IDENTIFICATION OF FACTORS LEADING TO THE SPOILAGE OF STERILIZED MILK

By

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DECLARATION

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AFFECTIONATELY DEDICATED TO
MY EVER LOVING PARENTS
&
TEACHERS

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ABSTRACT

Milk and milk products are an important as they gain demand because of its nutritional and organoleptic properties. Milk is perfect medium for microorganism to grow that favours it's resulting in subsequent spoilage. Thus, bacteria can easily be attracted and can rapidly multiply. Hence, in milk industry milk processing plays a major role leading to the enhancement of keeping quality of milk.

The microbial count of 1ml of raw milk is normally uncountable. Heat processing is done to bring this count to a lower safer level. Sterilized milk bottles shelf life is about 5 months. Normally commercial sterilization, spores are present 1 bottle out of 100. If milk bottles retain, bacteria it leads to the curdling of bottles. They may be post contaminated or process operation problem may retain bacteria spores in sterilized bottled.

Curdled bottles were taken and identified the presence of bacteria by using culture techniques. After that gram staining test was done for differentiation of bacteria either gram negative or gram positive. Degree of contamination was determined by drawing sample from all stages in process line and through following enumeration of TCC, Td and Tp bacteria. Sterilized bottles were incubated various temperature and identified the optimum temperature for curdling of bottes.55-60 ^{0}C range is considered to this most suitable temperature.

Changing of the pH acidity in curdles bottles reflects presence of bacteria in bottles. This variation was identified by measuring the pH and acidity in curdle and normal bottles.

The bottles may contaminated by remaining spores if washing process is inefficient. Therefore, the strength of washing tanks (pre soak and main soak) has been calculated. These calculations were done every testing day. After washing the remaining causticities were identified by adding the drop of phenolphthalein.

Samples were collected to be determining the process temperature variability this was done 3 times per day. The Changing in bottle sterilization method using autoclave lead to the poor time- temperature combination exposure to all the sterilized bottles. After the incubation at 55^{0}C period autoclave sterilized bottles were not curdle that identified. The heat treatment of milk causes some changes in the properties of milk depending upon the time temperature combination.

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LIST OF ABBREVIATIONS

B.	*Bacillus*
Eg.	Example
Hrs.	hours
Km	Kilometer
Ltd.	Limited
min.	minutes
N	Molarities
TCC	Total Colony Count
Td	Thermoduric
Tem.	Temperature
Tp	Thermophilic
spp.	Species
sterili.	Sterilization
UHT	Ultra High Temperature
GMP	Good Manufacturing Practices
HACCP	Hazard Analysis critical control point

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CHAPTER 1

Introduction & objectives

1.1Introduction

Milk is used for human consumption in the form of milk as such, or as an ingredient of different food items or in the form of various products. Purpose of converting milk in to its products can be,

- The Improvement of keeping the quality of milk
- In Minimize of storage facilities
- Decreasing storage space
- Avoid marketing problems
- Utilization of seasonal surplus
- Minimizing the Possibility of recontamination
- Solving the problems of unforeseen market failure
- Better nutritive value
- Preference, diversification, employment and enabling the export

Microbiological methods have an important role to play in the dairy industry. They are used to protect the public health and can reduce economic losses by the early detection of inadequate processing, packing or refrigeration. This can be achieved by monitoring the microbiological quality of raw milk supplies, bulk milk, and finished milk products immediately after production and during storage.

Sterilized milk must be sterile that is, it should contain neither bacteria nor bacterial spores (Burton et al., 1965). The main purpose of milk sterilization is to provide a food product whose properties immediately after processing will remain stable as long as possible.

In bottles, heat treatment destroys at least half of the vitamin C and there is significant lossof thiamin (30-40%).Vitamin B12 is almost completely destroyed. The indications at the present are that there is some loss of Vitamin B6 during sterilization; the

process has no effect on Vitamin A, Carotene, Riboflavin and its effect on the remaining Vitamins studied is doubtful (FAO, 1975).

The cooked taste in sterilized milk most predominant during the first few days after sterilization. But during storage, the sulfur compounds responsible for off flavour gradually are oxidized and the tastes of the milk improve; however during further storage, a taste similar to the initial cooked tástes appears (FAO, 1975).

Milk stored for long time at low temperature may contain high numbers of psychotropic bacteria, which can produce heat-resistant enzymes that are not completely inactivated through sterilization. During storage they can cause taste changes such as rancidity, bitterness or even gelation problems (age-thickening or sweet curdling).

The microorganisms most likely to endanger the stability of sterilized milk during the storage period are obviously those, which, for a variety of reasons may escape destruction in the sterilization stage. Except in those cases where the heating process is distinctly inadequate, these microorganisms consist of spores with a particularly high heat resistance level (which not all sterilization are equally able destroy).

Not all of these spores are contained in the raw milk. Some of them, the most heat resistant to heat and some times the most numerous, are introduced by the processing apparatus (e.g., containers or plant) or develop under the influence of processing conditions (such as temperature, or length of storage time before filling). This means that, if we are to deal more particularly with the characteristics of raw milk intended for sterilization, some of these factors of instability in sterilized milk must be left aside. Because of that, bottles were produced, as a result customer complain were raised. There fore completely sterile milk bottled production for the process was needed for the factory.

The research was related to a practical problem, curdling of some sterilized milk bottles incubated at 55^0C, identified at the milk processing factory of Milco Lanka (pvt) Ltd. at Narahenpita.

1.2 Objectives

1.2.1 Main Objective

Decreasing the risk of milk and milk products spoilage through identification of dormant micro flora and related factors.

1.2.2. Specific Objectives

- Identification of types of bacteria and enumerating microbial population in milk at each step of the manufacturing process.
- Identification of sources/places of contamination in the industrial processing.
- Application and evaluation of preventive measure effectiveness reduce the growth of spoilage microorganism.
- Studying the risk (probability) of recontamination.

CHAPTER 2

Literature survey

2.1. Importance of microbial spoilage of milk

Milk is the lacteal secretion, practically free from colostrums, obtained by the complete milking of one or more healthy cows, which contains not less than 8.24 % milk solids not fat and not less than 3.25 % milk fat (Atherton and Newlander, 2000).

There has been an increasing demand for milk and milk products during the recent years, which could be attributed to the higher standard of living and heath consciousness of people. Processing and preserving of milk has become essential due to its susceptibility to rapid spoilage (milk is contaminated very fast when exposed to air) and to prevent wastage of surplus milk in the producing areas due to lack of facilities to transport fresh milk to cities with in a short period. (Lampert, 1970)

2.2. Food safety as a function of the milk board

The main functions of the board to establish and maintain facilities and serve for the efficient and cheep production to marketing of milk to promote the establishment of maintenance of such facilities and services by local authorities and other bodies and person approve by the Ministry of Agriculture for the purpose of ensuring that an adequate supply of milk of good quality at reasonable prices is evaluate to consumer of milk in Ceylon. The milk board has its head office at Narahenpita, Pallekalle and Ambewella. The factory at Narahenpita introduced pasteurized milk firstly in to the market in August 1980.

In sterilized and pasteurized milk the factory at Narahenpita makes flavored milk. It was started in September 1981.

2.3. Milk Production and Utilization in Sri Lanka.

Milk production has some decline than previous years due to various reasons. The national production of milk in Sri Lanka in the year 2003 was 156 millions of liters. It accounted for 15% of national requirement and rest was imported as milk powder and other products. (Central bank report, 2003)

The gap of 390,000 liters was filled by the imported milk products such as milk powder, cheese etc. The milk import is about 50% of the requirement of the country. Per capita consumption of milk per day is approximately 68 ml. The comparison of per capita consumption of milk with that of other countries shows that it is very low in Sri Lanka. This may be due mainly due to the fact that the dairy sector in Sri Lanka is very small. In Sri Lanka the milk from cows and buffaloes are used as the raw materials in the production of market milk. (Pasteurized and sterilized), cheese, yogurt, butter, toned milk, condensed milk, dairy ice-cream, dry whole milk (full cream milk powder), non fat milk powder etc (De Silva, 1991).

Table.2.1 Total milk production of Sri Lanka.

year	Total milk production (million liters)	Total milk import (metric tones)
2003	156	67,941
2002	266	65,821
2001	266	55,471
2000	263	61,194
1999	260	54,000

Source :(Central Bank Report, 2003)

2.4. Definition of Sterilized Milk.

Strictly speaking, sterilized milk must be sterile that is it should contain neither bacteria nor bacterial spores, however, sometimes less stringent definitions are used that can be of practical value in many circumstances. Although, the theoretically correct definition of sterilized milk is that it must be completely sterile. The more practical definition is that sterilized milk is one that must have, at ambient temperatures, an unlimited keeping quality from the bacteriological point of view. This later definition does not always mean that sterilized milk must be completely free of bacterial spores, for there are spores (such as those of the obligate thermophilic bacilli) that under normal circumstances do not develop in milk (FA0, 1975).

2.4.1. Sterilization Techniques

The sterilization techniques most widely used today do not enable the spores of all species to be destroyed consistently. Those of thermophilic species are the most resistant, which caused to comment that absolute sterility was sometimes impossible to achieve in current practice. In the case of milk, the processor must always bear in mind that heating causes physicochemical changes which should be avoided as for as possible. The heating times and temperatures necessary to all spores are not always compatible with the avoidance of browning or modification of organoleptic and nutritive properties.

Once allowed to remain in the sterilized milk, surviving spores can germinate rapidly. Those of strictly aerobic Bacillus species are not hindered by the partial vacuum in the container to inhibit their development, an oxygen pressure of less than 0.2cmHg would be necessary.

Sterilized milk should therefore meet two requirements.

1) After processing, it should remain as close as possible to its original state.(e., it should have most of the properties of raw milk);
2) During storage, it should remain exactly as it was immediately after processing.

In other words, sterilized milk must be stable from the outset and remain stable.

