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THERMODURIC & PSYCHROPHILIC BACTERIA
FROM MILK OBTAINED FROM THE ADDIS ABABA DAIRY DEVELOPMENT
ENTERPRISE

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ABSTRACT

An attempt has been made in this study to isolate & identify thermophilic & psychrophilic bacteria, which are the most serious spoilage organisms, from milk obtained from tin? Addis Ababa Dairy Hovelpoment Enterprise (AADDE). An effort was also made to trace the sources & origin of these organisms so as to prevent or at least reduce their access into milk & hence increase the shelf-life of the pasteurized milk & milk products.

The standard plate count (SPC) ranged between 4×10^7 & 3×10^9 bacteria/ml for raw milk, between 6×10^4 & 3×10^6 for plant pasteurized milk & between 3×10^3 & 2.5×10^5 for laboratory pasteurized milk. The high standard plate count for the plant pasteurized milk, as compared to that of the laboratory pasteurized milk, has been found to be due to the ability of many organisms to gain access to the pasteurized milk as post pasteurization contaminants.

Psychrophilic bacteria were the most dominant organisms in both raw (98.1%) & plant pasteurized (53.0%) milk samples. Laboratory pasteurized milk, on the other hand, contained predominantly thermophilic bacteria.

During the course of study, bacteria belonging to 14 genera were isolated, the most dominant being: Streptococcus (27%), Staphylococcus (19.5%), Micrococcus (13.9%), Bacillus (12.9%) & Arthrobacter (11.3%). Out of the 185 representative isolates identified 50% were cocci & 40% rods.

As many as 16 species of bacteria belonging to 9 genera were isolated from raw milk, while 20 species of bacteria belonging to 14

gonora were isolated from the plant pasteurized milk. This indicated that bacteria, other than those found in raw milk have been introduced into the pasteurized milk as post pasteurization contaminants from the bulk tanks, fillers & their pipelines and the plastic bags that contain pasteurized milk. This fact has also been supported by data from keeping quality tests performed at different sampling sites.

Sodium hydroxide (NaOH) & sodium carbonate (Na₂CO₃), chemicals regularly used for rinsing & disinfecting utensils at the Addis Ababa Dairy development Enterprise, were found to be ineffective in inactivating bacteria at concentrations as high as 1:100 at 50-90 minutes holding time. Other disinfectants tested such as Savlon, & Chlorox, on the other hand, were found to be effective in eliminating the same group of bacteria at lower concentrations (1:100) at 15-30 minutes holding time.

Based on this study, therefore, recommendations are made by which the Dairy Plant can improve milk handling & processing conditions & produce milk & milk products with improved shelf-life.

1. INTRODUCTION

Milk and milk products have been consumed by man even before the dawn of civilization. Because of its high nutritive value, milk is considered as one of the most important diet items of many people. In addition to protein, fat, carbohydrate & Ca, milk serves as a significant source of many vitamins & trace elements (Pederson, 1979; Scott et al, 1981).

Bovine milk contains specific & non-specific antibacterial factors which may play a role in protection of the neonate from enteric infections (Reiter, 1978). One of the most potent antibacterial factors of milk is the lactoperoxidase system. This system has been activated for use in milk preservation (Bjorck, 1978) & to improve performance of calves fed on raw milk. Activation of the lactoperoxidase system results in generation of intermediate oxidation products of thiocyanate, including the hypothiocyanate, which are bactericidal to many Gram-negative bacteria (Ekstrand, Ertugan & Waterhouse, 1981). This activity is strong in fresh milk but the inhibitory effect is lost after a few hours.

Species belonging to lactic acid bacteria such as Streptococcus lactis, Streptococcus cremoris, Lactobacillus casei and the higher acid producing species of the genus Lactobacillus are important microorganisms which bring about desirable changes. On the other hand, species of the genera Escherichia, Enterobacter, Micrococcus, Bacillus, Serratia, Pseudomonas, Alcaligenes and others are undesirable because they are responsible for various forms of spoilage such as gassiness, putridity, fruitiness, bitterness,

proteolytic conditions, rancidity, ropiness or other forms of spoilage. These bacteria act on the different components of raw milk as soon as it is collected from the animal if unless the milk is maintained at reduced temperature or boiled, it spoils right away.

Post pasteurization contamination (PPC) due to psychrotrophic Gram-negative bacteria is the most significant factor responsible for reducing the keeping quality (KQ) of refrigerated pasteurized milk if cream (Phillips, Griffiths if Muir, 1981; Schroder, Cousin if McKinnon, 1982; Schroder, 1904).

Thermotolerant organisms that survive pasteurization become important only when they are psychrotrophic or if the product is kept at ambient temperature for several hours (Cox, 1975; McKinnon if Pettipher, 1983).

Normal milk contains low bacterial counts as it leaves the udder of a healthy animal, but gets contaminated later from the environment, exterior of udder, manure, water, soil, milker's hands if utensils. Contaminated milk can serve as a source if/or vehicle for the spread of certain bacterial diseases like tuberculosis, diphtheria, brucellosis, salmonellosis if food poisoning if intoxication if it is consumed raw (Isani, Sheikh if Arain, 1936). Milk at most places in Ethiopia is consumed raw. Milk products such as yoghurt, butter and butter milk are also produced using raw milk as starting material. Hence, there exists the possibility of consuming milk which has been contaminated with disease causing organisms.

According to Gebhardt if Nicholas (1975), rami milk may contain not more than 7.5×10^4 to 2×10^5 bacteria per ml by the Standard plate count (SPC). Pasteurized milk may not have more than 1.5×10^4 to 2.5×10^3 bacteria per ml by the SPC at the time of delivery. As Brock

ft Brock (1978) put **it**, the bacterial count of pasteurized milk generally ranges from 2,000 to 20,000 bacteria per ml, whereas the bacterial count of raw milk will generally range from 500,000 to 3,000,000 bacteria per ml. Standards based on long term research findings in Ethiopia are lacking. The Addis Ababa Dairy Development Enterprise (AADDE) doesn't have standards for raw ft pasteurized milk. And without such bacteriological standards **it** becomes impossible to implement quality control programs.

f In countries with poor milk production ft marketing practices, one can expect high **initial** viable bacterial counts posing as a health hazard as well as spoilage of large quantities of this important food (Bari ot al., 1986). The actual methods of storage on the farm ft mode of transportation to the processing plant do not prevent nor delay microbial activity in the raw milk in our country. Pasteurization ft post pasteurization handling ft marketing of milk has **its** own problems, hence the KQ is also low. Due to either surviving thermophilic bacteria which are also psychrotrophic ft/or post pasteurization contamination, the shelf **life** of pasteurized milk at ambient or refrigeration temperature decreases through time.

It was those important reasons, then, that have led to the need of the present study. The primary objectives of **this** study, therefore are :

1. To investigate the sanitary aspects of the milk processing equipment, containers, pasteurization ft storage parlours in use at the AAOE.
2. To follow up the overall processing of milk in the AAOE, starting from the collection of raw milk until the processed product reaches the consumer ft investigate the sources of contamination.

3. To monitor the contributions made by the different types of equipment used in the pasteurization process to the final microbial load in the milk.
- d. To estimate the range in microbial load of : a) raw milk collected from collection centers b) raw bulk milk which is about to be pasteurized c) pasteurized milk processed at the Dairy Plant.
5. To isolate ft identify the major contaminating microorganisms which are responsible for reducing the KQ of the milk.
6. To propose ways ft means of increasing the shelf-life of pasteurized ft raw milks after identifying the sources of contamination.
7. To recommend standards which are attainable for raw ft pasteurized milks based on this study,
8. To recommend on the use of certain chemicals at specified concentrations for use at the Dairy Plant as disinfectants which might help **it** to produce milk ft milk products with longer shelf-life ft acceptable flavor to its consumers.

2. LITERATURE REVIEW

2.1. MILK & ITS KEEPING QUALITY

Many industries have gone to great lengths to develop milk products with long shelf-lives. Changes in on-farm procedures of milk collection, longer refrigerated storage of raw milk at the farm, plus additional storage periods in bulk silos prior to processing, may contribute to decreased shelf-life of pasteurized milk.

Previous investigators have found that taste defects of pasteurized milks occur when bacterial counts reach 10^8 /ml. Other workers have suggested a count of 10^7 /ml for similar results (Langveld *et al.*, 1976; Schroder, Cousin & McKinnon, 1982; Schroder, 1984). Rodrigues & Pattipher (1984), on the other hand, defined KQ as the time taken for the bacterial count of the refrigerated milk to reach an intermediate count of 5×10^6 /ml.

The KQ of milk is influenced by a great number of factors, many bearing upon the milk's microbial content & the conditions that enhance or inhibit growth of the milk's microbial flora. In spite of our knowledge of the microbiology of milk & milk products, KQ is difficult to predict on the basis of laboratory estimates of the microbial content of freshly pasteurized milk (Hankin & Stephens, 1972).

There is continuing interest among milk processors who wish to extend the shelf-life of milk & milk products through the use of higher processing temperatures & also prevent recontamination by psychrotrophic bacteria after pasteurization. Maintaining the quality of raw milk to extend the shelf-life of milk & milk products is of greater importance. It has been said that "You cannot make a better finished product than the original starting product." Controlling

microorganisms in raw milk is of prime importance because this is the starting product (Janzen et al., 1982), Janzen (1972) suggested that **shelf-life** of pasteurized milk is influenced by somatic cell concentration in raw milk. High somatic cell concentration in raw milk is indicative of abnormalities in the cow that is frequently associated with increase in microbial population.

The KQ of milk & dairy products is increased by a reduction in storage temperature. This is because of the reduction in the growth rates & biochemical activities of most bacteria at reduced temperatures. At low temperature storage, however, psychrophiles increase markedly & ultimately are the cause of spoilage. These observations have led to the conclusion that KQ & psychrotrophic count are closely related, because of these reasons, the SPC (at 32°C) has been shown to be of **little** value for predicting the KQ. At times the psychrotrophic count at 7°C for 10 days may also be of **little** value.

This is because a low level of contamination with a biochemically active, rapidly growing psychrotroph results in a poorer KQ than that resulting from a relatively large number of biochemically inert or slowly growing psychrotrophs. Many workers give emphasis to the type of contaminating organism rather than to the number in evaluating KQ (Witter, 1961).

Product temperature is a major factor influencing **shelf-life**. The relatively high temperature of fluid dairy products in many store coolers, dairy cases & home refrigerators is responsible for reduced KQ. Product temperature must remain between 4-5°C for maximum **shelf-life**. As a general rule, for every 2.8°C rise in temperature, **shelf-life** is reduced by about 50% (Parnard, 1972).

2.2. MILK QUALITY EVALUATION

While there is almost universal agreement concerning the desirability of having high quality raw milk, there appears to be a decided lack of agreement as to the best way of determining the quality of this milk. Because of the variability of psychrotrophic bacteria that can get into milk, & the enzymes they produce, as well as the interacting effects of other microorganisms, somatic cells, inhibitory substances & temperature, it is probably impossible to devise a single test of milk quality that can have all the desirable attributes (Marshall & Harmon, 1978).

The SPC has been used as an index to ascertain the bacteriological acceptability of raw milk. Johns (1975) argues that the psychrotrophic bacterial count (PBC), serves as a better indicator of whether or not milk has been produced under sanitary conditions. However, the PBC, which calls for a 10 day incubation at 7°C, is considered by most to be impractical from a time standpoint. But they still regard it as the "standard" to which the newer, more rapid tests could be compared (White et al., 1978; Smith & Witter, 1979).

Two of the more promising tests, which are basically designed to measure the number of psychrotrophs in milk are : a) Preliminary Incubation (PI) of samples at 13°C for 18 hours followed by SPC, & b) Microscopic Colony Count (MCC) - a test perfected by Juffs & Pabel (1975).

A third test known as the Roseley test has also been widely applied & generally accepted as an indicator of KQ of milk. In this test containers of freshly processed milk are stored in a refrigerator

at 7°C for 5 or 7 days after making an SPC & then replated. Large increases in bacterial count between the first & second plating suggest that KQ problems can be expected during refrigerated storage of the product. However, the time needed to obtain results renders the Moseley & other tests rather impractical for monitoring the quality of a perishable product (Bishop, White & Firstenburg-Eden, 1984).

The microbiological techniques discussed earlier cannot be employed for immediate use as test results are not available until several days after the milk products have been shipped to consumers (Johns, 1969; Hartley, Vadamuthu & Reinbold, 1969; Blankenagel, 1976). There is obviously a need for a rapid test that would provide reliable estimates of milk shelf-life within a time period which would allow for effective corrective measures to be taken. The time frame should preferably be less than 48 hours (Patel & Blankenagel, 1972; White & Little, 1983).

Continuous impedance measurements during the incubation period of microorganisms has permitted dairy microbiologists to evaluate the degree of bacterial contamination (Bossuyt & Maes, 1983). Impedance change is brought about as a result of chemical changes which alter the electrical resistance (impedance in an AC circuit) of milk (Cady et al., 1978). The time required to produce a significant impedance change, i.e. the detection time is inversely proportional to the initial number of bacteria.

The impedance monitoring technique has three advantages over the previous tests : a) it predicts shelf-life better than others b) it is less labour intensive & c) it requires only 1-2 days; as opposed to 7-9 days to complete (Bishop, White & Firstenburg-Eden, 1984). Furthermore, since impedance measurements detect activity not only from

organisms present in the milk but also enzymes remaining from bacteria killed by pasteurization, impedance monitoring provides a new means of predicting KQ (Cady et al., 1978; Gnan & Luedecke, 1982).

