sample but the E.T.P. was very low, therefore, indicating improper heating and electrical exposure. Most samples had C.F.U./mL varying within zero to two log cycles. This was consistent with the trend (Table 4).

5.4.1.3 Day Nine of Stored Raw Milk

The storage of milk for nine days changed the condition of the milk dramatically. The mesophilic population had dwindled to one log cycle where the number of survivors was 80 mesophiles compared to a control of 26 million C.F.U./mL (Table 5). The best kill rate was illustrated in the first trial where the mesophilic isolated microorganisms were six, eleven and thirteen organisms per mL. These survivors came from a control sample of raw milk which contained 140 million microorganisms. The results were excellent and illustrated the ability of the electrical current to inactivate microorganisms very effectively even under high microbial loads.

5.4.2 Psychrotrophs

5.4.2.1 Day One of Stored Raw Milk

The bacteria that illustrated psychrotrophic characteristics were difficult organisms to isolate. Most trials showed no indication of growth for psychrotrophic microorganisms. The milk stored for one day had a trial with 15 microorganisms but the other trials on that particular day had no growth (Table 3). This could be an overestimation of the survivors. The overestimation could be due to mesophiles growing at low temperatures or human error. For freshly stored milk the kill rate for psychrotrophic bacteria was 100%.

5.4.2.2 Day Three of Stored Raw Milk

Similar patterns were shown for milk that had been stored for three days. The increase in storage time did not affect the ability of the electrical unit to reduce the bacteria. The surviving populations were similar to the milk that had been stored for one day. The highest survival rate was an average of three psychrotrophic microorganisms compared to a control of 4000 C.F.U./mL (Table 4). This was the same trial, indicated previously, that had been

inadequately treated. It was difficult to select a high kill rate because most were single survivors. A reduction of the survival rate to zero was the ideal goal. Therefore, the electrically treated samples were effectively reduced from a larger population in the control sample.

5.4.2.3 Day Nine of Stored Raw Milk

There were no surviving psychrotrophic bacteria when the milk was stored for nine days. One trial did show survivors of 500 C.F.U/mL but this was a poor example of how well the electrical current can perform for aged milk (Table 5). The rest of the trials showed no survivors which is in agreement with previous experiments shown in Figures 4 and 5. Therefore, excluding that one trial, it can be stated that the electrical current was able to reduce the psychrotrophic population to zero.

5.5.0 ANALYSIS OF TREATMENTS FOR SENSORY EVALUATION

5.5.1 High Temperature Short Time Treatment

Milk treated by HTST was obtained the same day the raw milk was batch pasteurized and treated with electricity. The ANOVA showed a significant difference between the treatment weeks because the f-value was below .05. The Duncan's test

showed that as the milk aged the panelists preferred the milk less. It also illustrated that weeks one and two were similar and weeks two, three, and four were similar. Therefore, weeks one, three and four were different when compared with each other.

5.5.2 Thermally Treated

The thermally treated milk was also studied over the four week period. The f-value indicated that the difference between weeks were not significant. The most preferred sample of milk was the second week. Week one, three and four were the next preferred, respectively. The Duncan's test showed that all four weeks were similar to each other over the four week period.

