ENUMERATION AND PREVENTION OF MICROBIAL CONTAMINATION OF PASTEURIZED MILK DURING THE MANUFACTURING PROCESS

By

P.A. Panditharatna

(00/AS/063)

A Research Report Submitted in Partial Fulfillment of Requirement for The Degree of

Bachelor of Science
In
Food Science and Technology

Department of Natural Resources
Faculty of Applied Sciences
Sabaragamuwa University of Sri Lanka
Buttala (91100).

March, 2004

DECLARATION

The work described in this thesis was carried out by me at the Swiss Cheese Company (Pvt.) Limited, New Town, Mulleriyawa, under the supervision of Miss Inakshi Amarasekara and Mrs. Deepika Priyadarshanie. A report has not been submitted to another university for another degree.

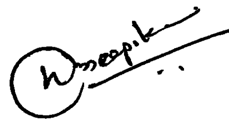
P.A. Panditharatna.
15/05/2004

Certified by,

External supervisor;
Miss I. Amarasekara.
Swiss Cheese Company (PVT) Limited,
No. 20, Sri Sumana Mawatha,
New Town,
Mulleriyawa.

15/05/2004

Internal supervisor;
Mrs. D Priyadarshanie.
Sabaragamuwa University of Sri Lanka,
Faculty of Applied Sciences,
Buttala.



15/05/2004

Prof. Mahinda Rupasinghe.
Head- Department of Natural Resources,
Faculty of Applied Sciences,
Sabaragamuwa University of Sri Lanka,
Buttala.

15/05/2004

Affectionately dedicated to my parents.

ACKNOWLEDGEMENT

I would like to take this opportunity to thank Prof. Mahinda Rupasinghe for arranging the place to carry out this project

I would also like to express my deep felt gratitude to Ms. Inakshi Amarasekara for helping me carry out this project successfully and Mrs. Deepika Priyadarshanie for the excellent guidance and support given throughout the project period.

I am also grateful to Dr. D.B.M. Wickramaratne and all who helped me in numerous ways for a satisfactory completion of the project.

ABSTRACT

Milk is a source of high nutrient content fortified with essential ingredients for a healthy growth and is thus a perfect medium for microorganisms to grow, resulting in subsequent spoilage. A frequently employed method of retarding spoilage of milk is pasteurization. This destroys all pathogenic organisms and guarantees a shelf life of 3 days. Pasteurized milk, to be up to Sri Lanka Standards, should be completely devoid of coliforms and should not contain more than 30,000 microbial colonies per ml. But these standards are not always met due to the cross contamination through equipment and people. Therefore a study was done to evaluate the effectiveness of oxonia as a sanitizer and hence to eliminate microbial contamination.

Degree of contamination was determined by drawing samples from all stages after pasteurization and carrying out Total Plate Count and coliform tests. Effectiveness of oxonia was evaluated by enumerating microbial counts before and after its application as a sanitizer. Workers, their hygienic practices and traffic flow within the factory was observed and examined thoroughly to identify causes of cross contamination.

Results indicated that the sources of re-infection were parts of the plant in which Clean In Place (CIP) was not sufficient to provide complete sanitation. Analysis of the test results proved oxonia to be a very good sanitizer that can be used on a permanent basis. Suggestions such as HACCP (Hazard Analysis and Critical Control Points) implementation and improving worker hygiene were made to further reduce the microbial count of pasteurized milk.

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ABBREVIATIONS

Avg.; average

et al.; and others

GMP; good manufacturing practices

HACCP; hazard analysis and critical control points

HTST; high temperature short time

IEP; iso electric point

Oxonia; P3-oxonia active

Sec.; seconds

TPC; total plate count

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CHAPTER 1

1.1 Introduction

Milk is the biological secretion, excluding colostrums, secreted by mammals for the nourishment of their young. (Adams and Moss, 2000). A number of animals are used to produce milk for human consumption, although the cow is by far the most important in commercial terms.