2.3. MILK SPOILAGE ORGANISMS & THEIR ENZYMES

Storage of milk for long periods at refrigeration temperature has created new problems related to KQ for the dairy industry. These problems are mostly related to the growth & metabolic activities of microorganisms. These microorganisms which are termed psychrotrophs, are ubiquitous in nature & are common contaminants of milk (Cousin, 1982). Psychrophilic microorganisms are responsible for one of the most important problems facing the dairy industry (Overcast, 1968).

In the dairy industry, & to some extent in other food industries, psychrotrophs are defined as those microorganisms which are able to grow at 7°C or below regardless of their optimum growth temperatures (Thomas, 1969; Collins, 1981).

Psychrotrophic growth is well distributed among the bacteria, yeasts & molds (Witter, 1961; Stokes & Redmond, 1966). These microorganisms may include long & short rods, vibrios, cocci, Gram-positive or Gram negative bacteria, spore formers or non-spore formers; & aerobic, facultative anaerobic or anaerobic microorganisms (Cousin, 1982). Many investigators have confirmed that soil, water, plant & animals form the natural habitats of these organisms (Stokes & Redmond, 1966; Thomas, Druce & Davies, 1966). Poorly cleaned & sanitized dairy farm & processing plant equipment probably constitute major foci for contamination of milk with the psychrotrophs (Thomas, 1966; Thomas & Druce, 1969; Thomas & Thomas, 1973; Thomas & Thomas, 1976; Thomas & Thomas, 1978).

Microorganisms that spoil milk at temperatures slightly above freezing are predominantly Gram-negative, non-spore forming, catalase positive rods (Thomas, 1958; Ingraham, 1962; Stokes, 1963). Bacteria belonging to genus Pseudomonas are most frequently encountered in the dairy industry & are the most troublesome psychrophiles. This is because of their pronounced ability to produce undesirable flavors, odors & pigments (Thomas, 1958; Stokes, 1963; Jones & Langlois, 1977). Other Gram-negative bacterial genera most commonly found to contain psychrophilic species are Achromobacter, Alcaligenas, Flavobacterium, Escherichia, & Enterobacter (Andrey & Frazier, 1959; Credit et al., 1972). Gram-negative facultative anaerobic rods of the family Enterobacteriaceae collectively referred to as coliform bacteria have been also the most frequently isolated from refrigerated raw milk (Thomas, 1970; Thomas & Bruce, 1972; Thomas, 1974; Thomas & Thomas, 1975). In addition to fermenting lactose to acid & gas at 37°C, the psychrotrophic coliforms are responsible for proteolytic spoilage of milk. Presence of coliforms is still being used to assess the sanitary production of milk & dairy products since these organisms are indicators of faecal contamination of milk & other milk products (Juven et al., 1981; Nunez, Chavarri & Nunez, 1984).

Considerable attention has been given to the incidence of heat-resistant spore-forming bacteria that can grow at 7°C or less in pasteurized milk. Spores of the genus Bacillus have been found in nearly all milk supplies & consequently, they are a major problem in heat treatment of milk (Martin, 1974; Donnelly & Busta, 1981; Shehata et al., 1983). Psychrophilic bacilli have been isolated from raw milk (Shehata & Collins, 1971) & pasteurized milk products (Grosskopf & Harper, 1969; Johnston & Bruce, 1982). Other workers (Chung & Cannon,

1971; Shehata, Duran & Collins, 1971; Wilkinson & Davis, 1973) have also reported on the presence & selected growth characteristics of psychrotrophic spore-formers in pasteurized milk. Occurrence of B. cereus in milk has been reported since 1916. The role of B. cereus in outbreaks of food-borne illness is well documented. A large number of viable cells of B. cereus is required to cause illness. Numbers in excess of 10^5 - 10^6 /g have been encountered in food suspected as causing illness (Goepfert, Spira & Kim, 1972). Multiplication of B. cereus in dairy products is not only of concern as a public health hazard but also as a cause of economic losses through spoilage of contaminated products (Ahmed, Noustafa & Warth, 1983).

Thermophilic organisms are those that grow at pasteurization temperature. These organisms occupy an important place in the dairy industry. Although they are less harmful to health, they are responsible for changes in the organoleptic properties of milk products.

The bacterial count varies with the sources of contamination, processing of milk & the type of milk. On farms, that use sterile utensils, the principal sources of contamination are poorly washed udders & defectively designed milking machines (Chattelin & Richard, 1981). Washing the udder & adjoining areas & cleaning the machines & pipelines with hot detergents or disinfectant solution can further reduce contamination (Marshall, 1982).

When milk samples are collected without washing the udder & milking machines, the bacterial counts averaged 2.2×10^6 /ml & 20,000 - 100,000/ml in milk collected on the farms that washed udders & milking machines (Piton & Richard, 1982).

Thermotolerant organisms do not grow at high temperatures (the

maxima are 35 to 40°C) but can withstand the pasteurization temperature. Many strains can survive a temperature at 80°C for as long as 15 minutes (Stainer, Doudoroff & Adelberg, 1970).

There has been much investigation concerning the incidence & significance of thermophilic bacteria in milk and dairy equipment. The healthy udder is not a primary source of thermophilic bacteria. Their presence in raw milk is always due to improperly cleansed milking & cooling equipment. Occasionally milking cans are also prolific sources (Thomas et al., 1967).

Various findings indicate that the predominant group of bacteria found on dairy equipment, which survive laboratory pasteurization of farm milk supplies are : certain species of lactic acid bacteria, Bacillus species, Micrococcus species & occasionally Gram-negative bacteria (Thomas et al., 1967; Martin, 1981).

The combination of thermophilic & psychrotrophic properties confers considerable spoilage potential upon a microorganism (Johnston & Bruce, 1982). According to Blankenagel (1976), it has been estimated that as few as 10 thermophilic psychrotrophs in a quart of milk can cause spoilage within a few days. The presence of heat resistant bacterial spores is a major concern in the production of high KQ milk. Recent findings, however, suggest that heat resistant enzymes present equally important shelf-life problems as the spore formers.

Most psychrotrophs produce extracellular enzymes in large quantities & some of these, mainly lipases & proteases affect the storage stability of milk (Juffs, 1976; Cliff & Law, 1982; Patel, Bartlett & Hamid, 1983; Gilliland & Ewell, 1983). Heat stable proteases of microbial origin have been reported by many investigators (Mayerhofer et al., 1973; Law, Andrews & Sharpe, 1977; Gebre-Egziabher,

Humbert & Blankenagel, 1980).

A property that distinguishes most psychrotrophic microbial enzymes from those of milk origin is their extreme heat resistance. This enables them to retain activities even after strong heat treatments & consequently affect the storage stability of milk (Law, Andrews & Sharpe, 1977; Anderson et al., 1981).

Ultra-high temperature (UHT) treatment of milk creates an environment suitable for protease activity. The proteases attack proteins & are responsible for the development of bitter flavor or gelation in milk (Adams, Barach & Speck, 1976; Gebre-Egziabher, Humbert & Blankenagel, 1980; Adams & Brawley, 1981; McKellar, 1981).

The rancid flavor produced in milk & milk products through the action of the microbial lipases is highly objectionable to man. The rancidity is mainly due to the liberation of butyric acid from triglycerides by the lipases.

In addition to the psychrophilic, thermotolerant & thermophilic organisms or their thermostable enzymes, other physical factors, such as light & oxygen, also cause defects in milk flavor. In evaluating flavor & quality of fresh & stored (sell-by-date) market milk, several investigators (Bandler & Wolf, 1979; Barnard, 1979; Shipe et al., 1980) reported that more than 50% of the stored samples had objectionable off-flavors.

The adverse effect of light on milk flavor is well known (Dimick, 1973; Hedrick & Glass, 1975). Other authors have also reported more rapid off flavor changes in milk packaged in plastic &/or clear glass than in paper board when subjected to sunlight or lights in milk display cases. In recent years milk is being packed in unpigmented polyethylene bags & paperboard cartons. Investigations have shown that

protection of milk quality offered by these containers differs. Their measurements indicate that milk is very susceptible to alteration by exposure to light. Flavor changes as well as loss of vitamins & other nutritional components are attributed to chemical reactions induced in the milk by light with wave lengths below 550 nm (Nelson & Cathcart, 1984). For unpigmented blow-molded plastic milk containers, light transmission of 25 to 50% were reported by Barnard (1972).

Milk contains little oxygen in the udder (Ford, 1967), but normally becomes saturated with it between milking & heat processing. Even though the solubility of oxygen in milk is not great, reactions of oxygen in milk generally lead to loss of quality which may be nutritional or organoleptic or both. Ascorbic acid & folic acid are readily oxidized. Losses of vitamins B12, A & E also depend on the oxygen content of milk (Hansen, Turner & Aurand, 1972; Schroder, 1982).

2.4. MILK PROCESSING : INHIBITION & CONTROL OF MICROORGANISMS

Application of heat is the most common method of destroying microorganisms in foods. Destruction of pathogenic organisms that may be present in milk is achieved through pasteurization. This requires heating the milk to a specific temperature, holding it there for a specified time period & subsequent cooling of the product rapidly. High temperature short time (HTST) pasteurization or continuous method consists of heating the milk at 72°C for 15 seconds while the low temperature long time (LTLT) or holding type of pasteurization requires treatment at 63°C for 30 minutes (Tuszynski, 1978).

The process of pasteurization is, however, not adequate for present day merchandising of milk products. A recent study at the

University of Florida revealed that leaving regular pasteurized milk in a shopper's automobile encouraged growth of microorganisms that survived the pasteurization process or were present as post-pasteurization contaminants. This in effect reduces subsequent shelf-life of the milk (Aggarwal, 1974).

Most processors exceed the time-temperature relationship established for pasteurization to increase the shelf-life of their product. Heat treatments considerably above pasteurization have been referred to as Ultra-High Temperature (UHT) processes. According to the International Dairy Federation, UHT processes refer to pasteurization techniques with temperatures of at least 130°C in a continuous flow & holding times approximately 1 second or more. Many UHT systems have the potential of producing a sterile, or at least a product with long shelf-life & unaltered flavor & texture (Westhoff & Poores, 1976).

The use of UHT process & aseptic packaging of non-refrigerated milk product has been in practice in many European countries & Canada for several years. More recent technical advances utilizing continuous flow UHT systems (both steam injection & steam infusion) have resulted in highly acceptable products. Processing with UHT of this type followed by aseptic packaging appears to be the only practical way at this time to produce a good quality product which only spoils occasionally. In most cases spoilage is due to contamination occurring during the filling operation (Westhoff & Dougherty, 1981). Studies made by Gebre-Egziabher, Humbert & Blankenagel (1980) have shown that complete destruction of heat resistant proteases in milk by UHT treatment is difficult to achieve.

Recently a device has been developed which automatically controls the quality of UHT milk. This device operates on the basis of

electrical registration of changes in hydrodynamic properties (eg. viscosity) due to microbial activity. It checks each package separately & automatically rejects faulty packages which have microorganisms in the milk (Hoisie & Kreula, 1973).

Shelf-life of 140 bottles (250 ml each) of milk processed with an indirect UHT system, constructed at Kansas State University at 135°C for 5 seconds, was prolonged to 4 months when held at 7 & 32°C (Wadsworth & Bassette, 1985).

The processing & distribution costs for UHT milk was found to be between 65 & 85 % of those for pasteurized milk. Based on this & many other studies, it appears that marketing UHT milk would be economically viable (Rama, 1978; Anon, 1979; Mehta, 1980).

2.5. MILKING & MILK PROCESSING EQUIPMENT

Many bacteria in natural waters & biological fluids are able to attach to available surfaces & proliferate (Corpe, 1970; Newman, 1974). Bacteria attached to inanimate surfaces are important not only as agents of biofouling & surface corrosion but also as potential contamination sources for any material which contacts the surface.

The generally accepted model of bacterial attachment is the two phase sorption suggested by Marshall, Stout & Mitchell (1971). In this model, bacteria are rapidly attracted to a surface, but are held only weakly. This reversible sorption may be followed by an irreversible phase of adhesion in which the bacteria can no longer be removed by washing. The second phase of attachment is often ascribed to synthesis by the cells of extra-cellular polymeric glue, although such synthesis may not always be necessary (Stanley, 1983).

Bacterial attachment is affected by the intrinsic properties of the attachment surface (Barszalek, Gerchakov & Udey, 1979) viz. topography, surface charge & wettability; & the nature & concentration of ions (Stanley & Rose, 1967; Marshall, Stout & Mitchell, 1971; Fletcher, 1976).

Proper cleaning & sanitizing of dairy farm equipment is vital for production of high quality milk (Bigalke, 1978). However, cleaning procedures in dairy plants are often inefficient in removing all bacteria from the equipment (Kaufmann et al., 1960; Haxcy, 1969).

2.6. MILK QUALITY IMPROVEMENT

There still are many opportunities to improve milk quality in general & manufacturing-grade milk quality in particular. Efforts made by field men, laboratory personnel, & milk sanitarians are starting to pay off in improved bacteriological quality of manufacturing-grade milk marketed from many dairy farms (Lagrange, 1979).