Immediate stability is unquestionably bound up with the physical and chemical composition of the raw milk. Even if it is not clearly apparent immediately after treatment and only takes on its final nature during storage, its causes are not to be sought in bacteriological process(except in the case of very poor quality raw milk) but in an abnormal composition of the initial milk.

Permanents stability, on the other hand, is essentially linked with the absence of bacterial growth in the sterilized milk. The occasional failure in this respect during storage is due to the presence in the raw milk of organisms with a high resistance to heat, the only ones, in fact, that are of any importance in sterilization.

The sterilization of milk involves both a raw material with its individual characteristics, and processing techniques with their advantages and drawbacks. The question now posed is whether the raw material, with its assets and disadvantages, does not also influence the result of the operation, e., and the properties of the finish

product. In other words, should we not demand that raw milk have certain well-defined qualities, or rather that it should not have failings, so that the sterilized product may conform to its definition and, in particular, have the stability that characterizes products intended for keeping? (Burton et al., 1965)

2.4.2. Requirements for raw milk

The most important demand to milk is its stability during sterilization. From a chemical point of view the stability of milk protein must be such that it does not clot when using an alcohol with a concentration of about 75 % for the alcohol precipitation test (ATP). From a bacteriological point of view, there must be no spores that survive the sterilization temperature. There is no knowing method for determining this rapidly. However, spores that can resist sterilization temperature are very rarely found in milk (FAO,1975).

2.4.3. Bacteriological Aspects of Milk Sterilization

When raw milk is heat-treated the spores are not shortly destroyed, their destruction is a process that needs time, whether they are of different species or of one species. It has generally been accepted that spores, which heated at a constant temperature, are destroyed in such a manner that a straight line-presents the relation between the logarithm of the surviving number and the time, the so called thermal destruction curve. (FAO, 1975)

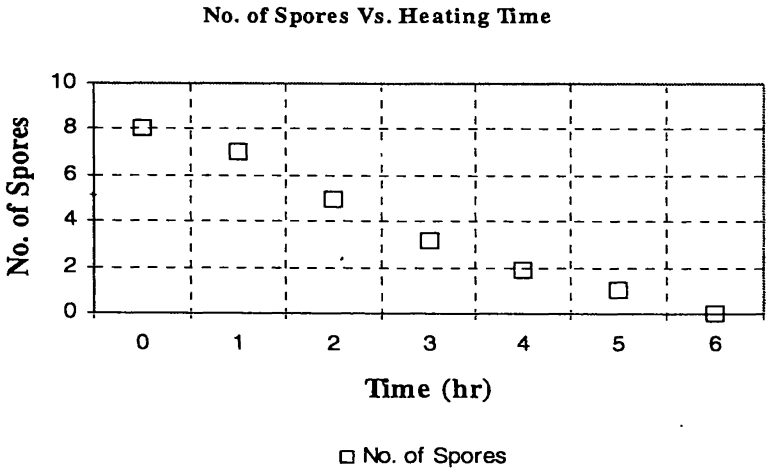


Fig.2.1 Semi logarithmic representation of the variation of the number of spores as a function of the heating time (FAO, 1975).

In dealing with sterilization, process the terms "decimal reduction" and "decimal reduction time" are often used. The latter indicates a reduction of the number of spores to one- tenth of the original concentration. The former reflects the time necessary for the performance of such a reduction. When spores destruction proceeds according to a straight curve the decimal reduction time is the same at all concentrations. From the course of the thermal destruction curve it follows that the initial spore concentration has an important effect upon the result of heat treatment. Heat treatment that reduces an initial concentration from e.g. 100.000 spores per liter to 1 spore per liter (5 decimal reductions) will reduce an initial concentration of 1000 spores per liter to 1 spore per 100 liters (also 5 decimal reductions). The term "sterilization effect" as employed here, refers to the number of decimal reductions that heat treatment is able to effect. A heat treatment with a sterilizing effect of 5 reduces the spore concentration from 100.000 per liter or from 1000 liter to 1 per 100 liters.

These theoretical remarks may be useful for a better understanding of the commercial sterilization process. When raw milk contains for example 10 spore per ml. and dairy manager wants to deliver a product whereby only one bottle, in 1000 may spoil, the heat treatment has to effect 7 decimal reductions). Commercial sterilization is not a term that can be precisely defined; the intensity of the heat treatment is dependent not only on the initial spore concentration but also on the final concentration acceptable to the milk plant in its product. The choice of this final concentration is greatly influenced by the conditions of the distribution and by the risk acceptable to the dairy manager. (FAO, 1975).

2.4.4 Chemical Aspects of Milk Sterilization

The higher the temperature of the heat treatment, the greater the sterilization effect and more marked the change in the colour and taste of the milk. When milk is heated off- flavours occur at first cooked flavour, caused by the production of volatile sulfur compounds, then, when intensity of the heat treatment is increased, a sterilization taste mainly caused by the reaction between the sugar and the protein constituents (Maillard reaction). This process influences not only the taste of the milk but also its colour, which become brownish.

One important phenomenon is that with increasing temperatures the spore-destruction rate increases more than influence on the taste and colour of milk. With every 10 ^{0}C

increase the rate of the browning reaction multiplies approximately 2.5 fold, while the rate of spore-destruction is increase of the heating temperature has on the browning rate as compared to the spore destruction rate (FAO, 1975).

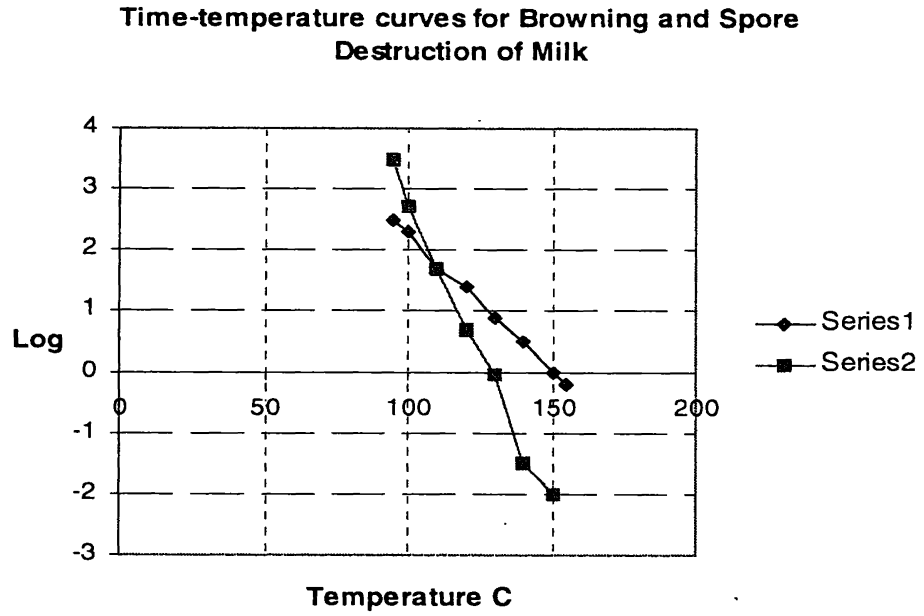
Table 2.2 Difference an increase of heating temperature has on the browning rate as compared to the spores' destruction rate (FAO, 1975).

Temp. Of heating, ^{0}C	Relative spore destruction rate	Relative rate of browning reaction
100	1	1
110	10	2.5
120	100	6.2
130	1000	15.6
140	10000	39.0
150	100000	97.5

It becomes more apparent when studying the number of temperature time combinations in table 2.2 with the same sterilizing effect and calculating the relative degree of browning caused by these heat treatments. All the time- temperature combinations produce the same sterilizing effect. (See Table 2.3)

Table.2.3 Time-Temperature combinations changing the Relative degree of Browning (FAO, 1975).

Temp. Of Heating, ^{0}C	Time, min	Relative degree of browning
100	100	100,000
110	60	25,000
120	6	6,250
130	36 sec	1,500
140	3.6 sec	390
150	0.36 sec	97



series1-brown discoloration

saries2-sterilization

Fig.2.2 Shows Time- Temperature Curves for Browning and Spores Destruction of Milk (FAO, 1975).

2.4.5. System of Milk Sterilization.

For the sterilization of milk, several different methods are now in use:

1). In bottle sterilization

The milk is bottled and sterilized at temperatures between 105- 120°C. The sterilization can be done either in an autoclave (batch) or in a tower sterilizer. (Continuous)

2). UHT (Ultra High Temperature for a Short Time)

The milk is sterilized in a continuous-flow at very high temperature (130-150°C) for very short Time (1-20sec.). These methods require aseptically packing in sterilized containers.

3) Two stage process sterilization.

The milk is first sterilized according to the UHT-process, then bottled and finally submitted to further heat treatment to destroy any spore, which may have entered during bottling (Khan,1998).

Figure 2.2 shows that at 110^0C the milk is sterile before it become brownish. This means that a sterile product is obtained that should be of normal colour. In practice, it is necessary to pay attention to the time of both heating and cooling. For proper sterilization there must be a certain safety margin in the duration of the heat treatment and this increase the possibilities of the heat-induced faults describes above. Heat treatments has however another influence upon the colour of the Milk. Intensive heat treatment will cause denaturing of the serum proteins with the result that milk reflects more light and thus appears whiter.