Cow's milk has been one of man's favorite foods since ancient days. Milk was either consumed immediately or processed into another form after milking, due to its high perishability. Its high water activity, moderate pH (6.4 – 6.6) and ample supply of nutrients make milk an excellent medium for microbial growth. This demands high standards of hygiene in its production and processing; a fact recognized in most countries where milk was the food to be the focus of modern food hygiene legislation (Adams and Moss, 2000). Fresh milk is bactericidal as well as bacteriostatic, but this activity disappears a few hours after the milk has been withdrawn. Therefore it is susceptible to deterioration by microorganisms.

A mixed flora of microorganisms can be found in milk, especially bacteria. The principal bacteria in milk are lactic acid bacteria, coliform bacteria, butyric acid bacteria, propionic acid bacteria and putrefaction bacteria (Bylund, 1995).

Family *Enterobacteriaceae* is a frequently encountered group of colifrms. This contains gram-negative, peritrichously flagellated or nonmotile, facultatively anaerobic, straight rods, with simple nutritional requirements. Members of the family, often called enterobacteria, can be divided into 2 groups based on their fermentation products. The majority (e.g., the genera *Escherichia, Proteus, Salmonella* and *Shigalla*) Carry out mixed acid fermentation and produce many lactate, acetate, succinate, formate (or hydrogen and carbon dioxide) and ethanol. In butanediol fermentation the major products are butanediol, ethanol and carbon dioxide. *Enterobacter, Serratia, Erwinia* and *Klebsiella* are butanediol fermenters. (Chakraborty, 1995).

Milk is susceptible to contamination by a wide variety of bacteria due to its very specific composition. Farm milk may contain anything from a few thousand bacteria per ml, if it comes from a hygienic farm, up to several millions, if the standard of cleaning, disinfection and chilling is poor. (Bylund, 1995). However, fresh milk, when reaching the processing area, does contain bacteria regardless of good hygiene and chilling. Louise Pasteur introduced a method of destroying most of this bacteria and prolonging spoilage. This method later became very popular and was employed frequently to improve the keeping quality of milk. Today it is universally known as pasteurization and is still being used as a method of extending the shelf life of milk without altering its taste and nutritive value. Pasteurization does not destroy all microorganisms in milk. It only destroys pathogenic forms. Milk, even when properly pasteurized, may later get contaminated again. This is a widely encountered problem in the milk pasteurization process in many dairy industries. Recontamination could happen at any stage of the process due to many reasons. Identification of sources of contamination and implementation of preventive measures is very important in order to make safe pasteurized milk.

1.2 Objectives

- Enumerating microbial population of milk at each step of the manufacturing process after pasteurization.
- Identification of sources of contamination.
- Application and evaluation of preventive measures to retard growth of coliforms and other spoilage microorganisms.
- To make suggestions in order to maintain a low count of microorganisms in pasteurized milk.
- Find evidence (if available) for recontamination of pasteurized milk.

CHAPTER 2

Literature review

2.1 Milk

Milk is the fresh, clean, lacteal secretion obtained by completely milking healthy dairy animals which are properly fed and kept, excluding that obtained within 15 days before and 10 days after calving. (Resubal, 1975).

The principal components of milk are water, fat, protein and lactose. The precise composition varies between species, for example, human milk has lower protein but higher lactose levels than cow's milk. Generally the protein content of the milk reflects the growth rate of the young animal – the higher the growth rate, the more protein the milk contains.

There can be considerable compositional differences between breeds of a single species – Jersey and Guernsey milks, for instance, are noted for their higher fat content which is reflected in a richer, creamier taste. Even within a single breed variations in composition can arise depending on factors such as the stage of lactation, the stage of milking, the intervals between milking, the time of day, the number of previous lactations and the general nutritional state and health of the cow.