A new possibility to improve a high bacteriological quality of raw milk at ambient temperature has been tested in many countries including Kenya & Sri-Lanka by the activation of the lactoperoxidase system. This is a naturally occurring inhibitory system in raw milk that can reduce the bacterial content of milk (Bjorck, 1978; Bjorck et al., 1975; Reiter, Marshall & Phillips, 1980). The lactoperoxidase system, inhibits lactic streptococci & Gram-negative rods especially pseudomonads. This new method is based on activation of the naturally occurring lactoperoxidase system in milk by increasing the concentration of the two reactants, thiocyanate & hydrogen peroxide, to optimum 0.25mM (Bjorck, 1978). In practice, activation of the

lactoperoxidase system can be made by adding H_2O_2 (or H_2O_2 donor) to the above concentration & increasing the natural content of thiocyanate (normally around 0.05mM) to the same level (Zajac et al., 1983).

Bjorck, Claesson & Schulthess (1979) experimented with this system as a temporary preservative for raw milk in developing countries. Results of these studies demonstrated that the length of bacteriostasis is temperature-dependent: 7 to 8 hours at 30°C, 11 to 12 hours at 25°C, 15 to 16 hours at 20°C & 24 to 26 hours at 15°C. The quality of treated milk, measured by the resazurin test showed a significant improvement over that of the untreated control (Cousin, 1982). Activation of the antibacterial lactoperoxidase system in milk results in a substantial reduction of the bacterial flora & prevents the multiplication of psychrotrophic bacteria for up to 5 days. This treatment has no effect on the physico-chemical properties of milk & doesn't lead to the accumulation of resistant bacteria (Bjorck, 1978).

Spoilage of refrigerated untreated whole milk was found to be inhibited by the addition of CO_2 & the effect was enhanced with increasing concentrations of CO_2 & decreasing storage temperature. Experiments with pure cultures of Pseudomonas fluorescens showed that the inhibitory effects of CO_2 were not due to increased acidity or to displacement of dissolved O_2 , but to the presence of CO_2 per se which induced an increase in the duration of the lag-phase of growth. Storage of milk containing 20-30 mM dissolved CO_2 at 10°C or below reduced the growth rate of psychrotrophs in raw milk (King & Mabbitt, 1982). The CO_2 added creates an acidic taste but can be removed from the milk by warming under reduced pressure.

3. MATERIALS & METHODS

3.1. SAMPLING & SAMPLE COLLECTION

Samples of raw &/or pasteurized milk were collected every week from November 1986 up to February 1988, from the Addis Ababa Dairy Development Enterprise (AADD), three privately owned & three government owned dairy farms around Addis Ababa. The AADD collects milk from farms around Addis Ababa and transports it to the plant in containers on open lorries unrefrigerated. It normally takes 3-6 hours between collection and pasteurization.

A sample in this study, consisted of 4 sub-samples each of 250 ml quantity. These were:

1. a) raw milk from the bulk tank containing all the milk obtained from different farms & collection centers (R) b) raw milk from privately owned farms or c) raw milk from government owned farms.
2. Pasteurized milk from a tap just past the cooling unit in the pasteurizer (P1).
3. Pasteurized milk contained in bulk tanks & ready to be packaged (P2)
4. Pasteurized milk collected from 500 ml capacity plastic bags, a few minutes after packaging (P3).

Unless otherwise specified, pasteurized milk in this study means P3.

The tanks, two of which have a capacity of 10,000 liters each & third with an 11,000 liters capacity, were all from Berry (The Berry Engineering Works Ltd., UK). The packing units, Prepac (Ville Juif 94-France) are three in number. The pasteurizing unit is Alfa Laval (Sweden) which pasteurizes by flash technique at 72°C for 15 seconds.

The milk samples had a temperature of 5°C. Samples for bacteriological analysis were collected in sterile bottles & immediately transported to the laboratory. Analysis was carried out in less than 3 hours.

3.2. CONTRIBUTION OF EQUIPMENT & UTENSILS TO THE TOTAL SPECTRUM AND MICROBIAL LOAD OF MILK

Two different but complementary techniques were employed to determine the contributions made by the equipment to the total microbial load & spectrum of microorganisms. The rinse solutions from the 3 different bulk tanks (F2) were taken to the laboratory for bacteriological analysis. One ml portions of the rinse solution were seeded in 3 sterile petri-dishes & 15-18 ml of sterile Tryptone Soya Agar (TSA) (Oxoid, England) was poured into each plate. The plates were gently rotated to bring about effective distribution of the samples. These were then allowed to solidify & incubated at 7°C for 7-10 days, 32°C & 55°C for 24-48 hours. Colonies were counted & results recorded.

In addition, the Replicate Organism Direct Agar Contact (RODAC) plate method was also used to estimate the microbial load of the equipment at the AMBE.

3.3. DETECTION & ENUMERATION OF MICROORGANISMS IN THE AIR IN THE PASTEURIZATION PARLOUR

Petri dishes containing sterile TSA were left open for 15 minutes at the sites of sampling. The plates were then carried to the laboratory & incubated at 32°C for 24-48 hours. Colonies were then

counted & recorded as colony forming units (cfu) / plate / 15 minutes. Representative colonies were also subcultured on to TSA slants for identification purposes.

3.4. ASSESSING CONTAMINATION OF THE PLASTIC BAGS

The microbial load of the plastic bags was also evaluated before & after the UV treatment of the bags using RODAC plates. The RODAC plates were then incubated at 32°C for 24-48 hours. Representative colonies were subcultured on to TSA slants for future biochemical work.

3.5. KEEPING QUALITY DETERMINATION

From the milk samples brought to the laboratory as well as the laboratory pasteurized milk, duplicate 20ml amounts were transferred to sterile test tubes & maintained at room & refrigeration temperatures to assess their KQ. The tubes were then checked for curdling & acid production. The earliest time at which the milk started to get spoiled or the earliest time at which the milk started to get an unusual odor or curdling was recorded as a criterion for assessing KQ.

Sniffing for unusual odors was performed by me throughout the investigation period.

3.6. DETERMINATION OF APPROPRIATE CONCENTRATIONS OF DISINFECTANTS

A total of 5 chemicals, two of which are regularly used by the AAPPE, for sanitization purposes were assessed for their bactericidal/

bacteriostatic activities. Soda ash (Na_2CO_3) & caustic soda (NaOH) used at the Dairy Plant regularly, were prepared at 10g/l and 26.5g/l concentrations (aqueous), respectively. Savlon, Dettol & Chlorox (SEHEX, Ethiopia) were used at 1:10, 1:100, 1:500, 1:1000 & 1:2000 dilutions.

To 9ml of chemical contained in a test tube at a specified concentration, 1ml of bacterial cell suspension whose OD_{540} was adjusted to 0.1 was introduced into it, mixed thoroughly & kept at room temperature for the duration of the test. Duplicate 0.1ml samples were removed from each tube following aseptic techniques & spread plated on TSA plates every 5 minutes for the first 15 minutes & thereafter every 30 minutes for upto 2 hours.

Several microorganisms which were found as the most predominant species were used to determine their sensitivity to these chemicals.

3.7. BACTERIOLOGICAL EXAMINATION OF MILK SAMPLES

The collected milk samples were serially diluted in a quarter Ringer's solution following recommended procedures (Narth, 1978). Six TSA plates were surface plated with 0.1ml portions from each dilution. Duplicate plates were then incubated at 7°C for about 10 days for psychrotrophic count, 32°C for 24-48 hours for SPC (aerobic mesophilic count), & at 55°C for 24-48 hours for thermophilic count.

3.8. LABORATORY PASTEURIZED MILK COUNT

To determine the thermoduric counts, ten ml amounts of each sub-sample were placed in sterile test tubes & pasteurized in a water-bath at 62.8°C for 30 minutes. Immediately following the heat

treatment, the milk samples were cooled rapidly in cold water & appropriate dilutions were surface plated on TSA & incubated at 32°C for 24-48 hours.

Representative colonies were picked & subcultured on TSA slants. These were restreaked on TSA plates to isolate pure cultures. The pure cultures were then maintained on TSA slants & used for biochemical tests.

3.9. IDENTIFICATION OF ISOLATES

Cultures were picked from each petri plate after checking the colony morphology, color in medium and Gram reaction.

Isolates obtained from milk samples, rinse solution, ROPAC plates, room contamination & plastic bags were identified to the species levels after conducting standard biochemical tests. The following were consulted for identification & naming the bacteria: Buchanan & Gibbons (1974), Collins & Lyne (1976), Buxton & Fraser (1977), & Harvey & Gilmaour (1985).

4. RESULTS

4.1. THE STANDARD PLATE COUNT

The standard plate count (SPC) is suitable for measuring bacterial populations in most types of dairy products. It is the most frequently employed index used for measuring the sanitary quality of milk & for determining the efficiency of pasteurization. It also gives enough information about the microbial load in the dairy product. The numbers & kinds of living bacteria present in any food also serve as a good index for measuring the shelf-life (KQ) of the food. In the case of dairy products, bacterial count is also one of the legally specified criterion by which the product is assigned a grade.

Because of these reasons, SPC was also determined for raw, pasteurized & laboratory pasteurized milks obtained from the RAJEE & other privately & government owned farms. As can be seen from Table 1, the counts for the milk samples fall between $10^7 - 10^9$, $10^4 - 10^6$ & $10^3 - 10^5$ for the raw, pasteurized & laboratory pasteurized milk samples, respectively.

As much as 13.3% of the raw milk samples had counts equal to or well over 10^9 bacteria/ml. However, 86.7% of the raw milk samples had bacterial counts greater than 10^7 but less than 9.9×10^8 /ml. On the other hand 33.3% of the plant pasteurized milk samples had counts greater than 10^6 bacteria/ml, while 53.3% had counts well over 10^5 bacteria/ml, Laboratory pasteurized milk contained fewer bacteria, but here too, 30% of the samples showed counts of 10^5 & greater. Therefore, the majority of the raw milk samples had counts between 10^7 - 10^8 bacteria/ml. Eventhough most samples from the laboratory pasteurized milk fell below 10^5 those pasteurized at the Dairy had counts less than or equal to 10^6 bacteria/ml (Table 1).

Table 1. Abundance of samples falling in different bacterial counts for raw, pasteurized & laboratory pasteurized milk samples.

Bacterial count range	RAW			PASTEURIZED			LAB. PASTEUR.		
	10^7	10^8	10^9	10^4	10^5	10^6	10^3	10^4	10^5
Number of Samples*	14	12	4	4	16	10	3	18	9
%	46.7	40	13.3	13.3	53.3	33.3	10	60	30

* number of samples equals to 30 throughout this study.

In addition to the total count, psychrotrophic, thermoduric & thermophilic counts were also obtained for the raw, pasteurized & laboratory pasteurized milk samples. This was basically done in order to compare the proportion of psychrotrophic to thermoduric & thermophilic counts in the different milk samples. The percentage abundance of these bacteria in the different samples is given in Tables 2 & 3. As can be seen from these tables, psychrotrophs make up the greatest proportion of bacteria in both raw (98.1%) & plant pasteurized (53%) milk samples. In the laboratory pasteurized milk samples, on the other hand, the dominant group are thermoduric organisms making up 70.4% of the population followed by the psychrotrophs & thermophiles.

In the raw milk samples the majority (86.6%) of the psychrotrophic bacteria had counts in the range of 10^5 - 10^6 /ml. In the plant pasteurized milk samples 90% of the psychrotrophic count ranges fall between 10^4 & 10^5 . In the laboratory pasteurized milk, on the other hand, 70% fall in the 10^3 bacterial count range & the rest 30% in the 10^4 range.

Table 2. Comparative proportions of the psychrotrophic, thermoduric & thermophilic organisms from raw (R), pasteurized (P), & laboratory pasteurized (LP) ml samples.

* Total count includes mesophilic count which is not given in this table.

	PSYCHROTROPHIC			THERMODURIC			THERMOPHILIC			TOTAL*	
Count range	10^5	10^6	10^7	10^5	10^6	—	10^4	10^5	10^6		
R	No. of Samples	10	16	4	25	5	—	18	10	2	5.7×10^8
	%	33.3	53.3	13.3	83.3	16.7	-	60	33.3	6.7	
Count range	10^4	10^5	10^6	10^4	10^5	10^6	10^3	10^4	10^5		
P	No. of Samples	12	15	3	15	13	2	10	15	5	1.9×10^6
	%	40	50	10	50	43.3	6.7	33.3	50	16.7	
Count range	10^3	10^4	—	10^4	10^5	—	10^3	10^4	10^5		
LP	No. of Samples	21	9	—	12	18	—	6	19	5	2×10^5
	%	70	30	—	40	60	—	20	63.3	16.7	

More than 80% of the thermoduric organisms in raw milk fall in the 10^5 bacterial count range, while in the plant pasteurized & laboratory pasteurized milk samples majority of the organisms (more than 90%) fall between the ranges of 10^4 & 10^5 bacteria/ml. The lowest count for pasteurized milk was 6×10^4 while the highest recorded was 3×10^6 /ml.

Table 3. Percentage distribution of bacteria in different milk types.