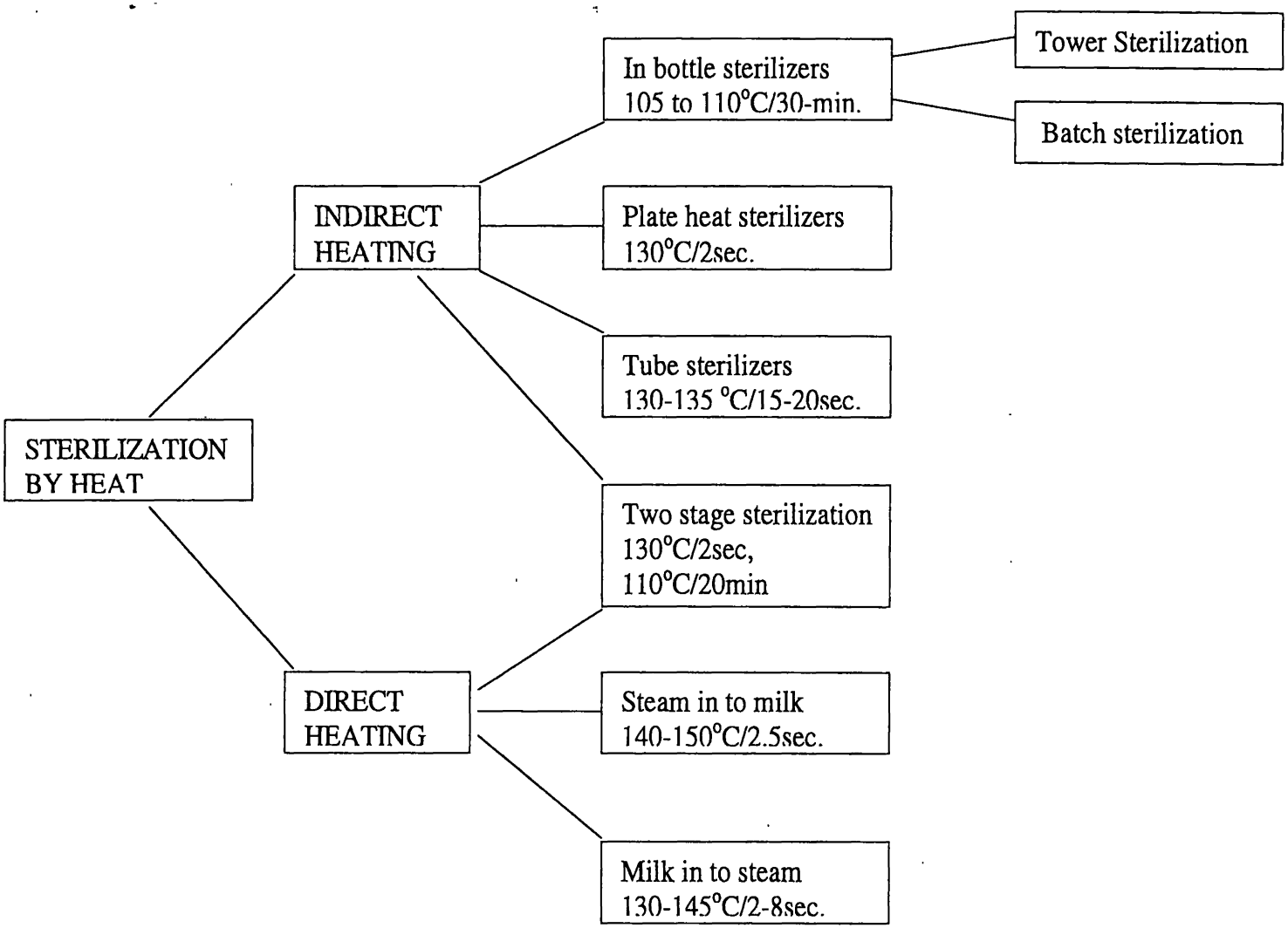


Fig. 2.3 Sterilization Methods (FAO, 1975)

2.5 Collection of milk

In Sri Lanka, milking is done twice a day. The morning (milk collecting in the morning) is normally processed or utilized during the same day and the evening milk, which has a higher percentage of fat (4 - 4.2%) is chilled and processed on the following day. Raw milk should be processed or chilled as soon as possible, after milking, as milk gets contaminated very easily and remove any dirty material, cooled and stored (FAO, 1975).

Milk is collected either in

a) Cans or

b) Bulk tanks twice a day

Small industrialists collect milk in aluminum cans. Large industries collect their requirement of milk in cans or in tanks from number of farms and individuals. The milk from the producers is brought to the collecting centers set up in close proximity to chilling centers or processing centers. For a cluster-collecting center, a milk-chilling center is 10 km radius of the processing center for the milk reach the cooling or processing center within two hours after milking to prevent spoilage and to ensure quality. Chilled milk from these centers is transported to processing industry in insulated bowzers, usually, of 400-600 tons capacity. The national milk board collects and utilizes about 30% of the country's milk production. The company has four milk processing centers at Narahenpita, Ambewela, Digana and Kilinochchi (De Silva, 1991).

2.6 Filtering of milk

The milk is first filtered in order to remove foreign bodies such as dust particles, hairs from the animal etc. In large dairies or farms the milk is piped directly from the milking until to a cool room equipped with a filtration unit. Small dairy holders and farmers having 2 or 3 cows, filter the milk through a sterilized kitchen strainer with a clean cloth as filter medium (De Silva, 1991).

2.7 Cooling of milk

When milk cannot be processed during the same day it has to be chilled to 4 ^{0}C to minimize the growth of microorganisms. Chilling is generally done in aluminum cans or jacketed stainless steel tanks.

In small scale chilling centers, milk is cooled in aluminum cans this is normally done by covering the milk cans with lids and placing them in a bath of cold running water. When a large volume of milk has to be cooled, jacketed bulk tanks fitted with agitators are used, where cold water from a refrigeration unit is circulated through the jacket. This process is generally takes more than 6 hrs (De Silva, 1991).

In large scale chilling centers and processing centers, this process is virtually automatic with rapid cooling facilities in the milk tank.

Transportation of unpasteurized milk should be done gently for the following reasons.

a) To avoid splitting of bacterial clumps;

b) To avoid alteration of fat globules;

c) To avoid admixture of air giving rise to oxidation problems (De Silva, 1991).

2.8 Storage of milk

Milk of good hygienic quality should be stored at 4 °C to prevent spoilage. Storage can be done in the bulk cooling tanks itself or in cans submerged in cold-water baths which is used for further processing.

2.9 Quality of Milk and Quality control.

Quality and quality control are very important aspects in the milk and milk processing industry. To check the quality, small-scale producers carry out simple spot tests such as lactometer test (flavour, colour, taste, and smell) etc. In order to minimize the capital outlay for testing facilities where as large milk processing companies have their own laboratories to do other elaborate testing procedures such as colony counts, special tests for added chlorine, salt Gerber test etc (De Silva, 1991).

2.10 Separation

The difference in specific gravity between the milk fat (of specific gravity, 0.93) and skim milk (of specific gravity, 1.035) causes the two components to separate when allowed to rest. The fat globules will raise to the surface and form a cream layer (FAO, 1975).

2.10.1. Cream Separation

Cream Separation is defined as the treatment that takes place in a centrifugal separator with one inlet for the milk and two outlets, one is for the heavy skim milk and the other for the light cream. The milk is channeled from the separator's inlet through the distribution holes in the disc stack and out in to the space between the disc where separation takes places. The interphase between the heavy and light phases is located at the same radius as the distribution holes. (FAO, 1975)

2.11 The Plate Heat Exchanger

Plate heat exchanger in which milk is rapidly heated to a temperature of not less than 72^0C and held for not less than 15 seconds and rapidly cooled down to a certain temperature depending on the size of regenerative and cooling sections.

The plate heat exchanger is made up of:

a. Fixed head (terminal head)
b. Plates
    1. Flow plates
    2. Intermediate plates
    3. Holder plates
    4. End plates
c. Movable plates
d. Closing plates

The plate's heat exchanger is used for HTST treatment especially for heating to temperatures, which are below the boiling point of milk. The plate heat exchanger is a

compact, and simple, unit, which is easy to clean and inspect. Its plates may be used for regeneration, heating, holding and cooling. The plates are supported in press between an intermediate plate in each heating and cooling section. The heat moves from the warm to the cool medium through stainless steel plates. The pates are constructed from 18-8 of what stainless steel, which is special steel alloy very resistant to cleaning chemicals. An approximately 3.5 mm space is maintained between the pates by a non-absorbent rubber gasket.

The pates are numbered and must be properly assembled. The plates ate normally mounted vertically in banks, from a carrying bar and held in place by a lower guide bar. The unit is usually closed by means of a central spindle enabling quick opening for inspection.

The plates are designed to provide uniform but not excessive turbulent flow of products resulting in rapid heat transfer. Raises sections on the plate in the form of knobs and channels help provide the turbulent action required. Greater capacity is obtained by adding more plates (FAO, 1975).

2.12 Standardization

When milk from number of producers with different fat content is collected in to a large tank, it has to be standardized. Standardization is the altering of the normal fat content of the milk or cream, by the addition of cream or skim milk to the desire fat content standardization of milk is done in standardization vat or tanks. This involves calculation to determine the amount of skim milk that has to be added if the fat content is too high

Pearson's square method is a very simple method to compute the amount of cream or skim milk to be added to arrive at the desired fat content (Burton et al., 1965).

2.13 Homogenizer

Milk is a very complex food containing a mixture of fat, protein, lactose, minerals etc. These constituents do not exist in perfect emulsion form and they separate out when allowed to stand over a period of time. Homogenization is the mechanical means of making a perfect stable emulsion of milk fat in milk serum. Homogenization is brought about by passing milk through a very small or face at a very high pressure, normally between 150 kg/cm^3. Where the fat globules, which are normally about 5 microns, are reduced to about 2 microns and suspended in the milk serum in a stable state.

The purpose of the homogenization of whole milk is to get a stable emulsion, which will not be liable to form a cream pluge or layer. The homogenization also makes it possible to reduce the fat content without affecting the flovour; this is an essential part of the production of sterilized milk, as otherwise the fat rises in to the neck of the bottle during heat treatment or during later storage and form a most unpleasant mass of solid or semisolid material. The homogenizer reduces the mean size of the fat globules in the milk, so that they no longer rise to the top of the milk but remain uniformly distributed (Burton et al., 1965).