The lipid content is the most variable feature. It is comprised mainly of C_{14}, C_{16}, C_{18} and $C_{18:1}$ fatty acids and is present in fresh milk mainly in the form of fat globules surrounded by a phospholipids rich layer known as the milk fat globule membrane. Typically these globules have a diameter of about 5μm and the milk contains about 10^{12} fat globules per liter. If fresh milk is allowed to stand, the fat rises to the surface of the milk to produce a distinct cream line. The tendency for this to happen is reduced if the size of the globules is reduced by passing the milk through a small orifice under pressure, a process known as homogenization.

About 80 – 85% of the protein in milk is present as caseins. These are milk specific proteins which are precipitated from milk by decreasing the pH to 4.6. This pH corresponds approximately to their IEP which is relatively low due to the predominance of acidic amino acids and the presence of phosphorylated serine residues in the molecules. There are 5 main classes of caseins, these aggregate together in association with calcium phosphate in milk to form colloidal particles known as micelles. Milk contains around 10 casein micelles/liter with an average diameter of around 0.2μm. The stability of the micelle is maintained by the presence of k – casein near or on the surface of the particle. Loss of this stabilizing effect occurs when k – casein is cleaved by chymosin during cheese production and leads to the micelles sticking together to form a coagulum.

The balance of the protein in milk is made up of the whey proteins, These mainly comprise the compact globular proteins β – lactoglobulin ά – lactalbumin but also a number of blood derived proteins such as serum albumin and immunoglobulins. The latter are present at higher levels in colostrums where they presumably confer some resistance to infection in the newborn calf (Adams and Moss, 2000).

2.2 Microorganisms in milk

When milk is secreted in the udder it is virtually sterile. But even before it leaves the udder it is infected by bacteria which enter through the teat channel. These bacteria are normally harmless and few in number, only a few tens or hundreds per ml. However, in cases of an udder inflammatory disease called mastitis, the milk is heavily contaminated with bacteria and may even be unfit for consumption. (Bylund, 1995).

Milk does posses a number of antimicrobial features, present either to protect the udder from infection or to protect the newborn calf. Generally these are present at a too low concentration in cow's milk to have a very marked effect on its keeping quality or safety.

In some cases the anti-microbial activity is antagonized by other milk constituents such as the effect of citrate and bicarbonate on lactoferrin activity. Stimulation of lactoperoxidase activity through the addition of exogenous hydrogen peroxide has been investigated as a means of preserving raw milk in developing countries, where ambient temperatures are high and refrigeration is not often available. In one trial in Africa, use of this technique increased the proportion of samples passing the 10 minute resazurin quality test from 26% to 88% (Prescott, et al., 1989).

Three sources contribute to microorganisms found in milk: the udder interior, the teat exterior and its immediate surrounds, and the milking and milk handling equipment.

Bacteria that get on to the outside of the teat maybe able to invade the opening and thence the udder interior. Aseptically taken milk from a healthy cow normally contains low numbers of organisms, and milk drawn from some quarters maybe sterile. The organisms most commonly isolated are *micrococci*, *streptococci* and the diptheroid *Corynebacterium bovis*. Counts are frequently higher though due to mastitis.

The udder exterior and its immediate environment can be contaminated with organisms from the cow's general environment. Contamination from bedding and manure can be a source of human pathogens such as *E. coli*, *Campylobacter* and *Salmonella* and *Bacillus* species maybe introduced from soil. Clostridia such as *C. butyricum* and *C. tyrobutyricum* can get into milk from silage feed to cows (Adams and Moss, 2000).

A number of measures can be taken to minimize milk contamination from the udder exterior;

- Providing enough clean bedding and replacing it as necessary.
- Removing slurry (feces and urine) from concrete areas at least twice daily.
- Preventing muddy areas whenever possible.
- Shaving udders and trimming tails.

- Washing teats with warm water containing disinfectant and drying individually with paper towels.
- Keeping the milking parlor floor clean during milking.
- Thoroughly cleaning teat cups if they fall off during milking and discarding foremilk.