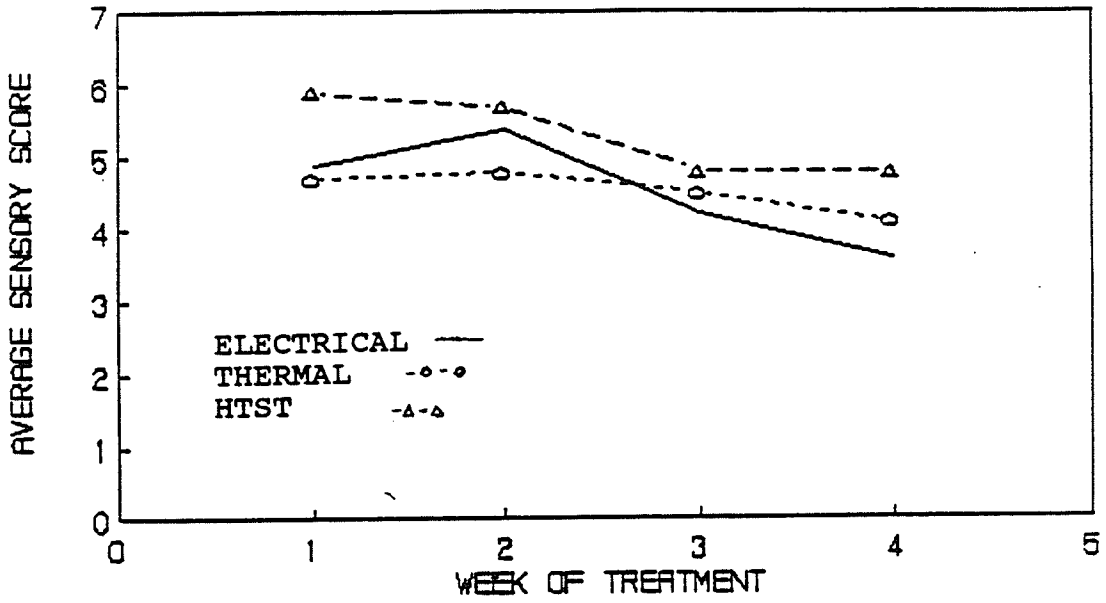
5.5.3 Electrically Treated

The electrically treated milk was similar to the HTST treated milk in that the ANOVA showed a significant difference among the four weeks. The f-value was below .05 making the values obtained for electrically treated milk different. The Duncan's test indicated that the most preferred results were from week one and the least preferred from week four. Weeks two and three were the second and third preferred, respectively. The Duncan's test also illustrated the similarity among weeks. Weeks one, three and four were similar. That meant week two was different from week three and four; and week one was different from week four. Therefore, as the milk aged there was a significant difference among specified weeks for the Duncan's test.

5.5.4 Comparison Among Treatments

The HTST treated milk was consistently the most preferred milk in each week that was tested. The batch pasteurized and electrically treated milk samples were less preferred. The batch pasteurized milk was the least preferred for the first two weeks, then the electrically treated milk was the worst for the last two weeks. According to the Duncan's test, all types of treatments were similar to each other. Therefore, at the end of the test period all three samples were spoiled. The only significant difference was between the electrical and HTST treatd milk samples. The HTST and electrically treated samples were preferred samples in the early stages of the experiment. The batch pasteurized samples were the least preferred throughout the experiment. The electrically treated milk did spoil at a faster rate than the other two samples (Figure 6). However, it should be noted that these conditions still did not make any treatment significantly different from one another. The microboilogical results were different from one another; therefore, the off odor must have been caused by the chemical breakdown of the components found in the milk. These off odors only occurred in the thermally treated milk.

Figure 6. Comparison Among Treatments Versus Average Sensory Score



6. DISCUSSION

6.1.0 THERMAL TREATMENT VERSUS ELECTRICAL TREATMENT

Many trials were rejected before reproducible results were obtained. The work represented here draws on the positive final results generated during this study. These results represent about 30 % of the total work effort.

The destruction of the mesophiles and psychrotrophs was the biggest advantage for pasteurization. The thermal treatment was able to reduce the spoilage organisms which were also the most prevalent microorganisms found in raw milk. The electrical treatment also inactivated most of the mesophiles and usually all of the psychrotrophic microorganisms. The other microorganisms isolated from these treatments were difficult to inactivate which could also indicate the highest survival rate. In an overall comparison of the two treatments, the electrical pasteurization process was able to reduce the survival of sporeformers and thermophiles more efficiently. This suggests a different mechanism of kill over thermal effects alone.

Milk stored for one day was considered fresh milk because the raw milk was obtained from the dairy on Monday or

Thursday afternoon, then processed the next morning. Each run had a duration of approximately six hours; therefore, the day after delivery was the starting point. The bacterial counts from the raw milk varied over a range of four to five fold, which represented a fairly normal range for raw material such as milk. Therefore, the calculation of percentages allowed for this discrepancy in the control. The percentages were calculated by the summation of all the microorganisms present within the milk. There was no treatment involved with these samples; therefore the survivors were the bacteria who outcompeted the other natural microflora.