2.14 Balance Tank

The maintenance of constant head on the suction of a dairy pump is essential to keep the product free of other gases and to contamination. For this purpose, a balance tank is fitted on the suction side of the pump. The balance tank is a stainless steel cylindrical tank, fitted with a float connected by a level to an ecentacally centrically rotating roller, which operates the inlet valve of the tank. When more milk is received, the float rises and the valve is closed when milk gets drained, the level of the float drops thereby the valve to let in fresh supply of milk (Burton et al., 1965).

2.15 Presterilizer

Milk, which has to be shelves for a long period, should be sterilized. For complete destruction of all the microorganisms, temperature as high as 135°C for 4 sec will have to be reached.

2.16 Buffer tank

Storage milk, before go to the reheated tank the temperature of the in milk in buffer tank is about 35°C. Purpose of the buffer tank is to germinate the spores, which are not destroyed in the presterilizer.

2.17 Washing of bottles

The bottle cleaning involves both a chemical and mechanical treatment to carry away solids, as protein and fat, and a bactericidal treatment, sanitation, to minimize the bacteria count in the bottle. Soaker type washer is constructed with an endless conveyor belt having receptacles in which the bottles are placed.

Pre soak section is a desirable feature when dealing with very dirty bottles, as loose solids can be removed or at least softened before the main detergent sections. This section's caustic soda strength is 1.5% and temperature is about 60-75°C (FAO, 1975).

2.17.1 Detergent soak tank

The duration of the soaking period is approximately 40% of the total time the bottles stays in the machine. They are actually exposed to the alkaline solution for nearly 70% of time. This section's caustic soda strength is 2% and temperature about 60-75°C (FAO,1975).

2.17.2 Pressure spraying

After the bottles, leave the soak tank. They travel upward and forward in the washer and are subjected to jet pressure spraying on the inner surface. This consists of pumping the warm alkaline solution with great force in to the bottle where by foreign matter previously loosened is thoroughly flushed out (FAO,1975).

2.17.3 Outside Rinsing

Jets spraying the hot alkaline solution on the outer surface perform the out side cleaning of the bottles (FAO, 1975).

2.17.4 Rinsing for alkali

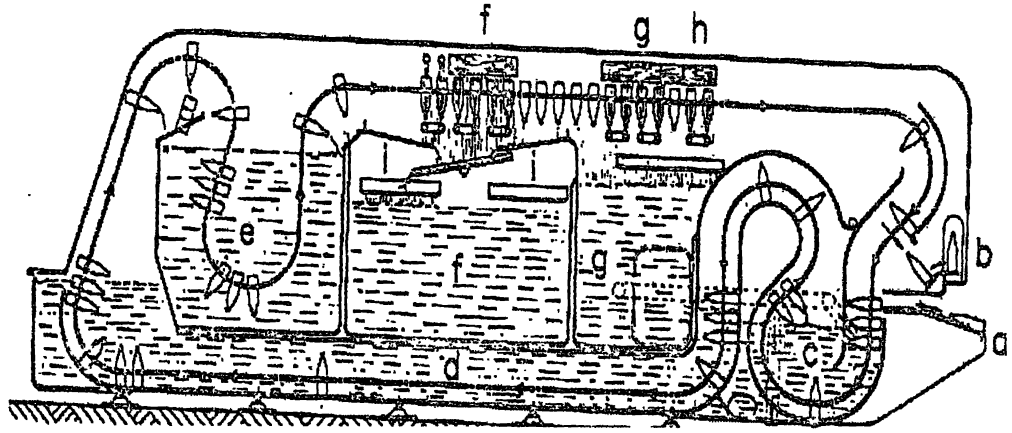
The bottles are subjected to a warm water rinse (under pressure) inside and outside to remove the alkali (FAO, 1975).

2.17.5 Fresh water rinse

The final inside treatment consist of a jetting of fresh tap water and renders the bottles perfectly clean provided the tap water is of a good quality. A finished bottle is no cleaner than the final rinse water used. The final treatment may therefore be with a chlorinated a further sanitizing treatment although the alkaline treatment alone gives a sterile bottle (FAO, 1975).

2.17.6 Draining period

A solution number of drain positions are provided when bottles are discharged (FAO,1975).



(a)Feed; (b) Discharge; (c) Presoak tank (25-30^{0}C); (d) Detergent soak tank (50-60^{0}C); (e)Detergent soak tank (70^{0}C); (f) Detergent jetting and tank (70-75^{0}C); (g) Recirculated Water jetting and tank (77-80^{0}C); (h) Hot fresh mains water rinse (80^{0}C); (i) Alternative Filter boxes.

Fig.2.4 Typical sterilized milk bottles washer of soaking –jetting type and com form.

2.18 Filling of bottles

The milk is filled on vacuum filler in to clean glass bottles, usually of 0.5 liters, with a narrow neck of 26mm diameter across the widest part of the sealing rim. The principle of vacuum filling is that the milk is sucked in to a bottle, which is maintain under vacuum through the vacuum tube, the filling will cease when level of the milk reached the lower opening of the vacuum tube. If the bottles are damaged, so no vacuum can be developed, milk will not be sucked in to it.

A considerable free space (head space) is left above the milk when the bottle is correctly filled, to allow for the expansion of the milk on heating, when might otherwise develop pressures high enough to burst the bottle or to force off the cap. Immediately after filling, the bottle is capped with a suitable airtight and pressure tight seal, to retain the pressure which is developed in the bottle during heat treatment, to prevent contamination from air or cooling water as the bottle cools and vacuum is formed within it is replace the pressure, and to give mechanical and bacteriological

protection during the handling of the sterilized milk between the processing dairy and the consumer. The bottled milk is then ready for heat treatment (Burton et al., 1965).

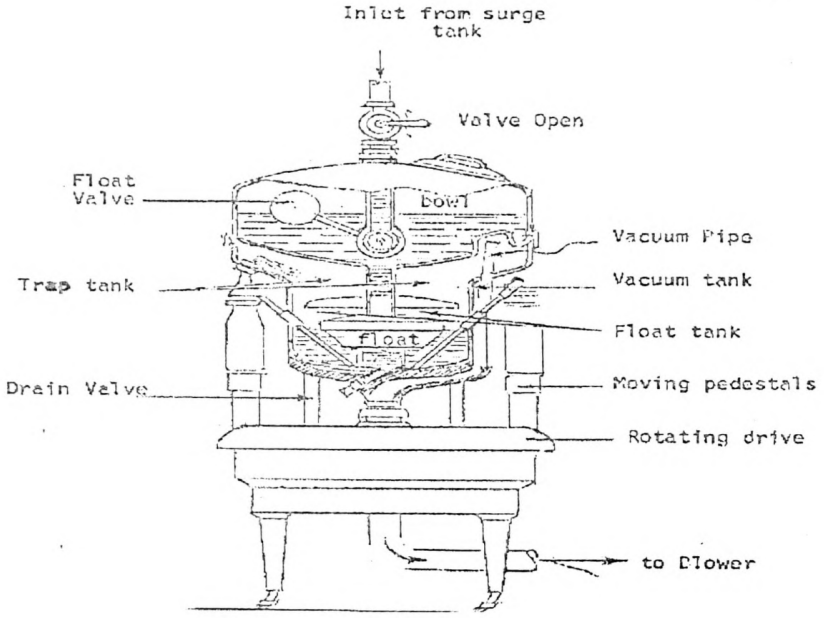


Fig.2.5 Vacuum type filler (Pien, Burton and Gthienlin, 1965).

2.19 Tower sterilization

The filled and closed bottles are then led by a chain conveyor in to a tower sterilizer consisting of 3 towers. The middle tower under steam pressure and it is where the sterilization takes place. The first and last towers are filled with water acting as seals for the steam so the pressure is allowed to rise to more than one atmosphere. The sealed bottles are then passed through a sterilizing tower where they are sterilized 118°C for 20min. The steam, tower comprises of vertical chambers which the bottles moves slowly, where the initial temperature of 60 °C is gradually increased to 118-120 °C, after which the temperature is gradually brought down to 60-35 °C The gradual increase and gradual decrease in temperature prevents cracking of bottles. The sterilized bottled are then packed in crates for storage (Burton et al., 1965).

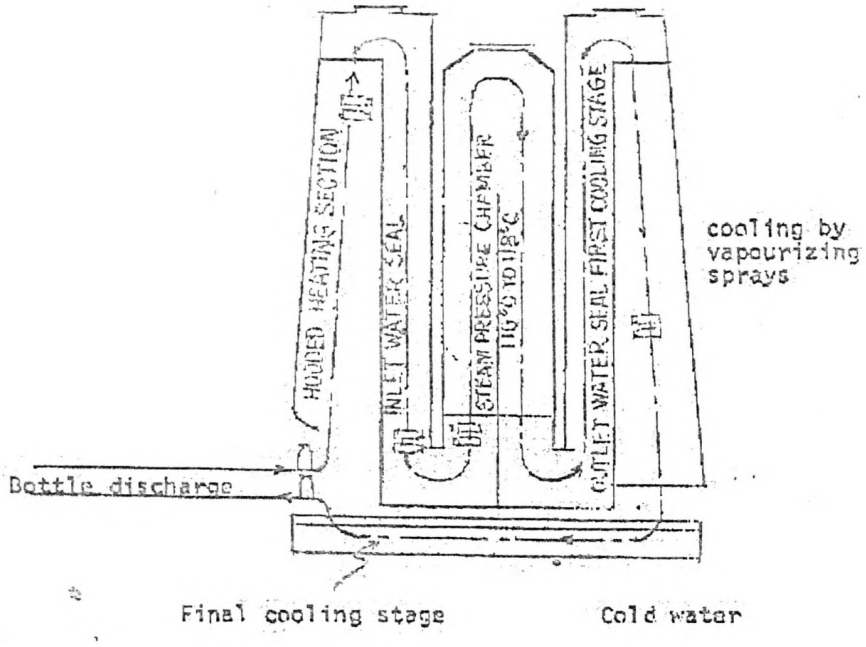


Fig. 2.6 Tower Sterilizer (FAO, 1975).