Types of Organisms	% Distribution of Bacteria		
	RAW	PLANT PAST.	LAB. PAST.
Psychrotrophs	98.1	53.0	4.1
Thermoduric	1.4	39.5	70.4
Thermophilic	0.5	7.5	25.5

4.2. SPECTRUM OF MICROORGANISMS ISOLATED & IDENTIFIED

In the course of this study 185 isolates were identified & their abundance in milk determined. The bacteria isolated & the relative proportions they occur in the milk is shown in Table 4. The isolates belong to 14 genera. These include both Gram-positive & Gram-negative rods as well as Gram-positive cocci. The rods & cocci appear to exist in a proportion of 40 to 60% respectively (Table 5).

Species belonging to the genera Streptococcus, Staphylococcus, Micrococcus, Bacillus, & Arthrobacter were found to be the most abundant (Table 4). Of these, the group with coccal morphology were found to be higher in abundance than those of the rods.

Table 4. Bacterial genera isolated from milk samples.

Isolated Genera	No. of isolates	%
<u>Streptococcus</u>	50	27.0
<u>Staphylococcus</u>	36	19.5
<u>Micrococcus</u>	25	13.5
<u>Bacillus</u>	24	12.9
<u>Arthrobacter</u>	21	11.3
<u>Aeromonas</u>	5	2.7
<u>Lactobacillus</u>	4	2.2
<u>Pseudomonas</u>	4	2.2
<u>Citrobacter</u>	4	2.2
<u>Streptomyces</u>	3	1.6
<u>Enterobacter</u>	3	1.6
<u>Escherichia</u>	2	1.1
<u>Alcaligenes</u>	2	1.1
<u>Corynebacterium</u>	2	1.1
Total	185	100%

The rod-shaped bacterial population was again classified into two depending upon their Gram's reaction. The Gram-positives were found to represent the highest proportion of the rod-shaped bacterial population, thus making up 73% & the rest, Gram-negatives, make up 27% (Table 6).

Of the Gram-positive rods 44% were found to be spore formers & the remaining 55.6% were non-spore formers (Table 7). The Gram-positive spore forming population comprises exclusively species which belong to genus Bacillus, the most abundant being B. subtilis & B. pumilis (Table 9). The Gram-positive non-spore forming population, on the otherhand, is dominated by species belonging to genus Arthrobacter, the most abundant being A. simplex (Table 8).

Table 5. Total number & relative proportions of rods to cocci.

Group of Organisms	No. of isolates	%
Cocci	111	60
Rods	74	40
Total	185	100%

Table 6. Total number & relative proportions of Gram-negative to Gram-positive rods.

Rods	No. of Isolates	%
Gram-positive	54	73
Gram-negative	20	27
Total	74	100%

Table 7. Total number & relative proportions of spore forming to non-spore forming Gram-positive rods.

Gram-positive rods	No. of Isolates	%
Spore Formers	24	44.4
Non-spore Formers	30	55.6
Total	54	100%

Table 8. Gram-positive non-spore forming bacteria.

Gram-positive Non-spore forming rods	No. of Isolates
<u>Arthrobacter simplex</u>	14
<u>Arthrobacter terregens</u>	6
<u>Lactobacillus brevis</u>	4
<u>Streptomyces spp.</u>	3
<u>Corynebacterium spp.</u>	2
<u>Arthrobacter globiformis</u>	1
Total	30

Table 9. Gram-positive spore formers.

Gram-positive Spore formers	No. of Isolates
<u>Bacillus subtilis</u>	6
<u>Bacillus pumilis</u>	6
<u>Bacillus licheniformis</u>	4
<u>Bacillus circulans</u>	3
<u>Bacillus cereus</u>	2
<u>Bacillus polymyxa</u>	2
<u>Bacillus macerans</u>	1
Total	24

The Gram-negative rod shaped bacteria which made up 27% of the total rod shaped bacterial population represented 6 genera. The most abundant was genus Aeromonas followed by Citrobacter, Pseudomonas & Enterobacter. The 7 different species which belonged to the six genera are listed in Table 10.

All bacteria with coccial morphology were found to be Gram-positive. They all fall into one of three genera, namely, Streptococcus, Staphylococcus, & Micrococcus (Table 11). As shown in Table 12, the Gram-positive coccial population was dominated by Streptococcus species followed by Staphylococcus & Micrococcus species.

Table 10. Gram-negative rod-shaped bacteria.

Gram-negative rods	No. of Isolates
<u>Aeromonas spp.</u>	5
<u>Citrobacter freundii</u>	4
<u>Enterobacter liquifaciens</u>	3
<u>Alcaligenes faecalis</u>	2
<u>Pseudomonas aeruginosa</u>	2
<u>Psuedomonas spp.</u>	2
<u>Escherichia coli</u>	2
Total	20

Table 11. Total number & relative proportions of the three coccal bacterial genera.

Coccal Genera	No. of Isolates	%
<u>Streptococcus</u>	50	45.1
<u>Staphylococcus</u>	36	32.4
<u>Micrococcus</u>	25	22.5
Total	111	100%

Table 12. Abundance of Gram-positive cocci.

Gram-positive Cocci	No. of Isolates
<u>Streptococcus lactis</u>	30
<u>Streptococcus durans</u>	20
<u>Staphylococcus epidermidis</u>	19
<u>Staphylococcus aureus</u>	17
<u>Micrococcus varians</u>	12
<u>Micrococcus luteus</u>	9
<u>Micrococcus roseus</u>	4
Total	111

An attempt was made to determine if differences existed amongst the bacterial species found in the three types of milk i.e. raw, pasteurized & laboratory pasteurized samples. The organisms isolated from the raw milk belonged to 9 genera & consisted of 16 species (Table 13). The most abundant of these organisms belonged to genus Streptococcus. It comprised 39% of the total raw milk isolates. This was followed by Staphylococcus comprising 21.7%, Bacillus, 11% & Micrococcus, 10.9%.

On the other hand, organisms isolated from pasteurized milk constituted 20 species which belonged to 14 genera. As can be seen from Table 14, the most abundant of these belonged to the genera Streptococcus (25.7%), Staphylococcus (18.5%), Arthrobacter (14.3%) & Micrococcus (12.9%).

Bacteria falling into 7 genera were also isolated from the laboratory pasteurized milk (Table 15). The most abundant organisms in this group belonged again to the genus Streptococcus which made up to 35%, genus Bacillus 20%, & genus Staphylococcus made up to 17.5% of the total thermophilic organisms.

Table 13. Total number & relative proportions of organisms isolated from raw milk.

Organisms	No. of Isolates	%
<u>Streptococcus lactis</u>	12	26.0
<u>Streptococcus durans</u>	6	13.0
<u>Staphylococcus aureus</u>	6	13.0
<u>Staphylococcus epidermidis</u>	4	8.7
<u>Micrococcus varians</u>	3	6.5
<u>Micrococcus luteus</u>	2	4.4
<u>Arthrobacter simplex</u>	2	4.4
<u>Citrobacter freundii</u>	2	4.4
<u>Bacillus cereus</u>	2	4.4
<u>Bacillus subtilis</u>	1	2.2
<u>Bacillus pumilis</u>	1	2.2
<u>Bacillus polymyxa</u>	1	2.2
<u>Pseudomonas aeruginosa</u>	1	2.2
<u>Pseudomonas sp.</u>	1	2.2
<u>Alcaligenes faecalis</u>	1	2.2
<u>Aeromonas sp.</u>	1	2.2
Total	46	100%

Table 14. Total number & relative proportions of organisms isolated from plant pasteurized milk.

Organisms	No. of Isolates	%
<u>Streptococcus lactis</u>	13	18.6
<u>Staphylococcus aureus</u>	8	11.4
<u>Arthrobacter simplex</u>	8	11.4
<u>Staphylococcus epidermidis</u>	5	7.1
<u>Streptococcus durans</u>	5	7.1
<u>Micrococcus varians</u>	5	7.1
<u>Aeromonas spp.</u>	4	5.7
<u>Bacillus pumilis</u>	3	4.3
<u>Enterobacter liquifaciens</u>	3	4.3
<u>Micrococcus luteus</u>	2	2.9
<u>Micrococcus roseus</u>	2	2.9
<u>Arthrobacter terreus</u>	2	2.9
<u>Lactobacillus brevis</u>	2	2.9
<u>Escherichia coli</u>	2	2.9
<u>Bacillus licheniformis</u>	1	1.4
<u>Citrobacter freundii</u>	1	1.4
<u>Alcaligenes faecalis</u>	1	1.4
<u>Pseudomonas aeruginosa</u>	1	1.4
<u>Streptomyces sp.</u>	1	1.4
<u>Corynebacterium sp.</u>	1	1.4
Total	70	100%

Table 15. Total number & relative proportions of organisms isolated from the laboratory pasteurized milk.

Organisms	No. of Isolates	%
<u>Streptococcus durans</u>	9	22.5
<u>Staphylococcus epidermidis</u>	7	17.5
<u>Streptococcus lactis</u>	5	12.5
<u>Bacillus subtilis</u>	4	10.0
<u>Bacillus circulans</u>	3	7.5
<u>Micrococcus luteus</u>	3	7.5
<u>Micrococcus varians</u>	2	5.0
<u>Arthrobacter simplex</u>	2	5.0
<u>Lactobacillus brevis</u>	2	5.0
<u>Bacillus licheniformis</u>	1	2.5
<u>Arthrobacter terragens</u>	1	2.5
<u>Corynebacterium sp.</u>	1	2.5
Total	40	100%

4.3. SOURCES OF CONTAMINATION

Studies were also conducted to determine the sources of contamination as well as the relative degree of contaminating microbiota contributed by each of the sources.

Raw milk brought from different collection centers: 3 privately owned & another 3 government owned farms were sampled before being introduced into the bulk cooling tanks. The SPC & psychrophilic counts of the milk samples obtained from the different farms were

determine) (Table 16). The spectrum of microorganisms found in the milk samples was not different from that found in the bulk cooling tanks. The microbial loads (SPC) as well as the psychrophilic counts of the raw milk samples collected from privately or government owned farms were very similar. However, state farms had fewer psychrophiles.

The second source of contamination that was considered, included the utensils used for holding raw & pasteurized milk. Both the RODAC plate method & the rinse method were used to determine the contributions made by the utensils to the total microbial load. Both methods demonstrated that the bulk tanks were heavily contaminated with a variety of microorganisms (Table 17), the majority of which were thermotolerant. These same organisms were also isolated from the pasteurized milk.

Table 16. Comparison of bacterial load in raw milk obtained from three state & three private farms.

COUNT	STATE FARMS				PRIVATE FARMS		
	1	2	3		1	2	3
SPC	5.4×10^9	5×10^8	4×10^8		8.6×10^9	4.1×10^9	7.4×10^9
PSY*	2.8×10^8	2×10^7	1×10^7		1.2×10^9	2.2×10^9	1.5×10^9

* Psychrophilic

The RODAC plate method was also used to study PPC in the plastic bags before & after UV treatment in the fillers. As can be seen from Table 19, UV treatment before packing greatly reduced the bacterial load in the plastic bags, even though complete sterilization was not possible.

Table 17. Organisms isolated from bulk tanks by the rinse & RODAC plate methods.

RODAC	RINSE
<u>Staphylococcus aureus</u>	<u>Staphylococcus aureus</u>
<u>Staphylococcus epidermidis</u>	<u>Streptococcus lactis</u>
<u>Micrococcus varians</u>	<u>Bacillus subtilis</u>
<u>Micrococcus luteus</u>	<u>Bacillus licheniformis</u>
<u>Streptococcus lactis</u>	<u>Micrococcus roseus</u>
<u>Bacillus pumilis</u>	<u>Citrobacter freundii</u>
<u>Bacillus licheniformis</u>	<u>Arthrobacter simplex</u>
<u>Arthrobacter simplex</u>	<u>Arthrobacter terregens</u>
<u>Arthrobacter terregens</u>	<u>Arthrobacter globiformis</u>
<u>Pseudomonas sp.</u>	<u>Streptococcus durans</u>

The rooms where milk is processed have also been examined to find out if they contribute to some extent to the bacterial count in milk. The organisms isolated were similar to those isolated from pasteurized milk (Table 18).

Table 18. Organisms isolated from the rooms where milk is processed at the AADDE.

PREDOMINANT ROOM CONTAMINANTS

Staphylococcus epidermidis

Streptococcus durans

Bacillus pumilis

Bacillus macerans

Micrococcus luteus

Micrococcus varians

Arthrobacter simplex

Arthrobacter terregens

Table 19. List of genera isolated from plastic bags before & after UV treatment by the RODAC plate method.

BACTERIAL GENERA ISOLATED FROM PLASTIC BAGS

Before UV treatment	After UV treatment
<u>Bacillus</u>	<u>Bacillus</u>
<u>Staphylococcus</u>	<u>Staphylococcus</u>
<u>Arthrobacter</u>	<u>Arthrobacter</u>
<u>Micrococcus</u>	<u>Micrococcus</u>
<u>Streptococcus</u>	<u>Streptococcus</u>
<u>Escherichia</u>	
<u>Corynebacterium</u>	
<u>Alcaligenes</u>	
<u>Pseudomonas</u>	

4.4. ASSESSMENT OF KEEPING QUALITY

The shelf-life (KQ) of raw, pasteurized (P1, P2, & P3) & laboratory pasteurized milk samples was also determined during this study period. The range of KQ at different storage temperatures is given in Fig. 1.