The age of the milk was the only variable in these experiments. Each incoming bacterial load was compared directly to the previous or next delivery. Therefore, all the microorganisms were calculated as percentage values to compare the raw milk used in each of the two treatments. Each day of storage had its own summation whereas the largest percentage of raw milk survivors was the longest storage time.

The percentage of total survivors was very deceiving and therefore the total bacterial count was useful. The percentage of survival rate was useful for a quick summation of raw milk. One misconception was the growth of the psychrotrophs, sporeformers and thermophiles. It seemed that

as the milk aged the growth of the three previously mentioned bacterial groups decreased. The reality of this statement was that the sporeformers and thermophiles decreased from day one to day three but increased by the time the storage period reached nine days. The psychrotrophs, sporeformers and thermophiles seemed to decrease because of the tremendous increase in mesophilic bacteria. The initial decreases in sporeformers and thermophiles were attributed to the shock of milk being stored at refrigeration temperature. The cold temperature probably inactivated some of the more heat resistant bacteria but as the storage time increased the population of the sporeformers and thermophiles slowly increased.

The percentage system was also established to compare the raw milk population with the survival rate of the thermally and electrically treated milk. The percentage of survivors of the total population was the only parameter used to compare the three types of treatments. The survival rates of the thermal and electrical pasteurization treatments were similar on a mean of fifteen trials each. The W mL^{-1} min^{-1} were different because the flow rate of the unit was varied. It was adjusted to illustrate that the unit could run at varying speeds. Therefore, to keep the temperature constant, the applied voltage was altered and thus the change in W mL^{-1} $min.^{-1}$ Each group of survivors was calculated as a percentage over its individual control. These percentages were averaged and

the survival rate of the total population was obtained.

Thermal treatments on raw milk were consistent, such that all samples were heated to 63°C for 30 min. All equipment was kept the same. The bacterial counts became so consistent that they were predictable. The figures show percentage of survivors and growth rate during a typical thermal treatment run of milk. The survival rate percentages were the average of the percentage for each individual run. The survival rate percentage of the total population gives a quick view of the trends for each particular treatment. The bacterial counts for a typical run must always be compared with the percentages to keep things in proper perspective.

The first day of storage resulted in a tremendous kill rate for the thermal treatment on the mesophilic microorganisms. The mesophiles were by far the most prominent bacteria found in raw milk; therefore, one would expect a good survival rate. The heat treatment by batch pasteurization was perfect for milk that maybe left out at room temperature for short periods of time. The mesophilic bacteria can grow at refrigeration temperatures; they also grow at a geometrical rate once the temperature climbs above 7°C. Therefore, if milk was left out of the refrigerator, the spoilage by mesophilic bacteria will be slower with milk treated by batch pasteurization than raw milk. The survival percentages became lower as the milk aged but this was the result of other organisms becoming more predominant. The

bacterial counts of a typical heat treated run indicated the consistency of the thermal treatment. The survivors never surpassed 3000 C.F.U./mL, even though the control for nine days of storage usually reached the millions or tens of millions C.F.U./mL. The fresher milk always showed a better reduction in survivors. There were two factors that were prevalent in the explanation of this occurrence. The fresher milk had a lower initial load and, therefore, had a lower number of survivors. However, if the raw milk received from the dairy had a higher initial load, then there were more survivors when compared to similar milk samples with the same storage period. Therefore, one would assume that a higher microbial load gave a higher survival rate. The results indicate that older milk spoiled more quickly with higher initial bacterial content, thus resulting in a harsher environment (e.g. more acidic), for the bacteria. The treatment of these organisms under this stress gave a better kill rate than other milk samples stored for nine days.