2.20 Microorganisms and fluid milk industry

The control of microorganisms in fluid milk product is one of essential functions of quality control. Control begins at the form and embraces all phases of processing from milking of cows to delivery of product to the ultimate consumer. Cooling, pasteurizing, packaging, storing and delivering procedures are all design to limit the growth of undesirable microorganisms. With the exception of cultured products such as curd, yogurt etc. All microorganisms are undesirable in the fluid milk industry (Bacteria yeast and moulds). Bacteria are by or the most prevalent and the most important from the standard point of public health and the keeping quality of fluid milk and many milk products (Robinson, 2002).

2.20.1 Thermophilic bacteria

Thermophilic bacteria are commonly defined as those, which will grow readily at 55°C (131 F). Upper limit for growth of most thermophlic bacteria is about 70 °C, and the standard procedure for the enumeration of thermophlic bacteria is to incubate the agar plates at 55 °C. The direct microscopic procedure is useful for detecting these organisms when present in large number (Robinson, 2002).

2.20.2 Thermoduric bacteria

The organisms that survive pasteurization, but do not grow at pasteurization, are considered by the dairy industry to be thermoduric (Robinson, 2002).

2.21 Bacteriological testing techniques

In the methods described up to the present, three phases can be distinguished:

1) Sampling;
2) Enriching the bacteria by incubation at suitable temperatures;
3) Inoculation in to appropriate nutrient media.

The purpose of these operations is to show up the microorganisms that most commonly affect the commercial valve of the product, namely, the Bacillus spp. They should also make it possible to detect the presence of all other pathogenic, toxinogenic, or saprophytic microorganisms, which indicate a gross failure of the sterilization process. When sterilized milk first began to be produced with techniques that were imperfect or ill applied, the number of samples contaminated by such bacteria was relatively important. The germ manifested their presence quickly by massive alteration: significant coagulation, intense proteolysis, and formation of gas. Today, such major effects are rare (Burton et al., 1965).

2.21.1 Sampling

The importance of sampling gives rise to much discussion in current practice. Referring to the statistical works of Thomas and Cheftel (1955), of Gould (1955), Mossel and Drion (1954), it will be seen that the number of samples to be taken is considerable if the quality of a batch is to be evaluated with sufficient certainly. But to accord with economic realities and the physical limitations of control laboratories, it must of necessity be reduced. The testers there for take, at random, a number of containers that is much too small: 4, 6, 8 or 10 per batch, depending on the total size of the batch. (Burton et al., 1965)

2.22 Incubation of the containers

There will be two separate incubation tests, at 30 °C and 55 °C, for milks intended both for hot and temperate climates. At 30 °C, incubation contains for at least 21 days

and preferably for 4 weeks, to obviate the possibility of prolonged latency of the spores. The temperature of 30 °Cis preferred to that of 37 °C for the following reason;

1). It allows the germination of spores treated by without preventing the development of vegetative forms of pathogenic or saprophytes food bacteria
It is also more favorable to multiplication of bacillus spp. At 55 °C, the incubation is not continued for more than 10 days, for fear of auto sterilization of the thermophilic bacteria
At the end of each incubation period, the milk is examined for alterations. These take two main forms in the case of multiplications of bacilli, "soft" coagulation and slight proteolysis. In the former, the milk is sometimes hard to distinguish but becomes more pronounced after a few minutes' immersion in boiling water; in the latter, the opalescence of the milk decreases and its color changes from white to dirty yellow. In case of doubt, centrifugal clarification will produce. A deposit with an upper layer of liquid that is more or less clear but always normal in appearance. Such altered milks should be examined under the microscope after gram staining. The presence of numerous vegetative stainable forms is proof of bacterial multiplication after processing, since micro- organisms in milk that is heated to high temperatures lose their property of fixing normal stains (Burton et al., 1965).

2.22.1 Incubation at 55°C

Thermophilic Bacillus spores more particularly, as they are for more resistant to heat than the mesophilic spores. Sterilized milk that is perfectly stable at 30 °C may not be so at 55-60 °C; in some commercial sterilized milk thermophilic spoilage appears in 55% of all samples taken. It might seem unreasonable to insist on the absence of these spores in products intended solely for countries in the temperature zone; such is the opinion of which make it possible, in the UHT process, to eliminate such spores, but this process is not as yet used on a very large scale and official product control should cover all products distributed (Burton et al., 1965).

2.23 Contamination of Milk

Leakage through caps can consequent post process contamination can lead to spoilage of in bottle sterilized milk, but this is rare and most problems result from the survival of heat resistant endospore.endospoes of *B. steorothermophilus* are present in varying

numbers. But germination and out growth do not occur order processes were, however, marginal with respect to commercial sterility and while modern processes have a higher safety margin, spoilage does occasionally occur as the result of the survival of end spores of mesophilic species of Bacillus. The pattern of spoilage is determined by the properties of the causative organism and typically involves gas production and various type of curdling (Varbam and Sutherland, 1994).

2.23.1 Contamination of Milk during Production

The contamination of raw milk by spores begins during milking; it is caused by fragments of litter or fodder, particles of feacal matter, epidermic debris, etc., to which should be added the influence of utensils are inadequately washed and disinfected. It is further promoted by contamination from air, the containers used to transports the milk, and the handling necessary when the milk reaches the plant.
It follows that all raw milk containers spores, in quantities, which vary greatly; most of these are mesophilic aerobes. It may be said that the presence of a large number of spores is commonly associated with abounded total flora. However, the converse is not true- a high total germ content does not necessary imply that milk contains a great many spores.

Many research workers have tried to determine the number and types of spores introduced in to milk in this way. As regards their number, results differ widely (as was only to be expected), depending upon the location studied, the reason, the degree of cleanliness and care taken in milking, the method of milking (hand or mechanical), etc (Burton et al., 1965).

Regarding the type of these bacteria, observations are more consistent: although most of them consist of spore- forming aerobes (of the genus *Bacillus*), the presence of spore-forming anaerobes (principally of the genus *Clostridium*) has also been noted. As is well known, the latter are less thermo- resistant than the former and hence are hardly ever found in sterilized milk, however, research workers do not agree as to the nature of the species most frequently founding raw milk. This may, of course, depend on real differences between the milks but also on the different methods used to identify certain species. Such would seem to be the case in the distinction between *B. subtilis* and *B.licheniformis*. Whether this is so not, raw milks raw milks are relatively

poor in the spores of highly thermophilic aerobes which are at the same time very resistant to heat (e.g.,*B.stearpthermophilus*). Thus, if we are to believe certain recent studied, the worst cases of instability in sterilized milk must sometimes be attributed to some other cause than the spores in the raw milk (Burton et al., 1965).

2.23.2 Contamination during processing

The contamination of milk during the sterilization process is generally a more serious affair; its extend depend on the techniques used. Thus, in UHT sterilization, when aseptic filling process is properly carried out, using sterile containers, contamination is infrequent or nil. But in process of the UHT type, and then an in bottle process, in hydrostatic continuous sterilizers or other systems- large scale contaminations can result from inadequate washing and disinfecting of the bottles, or storing the "pre-sterilized" milk at unsuitable temperatures, with the result that thermopilic aerobic bacteria grow in the tanks and fillers. But these types of contamination, sometimes on a very large scale, consisting of the highly thermo-resistant bacilli (and which are all the more serious as second, in bottle, sterilization is generally less severe than the first,) have nothing whatever to do with the quality of the original raw milk, and it is no our purpose to discuss them here (Burton et al., 1965).

What we must remember in this brief review of well-known principle is the following. Raw milk contains a variable quantity of spores depending on the conditions in which milking is carried out. Among these spores, a number represent no threat to the sterilized product because they are relatively easy to destroy (anaerobic spores). The spores of aerobic species are generally mesophilic (and medium degree of resistance to heat), whereas highly thermo-resistant spores of thermophilic species are generally found in small or very small quantities in raw milk. We can say that when carefully observe cleanliness and a minimum of aseptic precautions during milking and when conveying the milk to the plant, the risk of really dangerous contamination with regard to the future stability of the sterilized milk, resulting solely from the raw milk, is on the whole very small or nil, particularly if sterilization is effected at UHT (Burton et al., 1965).

2.24 Bacteria staining

Some bacteria species, such as *Bacillus* and *Clostridium*, form spores in response to unfavorable environmental conditions these spores are resistant to high temperatures,

numerous chemical, ionizing radiation, and extreme desiccation. The spore begins with in the bacterial cell (terminal, sub terminal or central) as development continues the sporangium. Eventually disintegrates, leaving a free spore. The size and shape of the spore is unique to each bacterial species and is often useful in identification. The nature of spore makes staining difficult. The primary stain, malachite green, is driven in to the cell by heat. However, care must be taken, as overheating will cause cells to expel the endospore. Once stained, the spore is relatively hard to decolorize, unlike the rest of the cell, which is counter stained using safranin (Wang,1994).

2.24.1 How stains work

Stains are chemicals containing Chromophores, groups that impart color. Their specificity is determined by their chemical structure. For example, a basic dye is a stain that is cart ionic (positively charged) and will therefore react with material that is negatively charged. The surface of bacteria at neutral PH is somewhat negatively charged and will therefore attract basic dyes. Some examples of basic dyes are crystal violet, safranin, basic fuchsine and methylene blue. Acid dyes have negatively charged chromophorem and are repelled by the bacterial surface. They stain the background and leave the microbe transparent. (Wang, 1994).