A comparison of the KQ for the raw, pasteurized & laboratory pasteurized milk samples at different storage temperatures is plotted in a bar graph in Fig.1. Laboratory pasteurized milk had a KQ of 180h (7.5d) at refrigeration temperature & 104h (4.3d) at room temperature. Raw milk has the shortest KQ, 48h (2d) at refrigeration temperature & 22h (0.9d) at room temperature.

The KQ of the plant pasteurized milk at different sites (P1, P2 & P3) has also been compared following the same procedures. It has, therefore, been found that plant pasteurized milk sampled from the pasteurizing unit (P1), has a better KQ at both storage temperatures as compared to milk samples taken from the bulk tanks (P2) & plastic bags (P3). As can be seen from Fig. 1, P1 has a KQ of 173h (7.2d) at refrigeration temperature & 74h (3.1d) at room temperature. The KQ of P2 & P3 milk samples, on the other hand, is very low at both storage temperatures as compared to the KQ of P1. Milk sample P2 has a KQ of 91h (3.8d) at refrigeration temperature & 54h (2.3d) at room temperature; & P3, 78h (3.6d) at refrigeration temperature & only 50h (2.1d) at room temperature. Milk samples P2 & P3, therefore, have comparable KQs at both storage temperatures as can be seen from Fig.1.

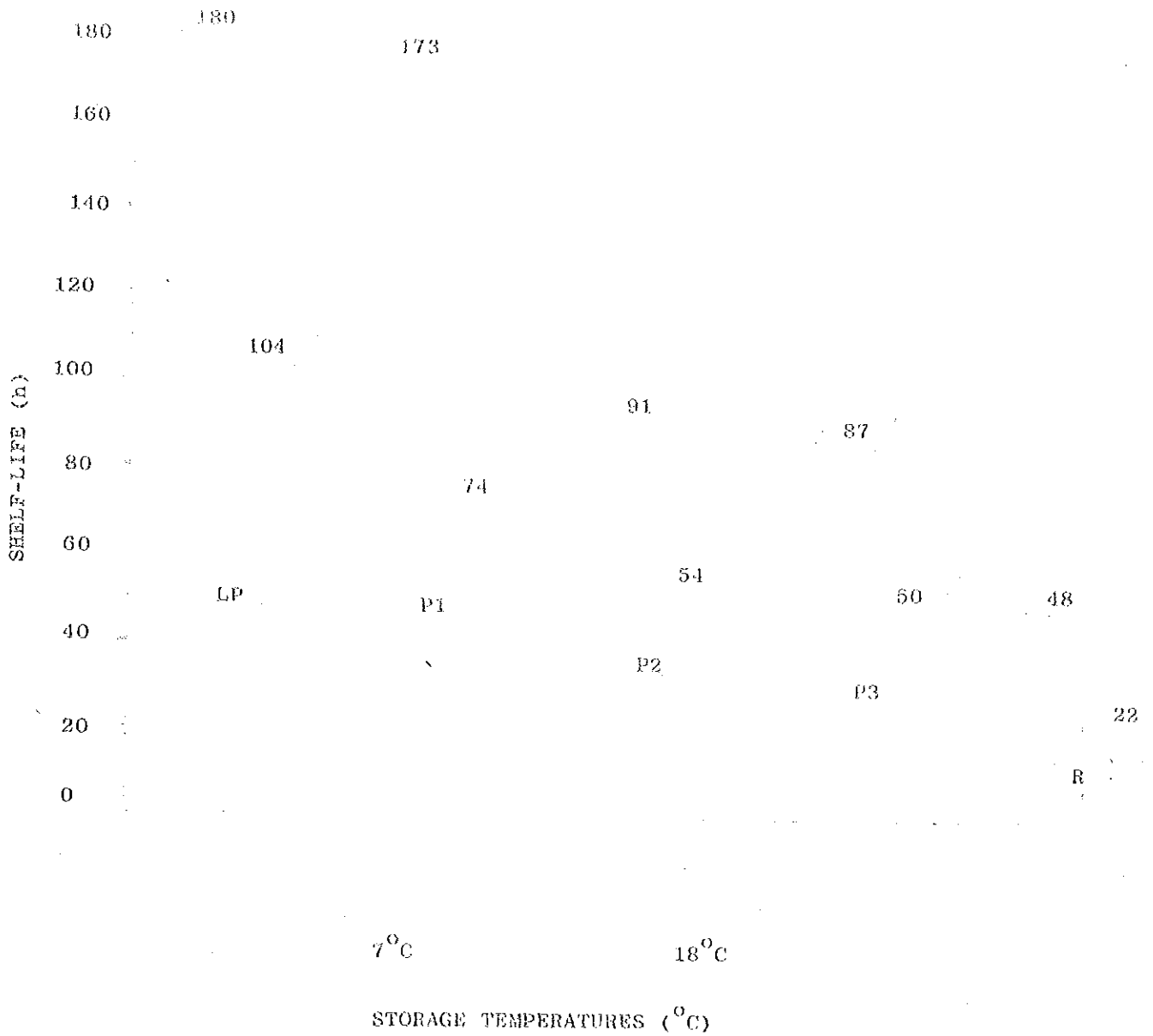


Fig. 1. Comparative shelf-life (h) of raw (R), laboratory pasteurized (LP) & plant pasteurized (P₁, P₂, P₃) milk samples at room (18°C) & refrigeration (7°C) temperatures.

4.5. DETERMINING THE APPROPRIATE CONCENTRATION OF DISINFECTANTS

The effectiveness of 5 disinfectants, two of which are regularly used by the AADDE, has been tested in the laboratory using the most predominant microorganisms isolated from the Dairy Plant's milk. Soda ash (10g/l) & caustic soda (26.5g/l) used at the Dairy Plant regularly were found to be ineffective not only at the concentrations used at the Dairy Plant but also at higher concentrations at 15-30 minutes holding time. Other disinfectants like Savlon & Chlorox, on the other hand, were found to be much more effective at a much lower concentration - as low as 1 : 500 at the same holding time (Table 20).

The bacteriostatic/bactericidal effect of these disinfectants decreases for some organisms like Bacillus. cereus. In general, however, the disinfectants are more effective on Gram-positive than on Gram-negative bacteria.

Table 20. Effectiveness of disinfectants at different concentrations to the major groups of bacteria isolated from the AADDE milk at 15 - 30 minutes holding time.

TYPES OF DISINFECTANTS	CONCENTRATION OF DISINFECTANTS			
	1 : 1000	1 : 500	1 : 100	1 : 10
Caustic soda	-	-	-	+
Soda ash	-	-	-	+
Dettol	-	-	+	+
Savlon	-	+	++	++
Chlorox	-	+	++	++

- Not effective + Effective ++ Highly effective

5. DISCUSSION

In most countries where the risk of contamination during milking, transporting, storage etc. is low, the standard plate count (SPC) for raw milk is also low. Gebhardt & Nicholes (1975) reviewing microbiological standards in milk, state that the SPC for raw milk in developed countries falls between 7.5×10^4 to 2×10^5 . This is at least 100 times less than what has been recorded for raw milk brought to the AADDE for pasteurization purposes.

During this study period the lowest SPC was found to be 4×10^7 while the highest was 3×10^9 bacteria/ml of raw milk. However, as much as 46.7% of the milk sampled had counts of 10^7 bacteria/ml of raw milk. Therefore, one could assume that under current milk handling procedures, here, the SPC of raw milk to be about 10^7 /ml. Of course with slight effort in improvements in collection of milk, this value could be reduced significantly. Pasteurized milk, depending upon the sampling site at which it was collected, showed SPC values ranging from 6×10^4 to 3×10^6 /ml. However, 86.6% of the pasteurized milk samples collected during this study period had counts between 10^5 and 10^6 bacteria/ml (Table 1). This could, therefore serve as a standard to quality assessment of milk. This is supported by the data obtained from the laboratory pasteurized milk samples. This again is higher than the allowable mesophilic aerobic count in pasteurized milk (Gebhardt & Nicholes, 1975). The raw milk which was pasteurized in the laboratory following the holding-type process, showed far fewer counts than the milk which was pasteurized at the AADDE. In fact a significant percentage (70%) had less than 10^5 & as much as 10% had counts well below 10^4 bacteria/ml.

This demonstrates the heat sensitivity of most of the microorganisms present in the milk at the beginning. The high bacterial count of the plant pasteurized milk was, therefore, found to be due to the initial high load of microorganisms in the milk processing equipment which are heat resistant & also microorganisms which were introduced into the milk just after pasteurization.

Psychrotrophs, which are considered to be the most serious spoilage organisms constituted the greatest proportion of the total bacterial count in both raw & pasteurized milks. If one considered the relative proportions of psychrophiles to thermoduric to thermophiles, the psychrophilic group made up 98.1% of the total microbial population in raw & 53% in pasteurized milk samples. Thermodurics constituted 1.4% of the total bacterial count in raw & 39.5% in pasteurized milk samples. On the other hand, it is the thermoduric group (70.4%) which predominated in the laboratory pasteurized milk (Table 3).

The psychrotrophs known to be present in milk belong to the genera of Pseudomonas, Alcaligenes, Bacillus, Streptococcus, Micrococcus & Arthrobacter. The most commonly occurring psychrotrophs in fresh milk are Gram-negative rod-shaped bacteria of which Pseudomonas species account for about 50% of the total Gram-negative bacterial species (Juffs, 1973). Although most of the psychrophilic isolates from the raw milk were Gram-negative rods (GNR), a significant number were also Gram-positive species. Similar types of isolates were recorded by Shehata & Collins (1971) & Masham, Olson & Vedamuthu (1977).

The prevalence of high numbers of psychrotrophic bacteria in milk appears to be due to gross contamination. In addition to this,

absence of cold storage facilities at the Dairy Plant & dairy farms from where the milk is collected seems to be responsible for the increased thermophilic psychrotrophic count. In milk stored & distributed under chilled conditions, psychrotrophic microbial growth limits KQ. In this group of organisms, GMR have the highest growth rates at low temperatures. The GMR found in milk are highly sensitive to heat treatment (Schroder, 1984).

Psychrotrophic bacteria are normally difficult to exclude from raw milk & many grow & produce proteolytic enzymes during refrigerated storage. Hence, if sufficient heat stable enzymes are in raw milk prior to pasteurization, defects may become more pronounced after pasteurization during refrigerated storage (Adams, Barach & Speck, 1975; Janzen et al., 1982). According to Adams, Barach & Speck (1975), all of the psychrotrophs isolated from raw milk produced heat resistant enzymes & 70 to 90% of the raw milk samples tested, contained psychrotrophs capable of producing these enzymes. The presence of these proteases in raw milk results in the development of bitter flavor (Gebre-Egziabher, Humbert & Blankenagel, 1980; Torrie et al., 1983).

Evidence suggests that such defects are produced by raw milk's proteolytic psychrotrophic bacterial populations. Psychrotrophs cause a variety of pronounced flavor defects when they occur in numbers exceeding $10^6/\text{ml}$ (Law, Andrews & Sharpe, 1977; O'Connor et al., 1986). Other organisms do not cause detectable defects at 10 times the population level. This is because of the difference in biochemical activities.

The majority of the raw milk samples analyzed had psychrotrophic populations exceeding $10^6/\text{ml}$ (Table 2). It is, therefore, highly probable that defects are produced in the raw milk

through the psychrotrophic proteolytic enzymes released during growth of the psychrotrophic group of bacteria.

Thermoduric count is often used as an index for measuring equipment sanitation. If milk utensils & milk processing equipment are mishandled & not properly disinfected, many kinds of bacteria especially thermoduric ones could survive & proliferate on the surfaces thus becoming potential spoilage organisms which will be responsible for the deterioration of the shelf-life of milk & its products. The proliferation of thermoduric bacteria on poorly cleansed dairy equipment has been amply documented (Parfitt, 1940; Murray, 1949; Cuthbert, Egdell & Thomas, 1953; Thomas et al., 1967; Nelson, 1981; Speers et al., 1984).

The thermoduric group which represented 1.4% of the total microbial population in raw milk had 39.5% share in pasteurized & 70.4% in laboratory pasteurized milk samples (Table 3). This truly reflects the decrease in psychrophilic population during pasteurization rather than an increase in population of the thermodurics.

With the exception of a few strains of enterococci & some Bacillus or Clostridium species, thermoduric bacteria do not grow at or below 7.2°C. Hence they do not play an important role in the spoilage of properly refrigerated, pasteurized milk. Nevertheless, above this temperature, they do grow & become a major factor in the spoilage of milk & other dairy products not properly refrigerated.

Since raw milk is transported unrefrigerated to the AADDE from long distances, it is likely that the thermoduric population will increase in number. This group also survived the pasteurization temperature at both the AADDE & the laboratory pasteurizers. Some thermoduric psychrophiles have also been isolated from milk (Law,

1979). Since they are also well known for their ability to produce extracellular proteinases, they tend to increase the seriousness of the thermoduric organisms as spoilage agents (Priest, 1977; Richter et al., 1979).

Pasteurized milk is transported to the market in open, exposed lorries, where the temperature is higher than ambient due to the exposure of the milk contained in plastic bags to direct sunlight. The retail shops also store the milk in shelves where the temperature is ambient (18-22°C). All these improper methods of milk handling, therefore, allow the thermoduric organisms to play a major role in the spoilage of the pasteurized milk. In this study the thermophilic group represented the least population in both raw & pasteurized milk samples.