The microorganisms that were isolated with sporeforming and thermophilic characteristics were the most consistent of thermally treated milk survivors yet were the most difficult to inactivate. The thermophilic microorganisms survived well at 63°C. The controls of raw milk were very similar to the treated samples. The control samples had been heated to 63°C once and the thermally treated samples had been heated to 63°C twice. It can be concluded that the organisms that

survived the first treatment at 63°C were thermophiles because a second treatment at the same temperature was non-effective for killing anymore bacteria with thermophilic characteristics. The survival rate percentage indicated that the thermophiles were the most prevalent survivors except for the longest stored milk. The highest rate of survival occurred when the milk was stored for three days in which 60.5% of the survivors were of thermophilic nature. The heat treatment for one specific experiment validated these findings in that the treated samples outnumbered the control sample. The treated sample contained 60 more thermophiles/mL than the control sample. This was explained as heat promotion or heat generation. When the samples were exposed to 63°C, the heat actually promoted the thermophilic organisms to grow. Therefore, each treatment at 63°C would increase the numbers of bacteria/mL that were isolated as thermophiles.

The sporeformers exhibit similar characteristics to the thermophiles but to a lesser degree. They were very difficult to inactivate because they were spores and sporeforming organisms which grew well above 63°C. Therefore, there was a minimal reduction in bacterial growth. Theoretically the sporeformers should survive the conventional batch pasteurization but some do not and others actually grow better. The sporeforming organisms were under stress and a

temperature of 63°C was enough to inactivate some of the organisms, possibly the vegetative forms. The stress may be from the age of the milk in which the environment had become harsher. The survival rate illustrated that as the milk aged the survival of spores increased. This was attributed to the growth of sporeformers during the storage of the raw milk.

The milk stored for one and three days both showed approximately a 40 % survival rate of the total population. The typical trial (a trial chosen at random) of milk showed that the treated samples were very similar to the control samples therefore indicating a excellent survival rate. The biggest change was when the milk was stored for nine days and the survival rate jumped to 75.6% of the total population. This was an indication that the sporeformers increased in numbers as well as resistance as the milk aged. This was possibly due to the vegetative organisms forming spores. The typical trial of milk illustrated that the sporeformers also underwent some growth promotion. The survival rate for milk stored for nine days was six to seven fold. Therefore, there was no kill established but a tremendous growth appeared which was common for the thermally treated sporeformers.

The psychrotrophs were easily destroyed by thermal pasteurization, but some interesting factors did arise from the thermally treated samples. The resistance of psychrotrophs in freshly stored milk was a major concern. The

raw milk which had been stored for one day had a low initial bacterial load. Therefore the number of survivors should be low as well. The survival percentage was 9.1% of the total population for the psychrotrophs. This may be misleading because it was a percentage of total survivors; however, one must consider the fact that the mesophilic and psychrotrophic controls were similar. The control samples for both groups of microorganisms were the same log cycle and yet approximately 10% of the psychrotrophs were not affected. The other interesting factor was the inconsistency of psychrotrophs when the milk reached nine days of storage. A typical trial of raw milk illustrated that the heat was relatively ineffective in inactivating the psychrotrophs. The numbers of bacteria in the control sample were infinitely large and a good kill rate was still accomplished. However, the number of survivors outnumbered the survivors from the other days of storage.

The electrical pasteurization system was able to reduce microorganisms below thermal pasteurization levels. The most significant group of organisms affected by the electricity were the psychrotrophs. The mesophiles were reduced tremendously by the electrical current but the sporeformers and thermophiles showed opposite characteristics. The sporeformers and thermophiles were usually reduced but still consisted of 94 to 99 % of the total surviving population.

The microorganisms that were isolated with mesophilic characteristics seemed to show a good kill rate. The percentage survival illustrated that the highest survival was when the milk was stored for three days. The mesophiles made up 6.1 % of the total surviving population. With the other days of storage, the mesophiles consisted of less than 2% of the total population. This was more significant considering the size of the initial population of the mesophiles in the control. The electricity did not reduce the survivors as well as in the thermally treated samples except when the milk became older. Fresh milk when electrically treated gave similar results to the thermally treated samples. The point of interest was when the milk was older.

It had been assumed that when the milk became older there were harsher conditions in the milk. Therefore, the exposure to heat and electricity may explain the increase in kill rate with older milk. The increase was deceiving because it was really the consistency of the electrical treatment trial that is important. The electrically treated samples reduced the survivors to a logarithm of one or two whereas the thermally treated samples were reduced to only two to four log cycles. Therefore, in addition to being more effective on high microbial loads, the electrically treated milk had less survivors as the milk aged.