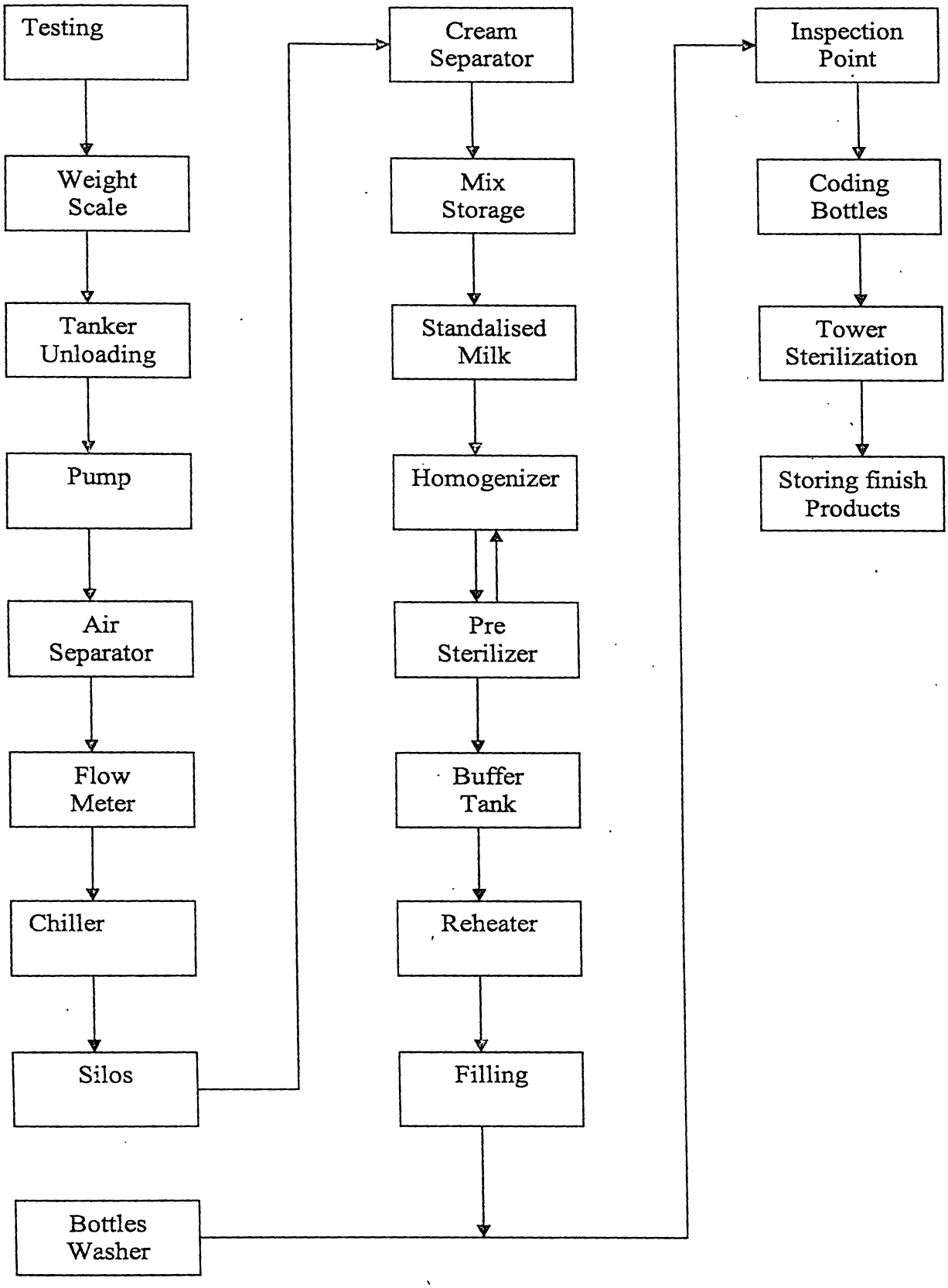


Fig.2.7 Milk bottles production Process Diagram

CHAPTER 3

3. Materials and Methodology

3.1 Location

The experiment was carried out at Milco (pvt) Ltd, Narahenpita and some other test were carried out at the Microbiological laboratory Faculty of Applied Science Sabaragamuwa University of Sri Lanka.

3.2 Preparation and Sterilization of laboratory wares

3.2.1 Sterilizing methods,

Direct flaming

(Metal equipments and glasswares)

1) Hot air oven

The glass wares (Pipettes, Petri dishes)

Used in experiment were thoroughly cleaned, dried and sterilized in an oven at 180^0C for two and half hours.

2) Autoclave

(Test tubes, boiling tubes, dilution bottles, culture media)

Chemical reagents and other materials used in experiment were sterilized in an autoclave at 121^0C for 15min.

3.3 Preparation of culture media and microbiological reagents.

The following Medias were employed for the enumerating of different organisms.

3.3.1 Preparation of culture media.

3.3.1.1 Milk agar

24g of milk agar powder was dissolved in 1Ldistilled water and kept in the water bath to dissolved with frequent agitation just sufficient to dissolve the all the crystals. 10ml quantities were distributed in test tubes, plugged with cotton wool. Covered with aluminum lids and autoclaved at 121^0C for 15mins.

3.3.1.2 Peptone water

1g of powder was added to 1L of distilled water and mixed well. 9ml quantities were distributed in milk dilution bottled and capped. They were sterilized at 121^0C P.S.I. pressure for 15 min.

3.4 Test for pH and Acidity in curdle bottles and normal bottles.

3.4.1 Test for pH

- pH of Curdle and normal bottles were measured using pH meter.
- pH was recorded for each bottle.

3.4.2 Test for acidity

- Curdle bottles were taken.
- 1ml of sample was taken in to titration flask using a pipette.
- Phenolphthalein was added as the indicator.
- N/10 NaOH was filled in to a burette and titrated the sample until the colour change in to pink.
- Added volume was recorded.
- Then this procedure was done normal bottle samples.

3.5 Observing spoiled milk bottles

- Curdle bottles were taken.
- Then bottles were opened and observed for flavour colour and aroma.

3.6 Determination of the strength of the washing solution.

- 1ml of samples were taken from main soak and presoak in a washing tanks.
- 1ml of each sample was placed in a small titration flask.
- Phenolphthalein was added as the indicator.
- N/10 HCL was filled in to a burette and titrated the sample until the pink co lour was change in the colorless.
- Added volume of HCl was recorded.

3.6.1 Calculation of the strength of HCL used for Na0H titration.

- 1ml of N/10 HCl used for 3:1 titration was taken in to titration flasks.
- Phenolphthalein was added as the indicator.
- N/10 Na0H was filled in to a burette and titrated the sample until the co lour change.
- Added volume of NaoH was recorded.

3.7 Bacteria count methods.

3.7.1 Total colony count and thermophilic bacteria count.

- Firstly sterile Petri dishes were labeled.
- Then 1ml samples were pipettes mixing thoroughly with 9ml. peptone water and a dilution series was prepared, above dilution technique.
- Next Petri dishes were inoculated with 1ml per dish by prepared dilution.

- Each inoculated Petri dished was poured with melted milk agar at the tem. 45C and mixed thoroughly with gently.

- As soon as the agar got hardened the inoculated Petri dishes were kept at in an incubator (30^0C) inverted for 48hrs. For TCC test.
- Other Petri dishes were kept at a 55^0C incubator for thermophilic bacterial.
- After the incubation period the colonies were counted using colony counter.

3.7.2 Thermoduric bacteria count

- First sterile Petri. Dishes and test tubes were labeled.
- Then 1ml sample was pipette mixing thoroughly with 9ml peptone water and a dilution series was prepared, using dilution technique.
- Next test tubes were inoculated with 1ml per dish by prepared dilution.
- Each test tube was poured with melted milk agar at the tem. 45^0C.
- Test tubes were kept in water bath 55^0C for 20min.
- After each test tubes were poured in to Petri dishes and mixed thoroughly with a gently.
- As soon as the agar got hardened the Petri dishes were kept at in incubator (30^0C) for 48hrs.
- After the incubation period the colonies were counted by using colony counter.

Total colony count, thermophilic, thermoduric bacteria count done following stage of processing lines in every testing day.

Table 3.place of sample collection

Sample taken Place	TCC	TP	Td
Pasteurized vat	X	X	X
Balance tank	X	X	X
Buffer	X	X	X
Filling ball	X	X	X
Nozzle	X	X	X
Empty bottle	X	X	X
After sterili.	X	X	X

3.7.3 Dilution procedure.

- 1ml of Milk sample was taken in to a 9 ml of sterile peptone water solution in dilution bottle.
- It was mixed properly A. diagrammatic form of a dilution procedure is given below.

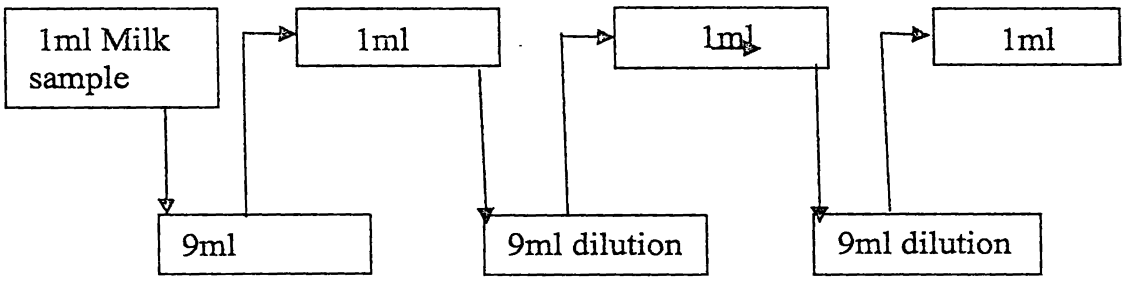


Fig.3 Dilution procedure diagram

Dilution	1:10	1:100	1:1000
Dilution exponential	10^{-1}	10^{-2}	10^{-3}
Dilution factor	10	100	1000

3.8 Changing for in bottle sterilization method

- Bottles were taken after th filling stage one by one in continuous line.
- Other bottles go through tower sterilization.
- Bottles were taken after filling stage put in to the Autoclave 120^0C for 20min.
- Steam was vented to atmosphere after the desired processing time of the steam.
- Autoclave was open and bottle were taken and kept in incubator (55^0C).
- Other bottles were kept incubator (55^0C).
- Then samples were observed (7days incubation test) was done 3 times in every testing day.
- Result obtained at the end of ach method.