In the course of this study bacteria belonging to 14 genera were isolated from raw & pasteurized milk & milking utensils. The most predominant genera were: Streptococcus (27%), Staphylococcus (19.5%), Micrococcus (13.5%), Bacillus (12.9%) & Arthrobacter (11.3%) (Table 4). Species in these genera include psychrotrophic & thermoduric organisms. The GNR bacteria belonging to the genera of Pseudomonas, Alcaligenes, Escherichia, Aeromonas & Citrobacter were fewer in number than the Gram-positive bacteria.

Out of the 185 representative isolates identified during the course of study, the cocci made up 60% & the rods 40% (Table 5). The Gram-positive rods constituted 73% of the rod shaped bacterial population & the Gram-negative rods 27% (Table 6). Similarly most of the isolates from pasteurized milk were Gram-positive bacteria. This indicates that they have greater heat resistance than the GNR. This finding supports previous reports made by investigators (Shehata &

Collins, 1971; Masham, Olson & Vedamuthu, 1977).

Out of the Gram-positive rods the spore formers constituted 44.4% & the non-spore formers 55.6% (Table 7). Spore formers are generally soil residents. Their presence in large numbers in milk is indicative of direct or indirect contamination with soils. The majority of the spore forming isolates were thermoduric & as a result survived the pasteurization temperature. These survivors are responsible for the spoilage of milk & its products. The spore forming population exclusively constituted Bacillus species (Table 9). Many workers have also identified this group as being responsible for the spoilage of milk & milk products in their investigations.

Bacillus species is known to account for about 95% of the total spore forming bacteria in milk with Clostridium species comprising the remainder (Martin, 1974). In this study all the spore forming isolates were Bacillus species, however, the anaerobic spore formers were not investigated. Previous findings indicated that B. licheniformis & B. subtilis are the most frequently occurring aerobic mesophilic spore formers in raw milk. B. subtilis tends to be more predominant in spoiled sterilized milk (Franklin, Williams & Cleg, 1956; Westhoff & Dougherty, 1981). It has also been demonstrated that spore formers in Indian milk were B. subtilis, B. cereus, B. pumilis, B. licheniformis, B. coagulans & B. stearothermophilus (Atwal et al., 1974). In this study, too, B. subtilis, B. pumilis, B. licheniformis, B. circulans, B. cereus, B. polymyxa & B. macerans have been isolated. It, therefore, appears that essentially the same species of spore forming microorganisms are involved world wide with the most common being B. subtilis, B. licheniformis & B. cereus. The only major difference appears to be the variation in the relative proportions of

these organisms in milk collected from different countries (Martin, 1981).

The Gram-positive non-spore forming rod-population was dominated by Arthrobacter species (Table 8). All the Gram-negative rods (Table 10) which were isolated were found to be psychrotrophs. The dominant organisms in this group included Aeromonas, Citrobacter & Enterobacter species.

The Gram-positive cocci comprised 60% of the total bacterial isolates. These belonged to 3 genera namely Streptococcus (45.1%), Staphylococcus (32.4%), & Micrococcus (22.5%) (Table 11). Of the Gram-positive coccal isolates Streptococcus lactis, Streptococcus durans, Staphylococcus epidermidis, Staphylococcus aureus & Micrococcus varians were the most predominant (Table 12).

As many as 16 species of bacteria falling into 9 genera were isolated from raw milk. On the other hand 20 species belonging to 14 genera were isolated from pasteurized milk. Laboratory pasteurized milk, however, yielded only 12 species belonging to 7 genera (Tables 13, 14 & 15). This study shows that more bacterial species are found in pasteurized milk than in the raw or laboratory pasteurized milk. This clearly indicates that organisms, other than those found in the raw milk have gained access to the pasteurized milk. These contaminants originate from the bulk tanks, rooms, fillers & their pipelines. Though of minor importance the plastic bags also contribute their own share of bacteria to the pasteurized milk.

The microorganisms in the laboratory pasteurized milk were either thermophilic or thermoduric bacteria belonging to the genus Streptococcus, Bacillus & Staphylococcus. Other thermoduric organisms including Micrococcus, Arthrobacter & Lactobacillus were also found in

the laboratory pasteurized milk (Table 15). In general, however, the microbial type & number in the laboratory pasteurized milk was by far less than that of the plant pasteurized milk. This, therefore, can explain the unsanitary & improper milk handling & processing in the Dairy Plant.

The pasteurization process, which is a flash technique (72°C for 15 seconds) is efficient enough to eliminate the majority of the heat sensitive organisms, except those that do survive the pasteurization temperature.

The bacterial load in the samples was greatly reduced just after pasteurization (P1) (Fig.1). However, the count as well as the number of bacterial genera present in the pasteurized milk increased as it was transferred to the bulk tanks. This was mainly due to PPC.

As described earlier, the different sources of contamination were identified & the degree to which each was responsible for this contamination determined. Study was also conducted to find out if milking conditions were also serving as major sources of contamination. This study, therefore, involved the determination of SPC & psychrophilic count of milk samples obtained from 3 state & 3 privately owned farms. Their total (SPC) & psychrophilic counts were more or less similar & fall well over the assumed average standards (Table 16). Most of the organisms isolated were not psychrophiles. However, more psychrophiles were isolated from privately owned than from state owned farms.

About 25-30 thousand liters of milk is received every day by the AADDE from state & private farms as well as collection centers. This value, of course, varies depending upon the season of the year. The state farms' contribution is slightly more than 50% of the milk

supplied to the Dairy Plant. The rest is supplied by private farms & through collection centers from individuals.

The contribution made by the storage bulk tanks, holding the pasteurized milk, to the post pasteurization contamination (PPC) was investigated. As can be seen in Table 17, more organisms are introduced into the pasteurized milk as it goes to the packing parlour. Data obtained using RODAC plates & rinse solutions clearly indicate that the tanks do contain several species of microorganisms (Table 17). Assessment of KQ of milk samples from the different sampling sites also revealed this condition, showing a decrease in KQ of pasteurized milk from these bulk tanks (P2) as compared to the KQ of milk sample from the pasteurizer (P1) (Fig. 1). Another important source for PPC occurred in the fillers. Marshall & Apel (1973) surveyed 12 modern fluid milk plants & concluded that relatively large numbers of bacteria are introduced from pipelines valves & filling machines into pasteurized milk. This has also been confirmed by Wilkinson & Davies (1973) & Eden, Rosen & Mannheim (1979).

Due to lack of milk containers, plastic rolls were fitted to hold milk after being treated with UV light in the fillers. The plastic bags which are used for packing the milk are made up of plastic rolls which originally served for storing sugar & other solid food items. These plastic rolls were not either properly sterilized or covered after UV treatment to avoid recontaminations. The sheets are carried to the packing parlor & left open until they are fitted to the fillers. The mishandling which occurred must have exposed the plastic rolls to microbial contamination from the surrounding environment & personnel. Hence, the microbial contamination of the plastic rolls also contributed to the final load of microbial mass in the milk. Assessment

of microbial contamination of these plastic containers by RODAC plates has shown that both UV treated & untreated ones contain many organisms (Table 19). UV treatment of packing materials has been found to decrease the microbial load to a certain extent. Zall (1981) pointed out that the action of UV on microorganisms depends upon the quality of radiation that reaches the organisms &, therefore, the system must be properly engineered & maintained. UV radiation will not work effectively unless environmental sanitation is also maintained properly.

Room contamination also contributes greatly to the entrance of spoilage microorganisms into the pasteurized milk & milk products in these areas. This has been assessed using TSA plates that were left open for 15 minutes at the different sampling sites. This assessment has, therefore, revealed that the air in these rooms contained a great variety of microorganisms which are potential contaminants of milk (Table 18). Many of these microorganisms isolated from the rooms are the same as those isolated from pasteurized milk.

Dimick (1973) exposed pasteurized milk to 100 foot candles of fluorescent light for 48 hours & examined the milk for off-flavor. There was a detectable off-flavor in milk in clear plastic & flint glass containers; however, there was no off-flavor in milk in regular paperboard containers. This has also been repeatedly reported by many workers. Light has been found, by many workers, to have an adverse effect on milk flavor. The transportation of milk by the AAPDE in transparent plastic containers in open trucks exposes the milk to light. This most probably will affect the quality of the milk to some extent.

Hansen, Turner & Aurand (1972; 1975) recommended that for minimum cost, oxidized flavor & vitamin destruction in fluid milk packaged in plastic bags can be reduced with gold & green filters in dairy cases. Plastic containers made with a patented green pigment (Rile Coe) gave 100% protection of shelf-life for milk. Nelson & Cathcart (1984) also reported that pigmented polyethylene milk containers coated with 4% titanium dioxide result in a substantial reduction in light transmission.

The KQ of the different milk samples was studied by storing them at different storage temperatures. Refrigeration increases the shelf-life (KQ) of a food or sample. The fewer the bacteria in the milk, the longer is the KQ (Lagrange, 1979; Schroder, Cousins & McKinnon, 1982). The laboratory pasteurized milk whether maintained at refrigeration or room temperature had the best KQ (Fig. 1). Since the rate of contamination was higher for the plant pasteurized milk, its KQ was also much lower than that of the laboratory pasteurized milk. Milk samples collected after pasteurization at various sites, identified as P1, P2 & P3, also showed progressive decrease in KQ. This complements the earlier findings made in this study concerning the high degree of contamination which emanated from the utensils & other sources after pasteurization (Fig. 1).

Psychrotrophic Gram-negative bacteria are responsible for reducing the KQ of refrigerated pasteurized milk (Phillips, Griffiths & Muir, 1981; Schroder, Cousin & McKinnon, 1982; Phillips & Griffiths, 1986; Kroll & Rodrigues, 1986). In pasteurized milk, psychrotrophic GNRs have been found to grow more quickly at 5°C than do psychrotrophic Gram-positive bacteria, but the reverse holds true at higher temperatures (Rodrigues & Pettipher, 1984).

The optimum control for psychrotrophic bacteria is, therefore, to have the least PPC followed by holding the product at the lowest practical temperature & certainly not above 5°C. Even relatively short periods of warm up may so accelerate growth that KD is reduced significantly. Admittedly the best way of reducing or retarding psychrotrophic bacteria is by preventing their entry into milk at the farm. After all, prevention is the best method & one which is technically feasible, but it is not easily achieved (White & Shilotri, 1979).

The chemicals used for rinsing & disinfecting the utensils at AADDE regularly are NaOH & NaCO₃ at 26.5g/l & 10g/l concentrations respectively. Investigation on the effectiveness of these two chemicals to remove or kill bacteria which are mostly associated with the utensils was carried out. As can be seen from Table 20, the chemicals were completely ineffective in inactivating bacteria at 1:100 concentration even if maintained for up to 60 & 90 minutes. On the other hand, disinfectants such as Savlon & Chlorox at 1:500 concentrations for 15-30 minutes killed the bacteria. The only group which withstood this treatment were those belonging to genus Bacillus. Post pasteurization contamination can be greatly reduced if such types of chemicals are used at the concentrations shown in Table 20 or at lower concentrations if disinfection is continued for a longer time period.

6. CONCLUDING REMARKS & RECOMMENDATIONS

Milk intended for consumption must be free from pathogens & must, if conditions permit, contain no or few bacteria. Clean milk could only be obtained if effective sanitary measures are taken starting from the point of milk withdrawn from the cow until it reaches the consumers. The shelf-life (KQ) of milk, be it raw or pasteurized, is only increased if the total microbial load in the milk is low at the start. Most nations have set up bacterial quality standards for milk & milk products. These standards are set based on experience. A regulatory agency then enforces the rules set.

An effective milk control program depends upon:

- 1) establishment of standards
 - 2) use of effective enforcement &
 - 3) education of producers, processors, distributors & consumers.
- Effective standards must apply to all aspects of production, processing & distribution.

Cleanliness involves washing & disinfecting the udder, milkers' hands & milking utensils. The aseptically collected milk must then be stored & transported cold to the Dairy Plant. Cold storage temperatures, minimal storage times & basic equipment sanitation are very important to milk quality.

The raw milk collected on the average has an SPC which ranges between 10^7 to 10^9 . On the other hand, the pasteurized milk has a SPC of 10^4 to 10^6 . The SPC of pasteurized milk is higher than that of other countries, hence, its KQ is much lower.

such as Savlon, Dettol, Chlorox or others which contain active ingredients such as Chlorhexidine & Cetriuride (Savlon), Chloroxyleneol (Dettol) & Chlorine (Chlorox) could be used at desirable concentrations to disinfect the utensils. This will ensure pasteurized milk whose SPC & KQ are acceptable.

4. It is recommended that the plastic rolls be UV radiated for longer duration & be maintained under aseptic conditions until use.
5. It is recommended that the clear polyethylene plastic bags be replaced with either pigmented polyethylene bags or paperboard cartons so as to reduce light transmission & hence prevent the adverse effects of light on milk. This can, therefore, increase the shelf-life of pasteurized milk & prevents its vitamin destruction.
6. The room sanitation must also be maintained. This could involve using wetting agents on the floors to avoid dust particles from hanging in the rooms.
7. In order to implement quality control programs & to make sure that the above recommendations are fulfilled, establishment of standards & the use of effective enforcement are essential.