The thermophilic bacteria were the most difficult organisms to inactivate when the electricity was applied. They had the greatest survival percentage and often showed heat promotion of the microorganisms. The temperature used for electrical pasteurization was similar to the optimum growth temperature of the thermophilic microorganisms. However, the electrical current seemed to show an effect on the microorganisms as well. The typical trial on raw milk showed lower numbers of bacteria in the samples which were treated with electricity. Results indicate that the electrical process reduced the bacterial load below the thermally treated samples in every situation. Therefore, the thermophilic microorganism, which were the major survivors and were difficult to inactivate, were more effectively reduced by alternating current than thermal treaments.

The sporeforming microorganisms consisted of the next largest group of survivors and were almost as difficult to inactivate as the thermophiles. The lowest survival percentage occurred when the milk was stored for three days but was approximately 50% of the total surviving population for the other days of storage. This was surprising in the fact that the sporeformers survived best in comparison with other bacteria when the milk was stored for fewer days. The sporeformers should have had a higher percentage as the milk aged, but the electricity played an important factor. There were more survivors as the milk aged but the control also

increased. Therefore, one can conclude that the factors that assisted the thermally treated sporeformers are not necessarily true for this scenario. The electrical current was able to inactivate more microorganisms than the thermally treated samples. This is probably based on the properties of membrane damage caused by electrical currents (Sale and Hamilton, 1967).

The last group of microorganisms that were isolated were the psychrotrophic bacteria which represent the greatest potential long term spoilage organisms for refrigerated milk. The psychrotrophs showed no growth when exposed to the electrical current; however, there were times when one or two colonies were found on the plate giving a survival percentage of 0.1%. But as the milk aged the survival rate decreased to lower the total percentage to 0%. The random treatment day showed growth of survivors but these may not be psychrotrophs. It was such a low survival rate that there was no concern. In refrigerated milk, mesophiles can grow at low temperatures. Therefore, it is possible that these few colonies were not psychrotrophs at all. Whatever the case, the low survival rate was significant and was less than the survival rate of the thermally treated samples.

The excellent kill rate established by the electrical system warrants further study on the psychrotrophic bacteria. The sporeformers and thermophiles had consistent results in both pasteurization processes and it was accepted

that the survival of these two groups were attributed to their optimum temperatures being at 63°C or higher. The mesophilic microorganisms were inconsistent throughout the various experiments and this caused some concern. Therefore, to obtain a better understanding of the mesophiles and psychrotrophs, more experiments on raw milk were carried out using electrical current only.

6.2.0 EMPHASIS ON ELECTRICAL PASTEURIZATION

Further research with electricity on raw milk gave similar results. It strengthened the theory of total destruction for psychrotrophs. There was an effective reduction in mesophilic organisms. They were usually reduced to two log cycles whether the raw milk control was four or nine log cycles. Therefore, there were some persistant mesophiles that were difficult to inactivate.

The raw milk that was stored for one or three days showed these characteristics, but the milk stored for nine days differed. The electricity was able to reduce the mesophilic microorganims more effectively as the storage time increased. This could have been due to the harsher conditions found in older milk or a different mechanism of inactivation. The mesophiles must have been under stress which made them easier to inactivate.

The psychrotrophic bacteria were easily destroyed by the electrical current. Most experiments showed there were no survivors that grew at refrigeration temperature. There were few microorganims shown on the plates but these were not psychrotrophs. The results clearly indicate the ability of the electrical current to totally reduce the psychrotrophs. Therefore, these survivors could be other types of bacteria such as mesophiles which can survive electrical pasteurization and grow at refrigeration temperature.

6.3.0 SENSORY EVALUATION DISCUSSION

The microbial assessment was performed every week on each treatment to compare with the sensory evaluation (Table 6). The thermally or batch pasteurized milk showed the lowest C.F.U./mL for each week. The raw milk was pasteurized in a steam kettle. The kettle kept inconsistent temperatures, plus or minus 5°C, which could have accounted for the variation in microbial assessment. After four weeks of storage the thermally treated milk had bacterial counts that were lower by two log cycles from the HTST and electrically treated milk.