Milk handling equipment such as teat cups, pipe work, milk holders and storage tanks is the principal source of the microorganisms found in raw milk. As the overall quality of the milk decreases so the proportion of the micro flora derived from this source increases. Milk is a nutritious medium and if equipment is not properly cleaned, milk residues on surfaces that are frequent left wet will act as a focus for microbial growth which can contaminate subsequent batches. (Adams and Moss, 2000).

In the course of handling at the farm, milk is liable to be infected by various microorganisms, mainly bacteria. The degree of infection and the composition of the bacterial population depend on the cleanliness of the cow's environment and the cleanliness of the surface with which the milk comes into contact, example the pail or milking machine, the strainers, or transport churn or the tank and agitator. Milk wetted surfaces are usually a much greater source of infection than the udder.

Many of the bacteria in milk are casual visitors. They can live, and possibly also reproduce, but milk is often an unsuitable medium for them. Some of these bacteria die when competing with species which find the environment more congenial.

The groups of bacteria which occur in milk can be divided into lactic acid bacteria, coliform bacteria, butyric acid bacteria, propionic acid bacteria and putrefaction bacteria (Bylund, 1995)

2.2.1 Coliform bacteria.

The family enterobacteriaceae is probably the most extensively studied among the kingdom of prokaryotes. These are Gram negative facultative rods. Some are motile by peritrichous flagella. The common intestinal species *Escherichia coli* is the single most extensively studied species of bacteria. *E. coli* and other lactose fermenting members of the family are commonly known by the name coliforms and are used as indicators of faecal pollution of water (Kshirsagar et al., 1997).

Coliform organisms are almost universally present in milk. The extent of their presence at the time milk is received in the plant is largely dependant on the sanitary conditions under which the milk was produced, and the bacterial growth between the time of milking and delivery to the processing plant. The occurrence of coliform in pasteurized milk and milk products may be attributed to:

- Improper pasteurization.
- Post – pasteurization contamination due to hygienic conditions of the utensils or to direct contamination from personnel handling the pasteurized milk and milk products.
- Heat resistance of certain species which allows them to survive pasteurization.

The group of coliform microorganisms includes the genera *Escherichia* and *Aerobacter.* The widespread distribution of coliform in nature, feeding itself on available food of its habitat, permits a minimal occurrence of these microorganisms in milk, milk products, surfaces of dairy equipment, air and water supply. A large number of these bacteria indicate poor hygiene and negligence. Proper heat treatment is sufficient to destroy the coliform bacteria, and its presence in pasteurized milk and milk products serves as an index of recontamination or post – pasteurization contamination, largely due to poor hygienic practices.

The coliform bacterium is a Gram – negative rod, and its ability to produce acid and gas as products of lactose fermentation is the prime basis of laboratory tests for detecting its presence in a sample(Resubal, 1975). Coliform bacteria break down milk protein, resulting in an off – flavor and smell.

Coliform bacteria are killed by HTST pasteurization. They are used as test organisms for routine bacteriological quality control in dairies. If coliform bacteria are found in milk and pipelines after the pasteurizer, this is a sign of reinfection which indicates that the cleaning and disinfection routines need to be improved. If no coliform bacteria are detected, the equipment cleaning procedures can be regarded as satisfactory. (Bylund, 1995)

2.3 Sri Lanka Standards Institution specifications for pasteurized milk.

SLS specifications for microbiology of pasteurized, homogenized milk are that the TPC should be less than 30,000 colonies per ml and that coliforms should be absent in 1ml. (appendix 1)

2.4 Heat processing

Proposals for the heat treatment of milk were made as early as 1824, 40 years before Pasteur's work on the thermal destruction of microorganisms in wine and beer.

Foods are subjected to thermal processes in a number of different contexts. Often their major objective is not destruction of microorganisms in the product, although this is an inevitable and frequently useful side effect.