REFERENCES

- Adams, D.M., Barach, J.T. & Speck, H.L. 1975. Heat resistant proteases produced in milk by psychrotrophic bacteria of dairy origin. J. Dairy Sci. **58** : 828.
- Adams, D.M., Barach, J.T. & Speck, H.L. 1976. Effect of psychrotrophic bacteria from raw milk proteins & stability of milk proteins to ultra-high temperature treatment. J. Dairy Sci. **59** : 823.
- Adams, D.M. & Brawley, T.G. 1981. Heat resistant bacterial lipases & ultra-high temperature sterilization of dairy products. J. Dairy Sci. **64** : 1951-1957.
- Aggarwal, H.L. 1974. Commercial sterilization & aseptic packaging of milk products. J. Milk Food Technol. **37** : 250-254.
- Ahmed, A.A.H., Houstafa, H.K. & Marth, E.H. 1983. Incidence of Bacillus cereus in milk & some milk products. J. Food Prot. **46** : 126-128.
- Anderson, R.E., Danielson, G., Hedlund, C.B. & Svensson, S.G. 1981. Effect of a heat resistant microbial lipase on flavor of UHT sterilized milk. J. Dairy Sci. **64** : 375-379.
- Andrey, J. Jr., & Frazier, W.C. 1959. Psychrophiles in milk held two days in farm bulk cooling tanks. J. Dairy Sci. **42** : 1781-1784.
- Anon. 1979. Ultra-high temperature milk successful in Canada. Amer. Dairy Rev. **41** : 22-25.
- Atwal, J.S., Shroff, D.M., Rattan Chand, & Srinivasan, R.A. 1974. Studies on aerobic spore forming bacteria in milk & milk products. I. Occurrence in raw & pasteurized milk. J. Dairy Sci. **27** : 22.

- Bandler, D.K., & Wolf, E.T. 1979. Characterization & extent of hydrolytic rancidity in fresh & aged pasteurized milk. J. Dairy Sci. **62** : 34.
- Bari, A., Awam, M.B., Sami, Z., Aktar, S. & Begum, M. 1986. Bacteriology of raw milk, meat & some ready-to-eat foods in Pakistan. Second World Congress : Food-borne Infections & Intoxications, vol. 1. Institute of Veterinary Medicine-Robert Von Ostertag - Institute, Berlin.
- Barnard, S.E. 1972. Importance of shelf-life for consumers of milk. J. Dairy Sci. **55** : 134-136.
- Barnard, S.E. 1979. Quality & flavor of store purchased milk samples. J. Dairy Sci. **62** : 34.
- Bigalke, D.L. 1978. Effect of low temperature cleaning on microbiological quality of raw milk & cleanliness of milking equipment on the farm. J. Food Prot. **41** : 902-906.
- Bishop, J.R., White, C.H. & Firstenberg-Eden, R. 1984. Rapid Impedimetric method for determining the potential shelf-life of pasteurized whole milk. J. Food Prot. **47** : 471-475.
- Bjorck, L. 1978. Antibacterial effect of lactoperoxidase system on psychrotrophic bacteria in milk. J. Dairy Res. **45** : 109-118.
- Bjorck, L., Claesson, O. & Schulthess, W. 1979. The lactoperoxidase /thiocyanate/ hydrogen peroxide system as a temporary preservative for raw milk in developing countries. Milchwissenschaft **34** : 726-729.
- Bjorck, L., Rosen, C.G., Marshall, V. & Reiter, B. 1975. Antibacterial activity of the lactoperoxidase system in milk against pseudomonads & other Gram-negative bacteria. Appl. Microbiol. **30** : 199-204.

- Blankenagel, G. 1976. An examination of methods to assess post pasteurization contamination. J. Milk Food Technol. **39** : 301-304.
- Bossuyt, R.G. & Waes, G.H. 1983. Impedance measurements to detect post pasteurization contamination of pasteurized milk: J. Food Prot. **46** : 622-624.
- Brock, T.D. & Brock, K.M. 1978. Basic Microbiology. Prentice-Hall, Inc., New Jersey.
- Buchanan, R.E. & Gibbons, N.E. 1974. Bergey's Manual of Determinative Bacteriology. 8th. ed. The Williams & Wilkins Co., Baltimore.
- Buxton, A. & Fraser, G. 1977. Animal Microbiology. Blackwell Scientific Publications, London.
- Cady, P., Hardy, D., Martins, S., Dufour, S.W. & Kraeger, S.J. 1978. Automated impedance measurements for rapid screening of milk microbial contents. J. Food Prot. **41** : 277-283.
- Chattelin, Y.H. & Richard, J. 1981. Sources of bacterial contamination of raw milk on the farm. Lait **61** : 80-94.
- Chung, B.H. & Cannon, R.Y. 1971. Psychrotrophic spore forming bacteria in raw milk supplies. J. Dairy Sci. **54** : 448.
- Cliffe, A.J. & Law, B.A. 1982. A new method for the detection of microbial proteolytic enzymes in milk. J. Dairy Res. **49** : 209-219.
- Collins, E.B. 1981. Heat resistant psychrotrophic microorganisms. J. Dairy Sci. **64** : 157- 160.
- Collins, C.H. & Lyne, P.M. 1976. Microbiological Methods. 4th. ed. Butterworth & Co. (Publishers) Ltd., London.

- Corpe, W.A. 1970. Attachment of marine bacteria to solid surfaces. In Adhesion in Biological Systems, pp. 73-87. Edited by R.S. Manly. New York & London: Academic Press.
- Cousin, H.A. 1982. Presence & activity of psychrotrophic microorganisms in milk & dairy products: A review. J. Food Prot. 45 :172-207.
- Cox, W.A. 1975. Problems associated with bacterial spores in heat treated milk & dairy products. J. Soc. Dairy Technol. 28 : 59-68.
- Credit, C., Hedeman, R., Heywood, P. & Westhoff, D. 1972. Identification of bacteria isolated from pasteurized milk following refrigerated storage. J. Milk Food Technol. 35 : 708-709.
- Cuthbert, W.A., Egdell, J.W. & Thomas, S.B. 1953. Recent investigations on thermophilic organisms in milk. Proc. XIII Int. Dairy Congr., The Hague. 2 : 139.
- Dimick, P.S. 1973. Effect of fluorescent light on the flavor & selected nutrients of homogenized milk held in conventional containers. J. Milk Food Technol. 36 : 383-387.
- Donnelly, L.S. & Busta, F.F. 1981. Anaerobic spore forming microorganisms in dairy products. J. Dairy Sci. 64 : 161-166.
- Eden, R.F., Rosen, B. & Mannheim, C.H. 1979. Seasonal & processing influences on bacterial count of raw & processed milk. J. Food Prot. 42 : 683-685.
- Ekstrand, B., Mullan, W.H.A. & Waterhouse, A. 1985. Inhibition of the antibacterial lactoperoxidase-thiocyanate-hydrogen peroxide system by heat-treated milk. J. Food Prot. 48 : 494-498.

- Fletcher, M. 1976. The effects of Proteins on bacterial attachment to polystyrene. J. Gen. Microbiol. **94** : 400-404.
- Ford, J.E. 1967. The influence of the dissolved oxygen in milk on the stability of some vitamins towards heating & during subsequent exposure to sunlight. J. Dairy Res. **34** : 239-247.
- Franklin, J.G., Williams, D.J. & Cleg, L.F.L. 1956. A survey of the number & types of aerobic mesophilic spores in milk before & after commercial sterilization. J. Appl. Bacteriol. **19** :46-53.
- Gebhardt, L.P. & Nicholes, P.S. 1975. Microbiology. The C.V. Mosby Company, Saint Louis.
- Gebre-Egzabher, A., Humbert, E.S. & Blankenagel, G. 1980. Heat stable proteases from psychrotrophs in milk. J. Food Prot. **43** : 197-200.
- Gilliland, S.E. & Ewell, H.R. 1983. Influence of combinations of Lactobacillus lactis & potassium sorbate on growth of psychrotrophs in raw milk. J. Dairy Sci. **66** : 974-980.
- Gnan, S. & Luedecke, L.O. 1982. Impedance measurements in raw milk as an alternative to the standard plate count. J. Food Prot. **45** : 4-7.
- Goepfert, J.M., Spira, W.M. & Kim, H.U. 1972. Bacillus cereus food poisoning organism. A review. J. Milk Food Technol. **35** : 213-227.
- Grosskopf, J.C. & Harper, W.J. 1969. Role of psychrophilic spore formers in long life milk. J. Dairy Sci. **52** : 897.
- Hankin, L. & Stephens, G.R. 1972. What tests usefully predict keeping quality of perishable foods? J. Milk Food Technol. **35** : 574-576.

- Hansen, A.p., Turner, L.G. & Aurand, L.W. 1972. Effect of fluorescent light on flavor & vitamins of milk packaged in plastic bottles & methods to prevent deterioration. J. Dairy Sci. 55 : 678.
- Hansen, A.P., Turner, L.G. & Aurand, L.W. 1975. Fluorescent light activated flavor in milk. J. Milk Food Technol. 38 : 388-392.
- Hartley, J.C., Vedamuthu, E.R. & Reinbold, G.W. 1969. Bacteriological methods for evaluation of raw milk quality. A review. J. Milk Food Technol. 32 : 4-15.
- Harvey, H. & Gilmour, A. 1985. Application of current methods for isolation & identification of staphylococci in raw bovine milk. J. Appl. Bacteriol. 59 : 207-221.
- Hedrick, T.I. & Glass, L. 1975. Chemical changes in milk during exposure to fluorescent light. J. Milk Food Technol. 38 : 380-385.
- Ingraham, J.L. 1962. Newer concepts of psychrophilic bacteria. pp. 41-56. In Proceedings Low Temperature Microbiology Symposium. 1961. Campbell Soup Company, Camden, New Jersey.
- Isani, G.B., Sheikh, B.A. & Arain, M.A. 1986. Bacteriological studies of market milk obtained from Hyderabad city. Second World Congress : Food-borne Infections & Intoxications, vol. 1. Institute of Veterinary Medicine-Robert Von Ostertag-Institute, Berlin.
- Janzen, J.J. 1972. The effect of somatic cell concentration in the raw milk on the shelf-life of the processed product. J. Milk Food Technol. 35 : 112.
- Janzen, J.J., Bishop, J.R., Bodine, A.B. & Cadwell, C.A. 1982. Shelf-life of pasteurized fluid milk as affected by age of raw

- milk. J. Dairy Sci. **65** : 2233-2236.
- Johns, C.K. 1969. Tests for estimating shelf-life of milk & milk products. J. Milk Food Technol. **32** : 301-303.
- Johns, C.K. 1975. Use of counts after preliminary incubation to improve raw milk quality for a Denver plant. J. Milk Food Technol. **38** : 481.
- Johnston, D.W. & Bruce, J. 1982. Incidence of thermophilic psychrotrophs in milk produced in the West of Scotland. J. Appl. Bacteriol. **52** : 333-337.
- Jones, F.T. & Longlois, B.E. 1977. Microflora of retail fluid milk products. J. Food Prot. **40** : 693-697.
- Juffs, H.S. 1973. Identification of Pseudomonas species isolated from milk produced in south eastern Queensland. J. Appl. Bacteriol. **36** : 585-598.
- Juffs, H.S. 1976. Effect of temperature & nutrients on proteinase production by Pseudomonas fluorescens & P. aeruginosa in broth & milk. J. Appl. Bacteriol. **40** : 23-32.
- Juffs, H.S. & Babel, F.J. 1975. Rapid enumeration of psychrotrophic bacteria in raw milk by the microscopic colony count. J. Milk Food Technol. **38** : 333.
- Juven, B.J., Gordin, S., Rosenthal, I. & Laufer, A. 1981. Changes in refrigerated milk caused by Enterobacteriaceae. J. Dairy Sci. **64** : 1781-1784.
- Kaufmann, O.W., Hedrick, T.I., Pflug, I.J., Pheil, L.G. & Keppler, R.A. 1960. Relative cleanability of various stainless steel finishes after soiling with inoculated milk solids. J. Dairy Sci. **43** : 28-41.

- King, J.S. & Habbit, L.A. 1982. Preservation of raw milk by the addition of carbon dioxide. J. Dairy Res. **49** : 439-447.
- Kroll, R.G. & Rodrigues, U.N. 1986. Prediction of the keeping quality of pasteurized milk by the detection of cytochrome C oxidase. J. Appl. Bacteriol. **60** : 21-27.
- Lagrange, W.S. 1979. Opportunities to improve milk quality. J. Food Prot. **42** : 599-603.
- Langveld, L.P.M., Cuperus, F., Van Breemen, P. & Dijkers, J. 1976. A rapid method for detection of post pasteurization contamination in HTST pasteurized milk. Neith. Milk Dairy J. **30** : 157-173.
- Law, B.A. 1979. Reviews of the progress of Dairy Science : Enzymes of psychrotrophic bacteria & their effects on milk & milk products. J. Dairy Res. **46** : 573-588.
- Law, B.A., Andrews, A.T. & Sharpe, M.E. 1977. Gelation of UHT sterilized milk by proteases from a strain of Pseudomonas fluorescens isolated from raw milk. J. Dairy Res. **44** : 145.
- Marshall, J.H. 1982. Cleaning & disinfection of large bore milking pipeline machine. Kieler Milchwirtschaftliche Forschungaberichte. **34** : 58-62. (Dairy Sci. Abst. **44** : 8620, 1982).
- Marshall, K.C., Stout, R. & Mitchell, R. 1971. Mechanism of the initial events in the sorption of marine bacteria to surfaces. J. Gen. Microbiol. **68** : 337-348.
- Marshall, R.T. & Appel, R. 1973. Sanitary conditions in twelve fluid processing plants as determined by use of the rinse filter method. J. Milk Food Technol. **36** : 237-241.