3.9 Identification of the proper time- temperature gets in to the all the bottles in tower sterilization stage.

- Bottled were numbered and marked (10 bottles at onetime) after filling stage in continuous line.
- Then bottled were go through a tower sterilizer.
- After sterilized bottled were taken and incubated at 55^0C.
- Specific batch was observed.
- This test was carried out three time in processing day.

3.10 Observing of Processing Plant.

Processing plant was observed every testing day.
Cleaning process was checked before starting the process.
Temperature was measured each processing step using infrared thermometer in every testing day.

3.11 Identification of bacteria in spoilage Milk bottles using gram Stain test.

3.11.1 Staining with Malachite Green.

- Spoilage Milk bottles were taken and prepared bacterial culture.
- Bacterial cultures were taken in slide and heat fixed.
- Smear was covered by Malachite green staining chemicals.
- Slide was exposed to the flame of a Bunsen burner gently.
- Slide was washed with tap water and drained smear was covered with safranin for 30S.
- Smear was wash with tap water, drained a blot dried gently prior to microscopic examination.
- Then bacteria were observed through a microscope.

3.11.2 Staining with Methylene Blue

- Slide was cleaned using cleaning material and dried gently.
- 2 or 3 loops of the culture were placed on a clean slide.
- Smear was spread over the entire slide to form a thin film.
- Then smear was allowed to air dried.
- Slide was passed through the flame of the Bunsen burner 3or 4 times to heat fixed.
- Smear was covered with Methylene Blue.
- It was allowed to remain dye for approximately 1 minute.

- Excess stain was washed by picked up slide one end and hold it at an angle over the staining tray using distilled water wash bottle.
- After it was washed tap water gently over the surface.
- Slide was examined under the microscope.
- Shape, arrangement and approximate size of the organisms was recorded.

3.11.3 Catalase test

- About 3% 1ml of H_2O_2 was poured over the selected colony and examined weather the gas was produced or not.

CHAPTER 4

4 RESULTS AND DISCUSSION

Milk is an excellent medium for fast growth of microorganisms. Processing of milk reduces the most of the microorganisms and increase the shelf life. Although invisible to naked, eye, the bacteria that may present in milk products become evident through their activities.

During the process lactic acid forming bacteria, convert lactose in to lactic acid and subsequently curdle milk. Other microorganisms produce gases and acids causing rapid spoilage resulting in undesirable flavours, odours and curdling of milk. Microbial methods have an important role to play in the dairy industry. The common procedure to measure the sanitary quality of milk is to determine its bacteria content.

The bacteria count usually is expressed as the number of bacteria found per milliliter.

4.1 Bacteria count of various stages of milk process

was identified using culture technique of the presence of bacteria in curdling bottles. TCC, Td and Tp count were taken in to curdling bottles within incubation days.

All stages of process line samples were taken and cultured for identifying the TCC, Td and Tp count.(table show bacteria count variation in each step.) There for we can get correlation with initial bacteria count and after sterilization bacterial count.

Table 4.1. Bacteria count of milk in the Vat

Place	Type	Amount									
Vat	TCC (10^9)	9	13	15	16	8	18	9	15	11	10
	TP (10^3)	6	5	8	6	6	3	2	5	5	3
	Td (10^3)	1	3	2	5	3	8	2	1	4	2

TCC- Total Colony Count, TP- Thermophilic, TD- Thermoduric

Table 4.2 Bacteria count of Balance Tank

Place	Type	Amount									
Balance Tank	TCC (10^3)	52	82	89	92	78	99	76	69	74	98
	TP (10^3)	35	41	59	38	41	60	38	36	48	43
	Td (10^3)	12	28	20	48	30	50	28	16	38	23

TCC- Total Colony Count, TP- Thermophilic count, TD- Thermoduric count

Duration or the and contact time influence the performance of sterilizer. Pre-sterilization stage temperature is 138^0C for 3 min. generally caramelization is occur in 138^0C. But 3 min. do not enough for the caramelization. To happen bacteria are destroyed at pre-sterilization temperature and resistance spores may remain after pre-sterilization. After the pre-sterilization. Stage, milk is transfer to the buffer tank. There the temperature is about 35^0C and after pre-sterilization stage resistant spores are germinated.

Table 4.3 Bacteria count of milk in the Buffer Tank

Place	Type	Amount									
buffer	TCC (10^3)	29	46	53	48	31	46	21	19	26	33
	TP (10^0)	3	4	11	3	6	6	9	21	10	9
	Td (10^0)	5	8	9	8	5	3	6	11	6	3

Milk is transfered to the nozzle from reheater. There the temperature is about 55-60^{0}C . reheater is used to heat the milk and it reachs to the temperature of washing. The principle of filling milk in to bottles, milk is sucked in to bottles, which maintained under vaccum through the vaccum tube. If the bottles are damaged, so no vaccum can be developed and, milk will not be sucked in to it.

Result listed in the table 3. that the nozzle Tp and Td bacteria count was increase than the reheater bacterial count. Reheater temperature is most suitable for the multiplication of the Tp and Td bacteria. Some time post contamination may be occur in the nozzle.

Table 4.4 Bacteria count of milk in the nozzle

Place	Type	Amount									
nozzle	TCC (10^3)	38	69	82	58	63	70	39	33	28	40
	TP (10^0)	4	11	15	6	9	16	22	31	15	8
	Td (10^0)	9	8	12	11	6	3	5	13	12	6

TCC- Total Colony Count, TP- Thermophilic count, TD- Thermoduric count

Table 4.5 Bacteria count of empty bottles

Place	Type	Amount									
Empty bottles	TP (10^0)	3	1	0	0	2	1	0	0	0	1
	Td (10^0)	1	1	0	0	1	0	2	1	0	1

TCC- Total Colony Count, TP- Thermophilic count, TD- Thermoduric count

In tower sterilization, the filing bottles cannot stand a sudden change in temperature the cooling, and heating of the bottles. It must be gradually changed to prevents the cracking of bottles.

Tower sterilization temperature is 118^0C for 13 min. If we further increase the temperature, it may affect to the quality of milk. Because milk retains at that temperature for a in longer time.

Table 4.6 Bacteria count after filling of bottles with milk

Place	Type	Amount									
After filling	TCC (10^3)	32	51	63	61	39	63	31	26	15	30
	TP (10^0)	9	13	13	8	14	19	24	20	9	13
	Td (10^0)	5	9	16	15	8	8	6	16	12	6

TCC- Total Colony Count, TP- Thermophilic count, TD- Thermoduric count

Batch process (autoclave) was used to Changing the in bottle sterilization method. after filling, bottles were kept in the autoclave and heated with steam under pressure corresponding to the required temperature (often 110-120^0C for 20 min.).Then observed after the incubation at 55^0C. most of the time after the autoclave process curdled bottles were not found after the incubation period. However, on some testing days tower sterilization bottles were curdled after theincubation period. This variation may be influence to the temperature fluctuation in the tower sterilizer.

But batch process (autoclave);

- economical for small quantities of milk.
- For less uniform products.
- Adjustment of sterilization time and temperature can easily be done.
- Lead to the development off- flavour and discoloration.

Table 4.7 Bacteria count after the sterilization of filled milk bottles

Place	Type	Amount									
After Sterilization of bottles	TP (10^0)	2	0	1	0	0	2	1	0	1	0
	Td (10^0)	2	1	0	3	0	1	0	0	1	0

TCC- Total Colony Count, TP- Thermophilic count, TD- Thermoduric count

Microbiological analytical methods are used to protect the public health and can be used to reduce economic losses by early detection of inadequate processing and strength of washing solutions. This can be achieved by monitoring, the quality of milk, and strength of washing tank solvents and the operation system.

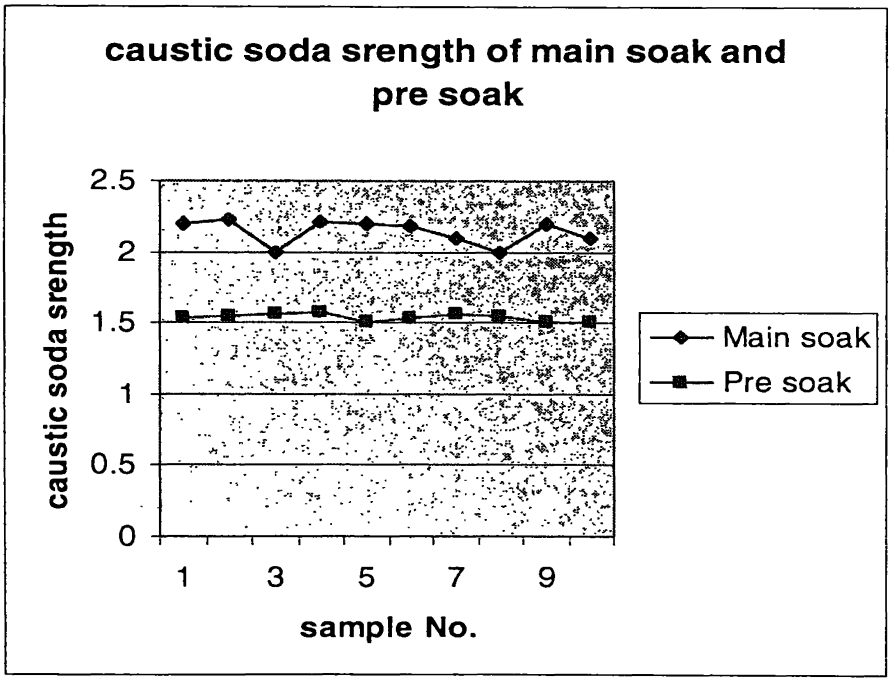


Fig. 4.1 Variation of caustic soda strength

4.2.Calculation

$$NaOH_{(aq)} + HCl_{(aq)} \longrightarrow NaCl_{(aq)} + H_2O_{(l)}$$

Volume at sample taken from washing tank = 1ml

Volume of HCl (N/10) Needed for titration = 5.5ml

Strength of caustic soda in the main soak = 5.5×0.4

= 2.2

Initial formula for the calculation.

$$A = \frac{BCD}{E}$$

There substitute values.