Credit for discovering the value of heat as a preservative agent goes to the French chef, distiller and confectioner Nicholas Appert. Appert held the view that the cause of food spoilage was contact with air and that the success of his technique was due to the exclusion of air from the product. This view persisted with sometimes disastrous consequences for another 50 years until Pasteur's work established the relationship between microbial activity and putrefaction. Today the 2 types of heat processes employed to destroy the microorganisms.

2.4.1 Pasteurization

When milk pasteurization was introduced by the dairy industry around 1890, it was as much to retard souring as to prevent the spread of disease. This had become an important commercial requirement since large quantities of milk were now being transported by rail into the large cities rather than being produced locally in cramped and insanitary cow houses.

Milk has long being recognized as an agent in the spread of human disease and within a few years it was appreciated that pasteurization was also providing protection against milk-borne diseases. Nowadays it is safety rather than spoilage considerations which determine the minimum legal requirements for pasteurization.

Pasteurization, the term given to heat processes typically in the range of 60 – 80°C and applied for up to a few minutes, is used for 2 purposes. First is the elimination of a specific pathogen or pathogens associated with a product. This type of pasteurization is often a legal requirement introduced as a public health measure when a product has been frequently implicated as a vehicle of illness. Notable examples are milk, bulk liquid egg and ice cream mix, all of which have a much improved safety record as a result of pasteurization. The second reason for pasteurizing a product is to eliminate a large proportion of potential spoilage organisms, thus extending its shelf life. This is normally the objective when acidic products such as beers, fruit juices, pickles and sauces are pasteurized.

When pasteurization is introduced to improve safety, its effect can be doubly beneficial. The process cannot discriminate between the target pathogen(s) and other organisms with similar heat sensitivity, so a pasteurization which destroys say *Salmonella* will also improve shelf life. The converse does not normally apply since products pasteurized to improve keeping quality are often considered as being intrinsically safe due to other factors such as low pH. This may be less true than was previously thought following recent outbreaks associated with unpasteurizd fruit juices.

Originally the main health concerns associated with milk were tuberculosis caused by *Mycobacterium bovis* and *M. tuberculosis* and brucellosis caused by *Brucella* species. In some parts of the world milk is still a significant source of these infections but in the UK and some other countries they have now been effectively eliminated from the national dairy herd by a program of regular testing and culling of infected animals. Enteric pathogens such as *Salmonella* and *Campylobacter* are still however prevalent in raw milk and pasteurization remains the most effective measure for their control (Adams and Moss, 2000).

The primary purpose of heat treatment is thus to kill all microorganisms capable of causing diseases in human beings. Pasteurized milk must be entirely free from pathogens.

Apart from pathogenic microorganisms, milk also contains other substances and microorganisms which may spoil the taste and storage life of manufactured products. A secondary purpose of pasteurization is therefore to destroy as much as possible of these other organisms and enzymatic systems to safeguard product quality. This requires more intensive treatment than is necessary to kill the pathogenic bacteria.

This secondary purpose of heat treatment has become more and more important as dairies have grown larger. Longer intervals between deliveries mean that despite modern cooling techniques, microorganisms have more time to multiply and develop enzymatic systems. The metabolism of microorganisms also produce by-products which in some cases are toxic. In addition, the constituents of milk are degraded, the pH falls, etc. To overcome these problems, heat treatment must be applied as quickly as possible after the milk arrives at the dairy (Alfa – Laval, 1999).

On its own, the contribution of pasteurization to extension of shelf life can be quite small, particularly if the pasteurized food lacks other contributing preservative factors such as low pH or water activity. Thermoduric organisms such as spore formers and some gram positive vegetative species in the genera *Enterococcus, Microbacterium* and *Arthrobacter* can survive pasteurization temperatures. They can also grow and spoil a product quite rapidly at ambient temperatures, so refrigerated storage is often an additional requirement for an acceptable shelf-life (Adams and Moss, 2000).