- Marshall, R.T. & Harmon, C.C. 1978. The automated pyruvate method as a quality test for Grade A milk. J. Food Prot. 41 : 168-177.
- Marszalek, D.S., Gerchakov, S.M. & Udey, R. 1979. Influence of substrate composition on marine microfouling. Appl. & Env. Microbiol. 38 : 987-995.
- Marth, E.H. 1978. Standard Methods for the Examination of Dairy Products. 14th. ed. American Public Health Association, Inc., USA.
- Martin, J.H. 1974. Significance of bacterial spores in milk. J. Milk Food Technol. 37 : 94-98.
- Martin, J.H. 1981. Symposium : Heat resistant microorganisms in dairy food systems. J. Dairy Sci. 64 : 149-156.
- Haxcy, R.B. 1969. Residual microorganisms in "Clean-in-place" systems for handling milk. J. Milk Food Technol. 32 : 140-143.
- Mayerhofer, H.J., Marshall, R.T., White, C.H. & Lu, H. 1973. Characterization of heat stable protease of Pseudomonas fluorescens. Appl. Microbiol. 25 : 44-48.
- McKellar, R.C. 1981. Development of off flavors in UHT & pasteurized milk as a function of proteolysis. J. Dairy Sci. 64 : 2138-2145.
- McKinnon, C.H. & Pettipher, G.L. 1983. A survey of the sources of heat resistant bacteria in milk with particular reference to psychrotrophic spore forming bacteria. J. Dairy Res. 50 : 163-170.
- Mehta, R.S. 1980. Milk processed at Ultra-high temperatures- A review. J. Food Prot. 43 : 212-225.
- Moisio, T. & Kreula, H. 1973. A method of checking liquid milk products in sealed packages. Karjantuote 56 : 8-9 (Dairy Sci. Abstr. 37 : 6582).

- Murray, J.G. 1949. The speed of build up of thermophilic organisms on farm equipment. Proc. Soc. Appl. Bacteriol. 2 : 20.
- Nelson, F.E. 1981. The microbiology of market milk. In Dairy Microbiology vol. 1 ed. Robinson, R.K., pp. 165-207, London : Applied Science Publishers.
- Nelson, K.H. & Cathcart, W.H. 1981. Transmission of light through pigmented polyethylene milk bottles. J. Food Prot. 47 : 346-348.
- Newman, H.N. 1974. Microbial films in nature. Microbios 9 : 247-257.
- Nunez, J.A., Chavarri, F.J. & Nunez, M. 1984. Psychrotrophic bacterial flora of raw ewes' milk with particular reference to Gram-negative rods. J. Appl. Bacteriol. 57 : 23-29.
- O'Connor, R.E., Ewings, K.N., Hayward, A.C., & O'Rourke, P.K. 1986. Numerical taxonomy of proteolytic psychrotrophs from Queensland raw milks. J. Appl. Bacteriol. 61 : 25-38.
- Overcast, W.H. 1968. Psychrophilic microorganisms & keeping quality of milk & its products. J. Dairy Sci. 51 : 1336-1338.
- Parfitt, E.R. 1940. Bacteriological studies on the cleaning of milking machines. Bull. Purdue Univ. Agric. Exp. Sta. No. 449.
- Patel, G.B. & Blankenagel, G. 1972. Bacterial counts of raw milk & flavor of the milk after pasteurization & storage. J. milk Food Technol. 35 : 203-206.
- Patel, T.R., Bartlett, F.M. & Hamid, J. 1983. Extracellular heat resistant proteases of psychrotrophic pseudomonads. J. Food Prot. 46 : 90-94.
- Pederson, C.S. 1979. Microbiology of Food Fermentations. 2nd. ed. The Avi Publishing Company, Inc., USA.

- Phillips, J.D. & Griffiths, M.W. 1986. Estimation of Gram-negative bacteria in milk : A comparison of inhibitory systems for preventing Gram-positive bacterial growth. J. Appl. Bacteriol. **60** : 491-500.
- Phillips, J.D., Griffiths, M.W. & Muir, D.D. 1981. Factors affecting the shelf-life of pasteurized double cream. J. Soc. Dairy Technol. **34** : 109-112.
- Piton, C. & Richard, J. 1982. Source of bacterial contamination in moderately contaminated milk from farms near Rennes. Lait **62** : 67-74 (Dairy Sci. Abst. **44** : 4066, 1982).
- Priest, F.G. 1977. Extracellular enzyme synthesis in the genus Bacillus. Bacteriol. Rev. **41** : 711-753.
- Ramm, G. 1978. Competition in the UHT milk market. Molkerei Zeitung Welt der Milch **32** : 465-470 (Dairy Sci. Abst. **40** : 7281).
- Reiter, B. 1978. Review of the progress of dairy science : antimicrobial systems in milk. J. Dairy Res. **45** : 131-147.
- Reiter, B., Marshall, V.M. & Philips, S.M. 1980. The antibiotic activity of the lactoperoxidase-thiocyanate-hydrogen peroxide system in the calf abomasum. Res. Vet. Sci. **20** : 116-122.
- Richter, R.L., Schmidt, R.H., Smith, K.L., Mull, L.E. & Henry, S.L. 1979. Proteolytic activity in ultra pasteurized, aseptically packaged whipping cream. J. Food Prot. **42** : 43-45.
- Rodrigues, U.M. & Pettipher, G.L. 1984. Use of the direct Epifluorescent Filter Technique for predicting the keeping quality of pasteurized milk within 24 hours. J. Appl. Bacteriol. **57** : 125-130.
- Schroder, M.J.A. 1982. Effect of oxygen on the keeping quality of milk. J. Dairy Res. **49** : 407-424.

- Schroder, M.J.A. 1984. Origin & levels of post pasteurization contamination of milk in the dairy & their effects on keeping quality. J. Dairy Res. **51** : 59-67.
- Schroder, M.J.A., Cousin, C.M. & McKinnon, C.H. 1982. Effects of psychrotrophic post pasteurization contamination on the keeping quality at 11° & 5°C of HTST-pasteurized milk in the U.K. J. Dairy Res. **49** : 619-630.
- Scott, K.J., Bishop, D.R., Zechalko, A., Edwards-Webb, J.D., Jackson, P.A. & Scuffam, D. 1984. The nutrient content of liquid milk. J. Dairy Res. **51** : 37-50.
- Shehata, A.E., Magdoub, H.N.I., Sultan, M.E. & El-Samragy, Y.A. 1983. Aerobic mesophilic & psychrotrophic spore forming bacteria in buffalo milk. J. Dairy Sci. **66** : 1228-1231.
- Shehata, T.E. & Collins, E.B. 1971. Isolation & identification of psychrophilic species of Bacillus from milk. Appl. Microbiol. **21** : 466.
- Shehata, T.E., Duran, A. & Collins, E.B. 1971. Influence of temperature on the growth of psychrophilic strains of Bacillus. J. Dairy Sci. **54** : 1579-1582.
- Shipe, W.F., Senyk, G.F., Ledford, R.A., Bandler, D.K. & Wolf, E.T. 1980. Flavor & chemical evaluations of fresh & aged market milk. J. Dairy Sci. **63** : 43.
- Smith, T.L. & Witter, L.D. 1979. Evaluation of inhibitors of rapid enumeration of psychrotrophic bacteria. J. Food Prot. **42** : 158-160.
- Speers, J.G.S., Gilmour, A., Fraser, T.W. & McCall, R.D. 1984. Scanning electron microscopy of dairy equipment surfaces contaminated by two-milk-borne microorganisms. J. Appl. Bacteriol. **57**:139-145

- Stainer, R.Y., Doudoroff, H. & Adelberg, E.A. 1970. The Microbial World. Prentice-Hall, Inc., New Jersey.
- Stanley, P.M. 1983. factors affecting the irreversible attachment of Pseudomonas aeruginosa to stainless steel. Canadian J. Microbiol. **29** : 1493-1499.
- Stanley, S.O. & Rose, A.H. 1967. On the clumping of Corynebacterium xerosis as affected by temperature. J. Gen. Microbiol. **48** : 9-23.
- Stokes, J.L. 1963. General biology & nomenclature of psychrophilic microorganisms. Pages 187-192. In N.E. Gibbons (ed.), Recent Progress in Microbiology. University of Toronto Press, Canada.
- Stokes, J.L. & Redmond, H.L. 1966. Quantitative ecology of psychrophilic microorganisms. Appl. Microbiol. **14** : 74-78.
- Thomas, S.B. 1958. Psychrophilic microorganisms in milk & dairy products. Part I. Dairy Sci. Abst. **20** : 355-370.
- Thomas, S.B. 1966. Sources, incidence and significance of psychrotrophic bacteria in milk. Milchwissenschaft **21** : 270-275 (cited in Cousin, 1982).
- Thomas, S.B. 1969. Methods of assessing the psychrotrophic bacterial content of milk. J. Appl. Bacteriol. **32** : 269-296.
- Thomas, S.B. 1970. Psychrotrophic microorganisms in market cream. A review. Part I. Dairy Ind. **35** : 79-84.
- Thomas, S.B. 1974. The microflora of bulk collected milk. Part 2. Dairy Ind. **40** : 279-282.
- Thomas, S.B. & Druce, R.G. 1969. Psychrotrophic bacteria in refrigerated milk. Part I. Dairy Ind. **34** : 351-355.

- Thomas, S.B. & Druce, R.G. 1972. The incidence & significance of Coli-aerogenes bacteria in refrigerated bulk collected milk. Dairy Ind. **37** : 593-598.
- Thomas, S.B., Druce, R.G. & Davies, A. 1966. The significance of psychrotrophic bacteria in raw milk. Dairy Ind. **31** : 27-32.
- Thomas, S.B., Druce, R.G., Peters, G.F. & Griffiths, D.G. 1967. Incidence & significance of thermoduric bacteria in farm milk supplies : A reappraisal & review. J. Appl. Bacteriol. **30** : 265-298.
- Thomas, S.B. & Thomas, B.F. 1973. Psychrotrophic bacteria in refrigerated bulk-collected raw milk. Part I. Dairy Ind. **38** : 11-15.
- Thomas, S.B. & Thomas, B.F. 1975. The bacteriological grading of bulk collected milk. Part 7 : Thermoduric, psychrotrophic & coli-aerogenes colony counts. Dairy Ind. **40** : 338-340.
- Thomas, S.B. & Thomas, B.F. 1976. The bacterial content of farm bulk milk tanks. Dairy Ind.Int. **41** : 162-164.
- Thomas, S.B. & Thomas, B.F. 1978. The bacterial content of milking machines & pipeline milking plants. Part V. Thermoduric organisms. Dairy Ind. Int. **43** : 17-21, 25.
- Torrie, J.P., Cholette, H., Froehlich, D.A. & McKellar, R.C. 1983. Growth of an extracellular proteinase deficient strain of Pseudomonas fluorescens on milk & milk proteins. J. Dairy Res. **50** : 365-374.
- Tuszynski, W.B. 1978. Packaging, storage & distribution of processed milk. Animal Production & Health Paper (FAO) No. 11.
- Wadsworth, K.D. & Bassette, R. 1985. Laboratory scale system to process Ultra-high temperature milk. J. Food Prot. **48** : 530-531.

- Washam, C.J., Olson, H.C. & Vedamuthu, E.R. 1977. Psychrotrophic bacteria & the effects of their enzymes on milk & milk products. J. Food Prot. **40** : 101.
- Westhoff, D. & Doores, S. 1976. Milk processed by a Falling-Film pasteurizer-sterilizer. J. Dairy Sci. **59** : 1003-1009.
- Westhoff, D.C. & Dougherty, S.L. 1981. Characterization of bacillus species isolated from spoiled ultra-high temperature processed milk. J. Dairy Sci. **64** : 572-580.
- White, C.H., Gillis, W.T., Simmler, D.L., Galal, M.K., Walsh, J.R. & Adams, J.T. 1978. Evaluation of raw milk quality tests. J. Food Prot. **41** : 356-360.
- White, C.H. & Little, R.D. 1983. Evaluation of tests to predict the shelf-life of fluid milk products. J. Dairy Sci. **66** : 268.
- White, C.H. & Shilotri, S.G. 1979. Inoculation of citric acid fermenting bacteria into raw milk in farm bulk tanks. J. Food Prot. **42** : 51-54.
- Wilkinson, G. & Davies, F.L. 1973. Germination of spores of B. cereus in milk & milk dialysates: Effect of heat treatment. J. Appl. Bacteriol. **36** : 485-496.
- Witter, L.D. 1961. Psychrophilic bacteria - a review. J. Dairy Sci. **44** : 983-1015.
- Zajac, M., Gladys, J., Skarzynska, M., Harnulv, G. & Bjorck, L. 1983. Changes in bacteriological quality of raw milk stabilized by activation of its lactoperoxidase system & stored at different temperatures. J. Food Prot. **46** : 1065-1068.
- Zall, R.R. 1981. Control & Destruction of Microorganisms. In Dairy Microbiology vol. 1 ed. Robinson, R.K., pp. 77-117, London: Applied Science Publishers.