The HTST treated milk was less efficient than the thermally treated milk at reducing mesophilic and psychrotrophic microorganisms. The HTST treated milk was also similar to the electrically treated milk.

The electrically treated milk was similar to the HTST treated milk but it did have the highest bacterial counts after the second week of storage. These figures were all within one log cycle and were not different from the HTST treated milk.

Overall all milk samples were similar throughout the week by microbial characterization. The batch pasteurized milk was slightly better but not significant enough to make a difference.

Table 6. Microbial Assessment of Batch, Electrical and High Temperature Short Time Treatments used for Sensory Analysis

DATE	TREATMENT	MICROORGANISMS	
		MESOPHILIC	PSYCHROTROPHIC
		COUNT (C.F.U./mL)	COUNT (C.F.U./mL)
FEB. 12			
	HTST	5,000	2,000
	THERMAL	70	3.0
	ELECTRICAL	110	5.0
FEB. 19			
	HTST	700	5,000
	THERMAL	500	30
	ELECTRICAL	19,000	24,000
FEB. 29			
	HTST	320,000	600,000
	THERMAL	3,000	1,600
	ELECTRICAL	5,600,000	2,500,000
MAR. 06			
	HTST	> 10,000,000	> 10,000,000
	THERMAL	700,000	690,000
	ELECTRICAL	> 10,000,000	> 10,000,000

6.3.1 Between Weeks

The quality assessment of the pasteurized milk was subdivided into two sections. The first section attempted to illustrate the difference between the storage times over a four week period for each treatment. The second section was the difference between the types of treatments over the same four week period.

The analysis of variance or ANOVA was performed on all trials to establish the f-value. The f-value, if below .05, indicated that there was a difference between the two parameters. In fact, if the paramenters were so diverse it was known as significantly different. This was the case for the milk that was treated by HTST and electricity. The thermally treated milk on the other hand was not significantly different throughout the weeks.

The significance was relative to the panelist's preference of the milk. The preference for older milk should be less than the fresh milk. The aroma of the electrically and HTST treated milk was noticeable and there was a significant difference. However, the thermally treated milk also developed off odors and these were noticed by the panelists. The odors were not significantly different from

the beginning of the evaluation and at the end of four weeks. Therefore, it could be deduced that the thermal treatment may have inactivated some of the flavor compounds.

It was shown that the panelists could tell the difference between new and older electrically pasteurized milk. This was also true for the HTST treated milk. Overall the electrically treated milk was similar to the other continuously processed milk.

6.3.2 Between Treatments

The analysis of the evaluation between types of treatments illustrated why the thermally treated milk was not significantly different between weeks. The thermally treated milk was the worst sample at the beginning of the trial and remained poor. Therefore, heating to 63°C for 30 min developed the worst sample as far as smell was concerned. The bacterial load was lowest for the thermally treated milk, therefore the heating must have been harsher for the batch pasteurized milk.

The HTST treated milk was very consistent and did not seem to spoil as quickly as the other two treatments.

The electrically treated milk was discouraging in the fact that it spoiled quicker than the other two treatments and quicker than expected. This does compare to the microbial

analysis in that there were more C.F.U./mL. The positive aspect was the ANOVA did not show a significant difference between all three treatments. Therefore, the panelists could distinguish only a slight difference between the treatments but not enough to make it significantly different from one another.

7. CONCLUSION

Electrical current has been shown to inactivate microorganisms in milk. The potential spoilage organisms, psychrotrophs, were reduced to zero in almost all cases. The orginal thought was to reduce bacterial counts in the milk to a level that would be suitable for secondary dairy products. It was shown that electrical pasteurization of milk could be equal to conventional HTST pasteurization. Therefore, this pasteurization procedure for milk would appear to have the potential, with further optimization, for providing sterile milk products.

The electrical process was especially effective in the control of psychrotrophs. These bacteria represent a continuing problem for the dairy industry. Mesophiles, sporeformers and thermophiles were also reduced effectively by the electrical current.

The effective use of electrical energy in the pasteurization of milk is much less dependent on the overall microbial load in the product than is thermal pasteurization.

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