HCl volume required for 1 ml of sample $= \frac{0.1 \times V \times 40}{1000}$

V = HCl volume

For 100 ml of sample $= \frac{0.1 \times V \times 40 \times 100}{1000 \times 1}$

$= \frac{0.1 \times V \times 40}{10}$

$= \underline{0.4V}$

Strength of caustic in washing tank = 0.4×volume of HCl

Finding the strength of HCl used for above titration

Above titration used HCl titrated with stranded NaOH (N/10).

$$H^+ + OH^- \longrightarrow H_2O$$

1mol 1mol 1mo

Volume of the HCl sample = 1ml

Volume of NaOH required for the titration = 1ml

HCl concentration (C)

$$\frac{C \times 1}{1000} = \frac{0.1 \times 1}{1000}$$

$$C = 0.1 moll^{-i}$$

PH value as an indicator of quality of milk.

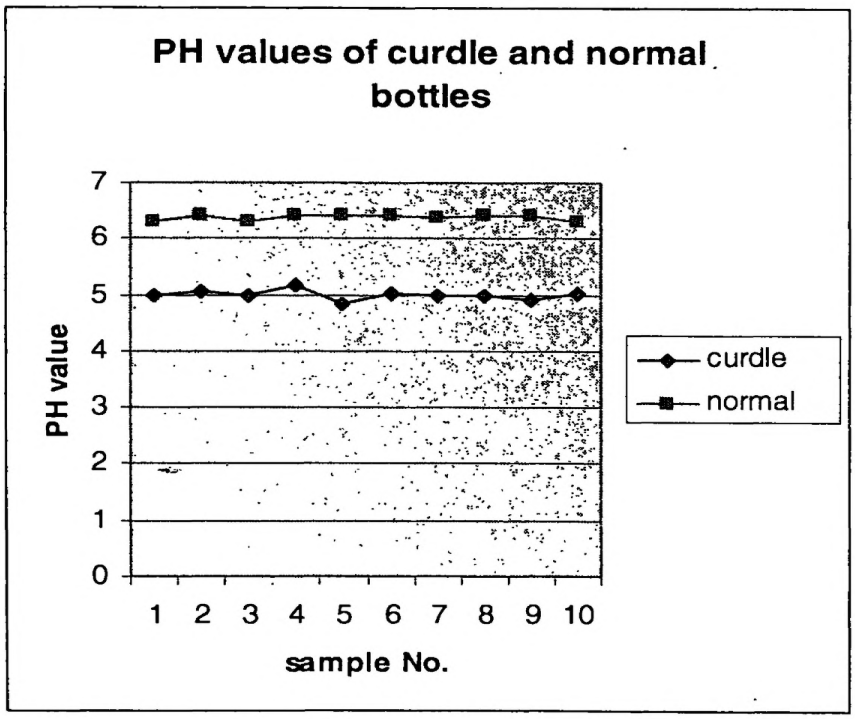


Fig. 4.2 Variation of pH in curdle and normal bottles.

If the microorganisms multiplication is occuring in sterilized milk bottles the pH tend to changed due to their activities. pH of the curdle bottles and normal bottles were taken using a pH meter with in the incubation period. Also the acidity of milk was measured by titration with N/10 HCl.Fig. 4.2 shows the variation of pH for each curdle and normal bottle. After sterilization pH was not change in normal bottles. Most of the normal bottles showed a pH of 6.4. But curdle bottles pH was about 5.0.

4.4 Curdling of sterilized milk at various incubation temperatures

various incubation temperature were used for finding the optimum temperature for the sporalation process 30^0C, 37^0C, 45^0C, 55^0C, and 60^0C. for this purpose bottles were taken from continuous line after filling stage. As fluctuation tower sterilization temperature may affect the sterilized the bottles. Incubation temperature. That ranges from 55-to 60 ^{0}C curdled the milk and other temperature range did not lead to the same. Therefore, the temperature range of 55 -60^0C is considered to be the most suitable temperature for the curdling of milk bottles.

Table 4.8 Sterilized milk bottles exposed to in various incubation temperatures

Incubation tem.^{0}C	Sample No.	observation
30	10	Normal
37	10	Normal
45	10	Normal
55	10	curdled
60	10	curdled

Table 4.9 Samples inspection for determination of the sterilization process temperature variability

Sample No.	Date	Time a.m.	Sample size	Observation
1	02/03/05	9.15	10	A
		10.15	10	A
		11.15	10	B
2	05/03/05	9.15	10	A
		10.15	10	C
		11.15	10	B
3	08/03/05	9.15	10	A
		10.15	10	A
		11.15	10	A
4	10/03/05	9.15	10	A
		10.15	10	B
		11.15	10	B
5	04/04/05	9.15	10	A
		10.15	10	B
		11.15	10	B
6	06/04/05	9.15	10	A
		10.15	10	A
		11.15	10	D
7	08/04/05	9.15	10	B
		10.15	10	B
		11.15	10	B
8	10/04/05	9.15	10	A
		10.15	10	B
		11.15	10	A

A – All bottles were curdled. C – 9 bottles were curdled.

B - All bottles were normal. D – 7 bottles were curdled.

Samples taken at 11.15 a,m. tend to be curdle though there was no strick correlation.

41.7% of samples were normal and 58.3% of samples were curdle.

4.5 Bacteria identification

4.5.1 Using Malachite green

Table 4.10 Result of Malachite green staining test

Sample No.	Colony morphology		Gram stain
	Shape	Colour	
1	Cylindrical	Purple	+
2	Cylindrical	Purple	+
3	Cylindrical	Purple	+
3	Cylindrical	Purple	+
5	Cylindrical	Purple	+
6	Cylindrical	Purple	+
7	Cylindrical	Purple	+
8	Cylindrical	Purple	+

4.5.2 Catalase test

Table 4.11 result of catalase test

Sample No.	Catalase test
1	-
2	-
3	-
4	-
5	-
6	-
6	-
8	-

4.5.3 Methylene blue test

Table 4.12 Result of Methylene blue test

Sample No.	Colour
1	blue
2	blue
3	blue
4	blue
5	blue
6	blue
7	blue
8	blue

After the filling stage bottles that in the continuous line were marked and then incubated after tower sterilization. This test was done about 3 times per day. After the incubation period, it was identified whether sample taken in to each batch either curdling or not.

This gives idea of how important the time temperature combination in sterilization and require more attention to the operation. The procedure require the condition to be maintained at constant level throughout the operation process to avoid any curdling.

Milk residues dry on to the inner surface of the bottle, giving an opportunity for bacteria to develop in conditions particularly suitable for the sporulation of resistant spore- forming organisms.

Efficient washing to remove as many of these resistant spores as possible has an important bearing on the sterilization process as a whole. The in bottle sterilization treatment reduces the total number of spores in the bottles milk by a definite proportion, which depends on the intensity of the heat treatment and the heat resistance of the spores which are present. The total number of spores is made up of the spores in the milk, plus the spores in the washed bottles. If the bottles contributes a large number of spores in the washed bottles. It reflects the washing process is inefficiency. The large number of spores surviving the treatment will higher the

spoilage level; in the absence of faults in the heat treatment process. Good control of the bottles washer is therefore of vital important.

The shape of the bottles is a further disadvantage, because the narrow neck makes it difficult to direct a let of water consistently and accurately in to the bottle.

The presoak section is a desirable feature when dealing with very dirty bottles, as loose soil can be removed or at least softened before the main detergent sections. The overflow from the pre soak tank goes directly to waste.

High detergent causticities are needed in the soak tanks to eliminate the build- up of spores which are washed out of the bottles and remain in the detergent. 2% caustic soda used to pre soak tank and 1.2% caustic soda used to pre soak tank. In the graph (Fig.5) was shown the changing caustic soda strength in main soak and pre soak. However, strength was not decrease in the washing tank. Nevertheless, it was possible to observe increase in the small values.

With these high detergent causticities, there is a risk of etching the glass of the bottles, and a suitable compound may be added to prevent this problem.

After washing bottles it was possible identified the presence of excess detergent causticities adding phenotheline.

After washing bottles were taken and Tp and Td bacteria counting it was identified the effectiveness of causticities. During the test period the Tp or Td bacteria count were not observed. Nevertheless, rarely found 1 or 2 bacteria spores in testing period.

CHAPTER 5

CONCLUSION AND RECOMMENDATICN

- The main constraining factor of milk curdling is that the spores may remain in the milk bottle after sterilization. The tower sterilizer is not maintained properly within the whole process time. There fore resistant spores retain in the bottles and they germinated within the incubation period.
- The milk that comes in to nozzles, bacterial count is higher than that in the buffer tank. There fore post contamination may be occurring.
- It was identified that the strength of washing tank used for bottles washing of bottles and it has no influence on the contamination process being studied.
- The increase efficiency of the tower sterilizer the speed should be decreased.
- Improving working hygiene; applying GMP and HACCP system implementation in the industry can further eliminate any remaining other microorganism or post contamination.

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APPENDIX I

Table 6.1 Caustic soda strength of main soak and pre soak tankS

Sample No.	Main soak	Pre soak
1	2.2	1.6
2	2.2	1.5
3	2.0	1.5
4	2.2	1.6
5	2.1	1.5
6	2.1	1.5
7	2.1	1.6
8	2.0	1.5
9	2.2	1.6
10	2.1	1.2

Table 6.2 pH of curdle and normal milk bottles after sterilization

Sample No	Curdle bottles	Normal bottles
1	5.0	6.2
2	5.0	6.4
3	4.9	6.3
4	5.1	6.4
5	4.8	6.4
6	5.0	6.4
7	4.9	6.3
8	5.0	6.4
9	4.9	6.4
10	5.0	6.3

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