Alongside correctly performed chilling, the pasteurization of milk is one of the most important processes in the treatment of milk. If carried out correctly, these processes will provide the milk with the best possible keeping properties. A further requirement is for scrupulous hygiene during processing, so that the pasteurized milk does not become re infected. Milk is of course a perfect substrate after it has been heat-treated and can be spoiled very rapidly by infectious microorganisms (Alfa – Laval, 1999).

2.4.2 Limiting factors of heat treatment.

From the microbiological point of view, then, intensive heat treatment of milk is desirable. But such treatment also involves a risk of adverse effects on the appearance, taste and nutritional value of the milk. Proteins in milk are denatured at high temperatures. This means for example that the cheese-making properties of milk are drastically impaired by intensive heat treatment. Harsh heating produces changes in taste, first cooked flavour and then burnt flavour. The choice of time and temperature combination is therefore a matter of optimization in which both microbiological effects and quality aspects must be taken into account (Alfa – Laval,1999).

2.4.3 Different degrees of heat treatment

The 4 types of heat treatment applied to milk are;

Table 2.1 Heat treatment applied to milk.

Low Temperature Holding (LTH)	62.8°C for 30 minutes
High Temperature Short Time (HTST)	71.7°C for 15 sec.
Ultra High Temperature (UHT)	135°C for 1 sec.
Sterilized	>100°C typically 20 – 40 minutes.

Source- Alfa-Laval,1999

The original type of pasteurization used in the dairy industry was a batch process. The milk was heated to 63°C in open vats and kept at that temperature for 30 minutes. Nowadays, milk is almost always pasteurized by the continuous HTST and UHT processes (Alfa – Laval,1999).

2.4.4 Effects of heat treatment

Milk is heat treated at the dairy to kill off any pathogenic microorganisms that maybe present. Heat treatment also causes changes in the constituents of the milk. The higher the temperature and the longer the exposure to heat, the greater the changes. Within certain limits, time and temperature can be balanced against each other. Brief heating to a high temperature can have the same effect as longer exposure to a lower temperature. Both time and temperature must therefore always be considered in connection with heat treatment.

2.4.4.1 Fat

Fat is not affected by temperatures below 100°C. Some coalescence of fat globules occurs at higher temperatures. Cream separability is, however, impaired somewhat if the milk is heated to 75°C or above.

2.4.4.2 Proteins

Casein does not undergo any detectable change at temperatures below 100°C, but the rate of coagulation of milk by rennet diminishes with increasing pasteurization temperature. The reason for this is that the casein losses its calcium when heated.

If milk is heated to about 140°C for 5 minutes, the casein is precipitated as a brownish mass. β- lactoglobulin and albumin begin to precipitate at temperatures as low as 70°C. After a minute or so at 75°C, milk begins to taste and smell cooked. This is due to the release of flavour substances, probably from β- lactoglobulin.

2.4.4.3 Enzymes

Enzymes can be inactivated by heating. The temperature of inactivation varies according to the type of enzyme.

2.4.4.4 Lactose

Lactose undergoes changes more readily in milk than in the dry state. At temperatures above 100°C a reaction takes place between lactose and protein, giving rise to a brownish colour.

2.4.4.5 Vitamins

Vitamin C is the most sensitive to heat, especially in the presence of air and certain metals. HTST pasteurization in a plate heat exchanger can, however, be accomplished with virtually no loss of vitamin C. The other vitamins in milk suffer little or no harm from moderate heating.

2.4.4.6 Minerals

The minerals in milk are not affected to any significant degree by heat treatment (Alfa – Laval, 1999).

2.5 Cleaning of dairy processing plants

Equipment cleaning techniques have undergone very rapid development over the past 10 – 15 years. Manual scrubbing of tanks, vats, etc. followed by hosing with water was formerly common practice. This method of cleaning was time-consuming, expensive and often unsatisfactory in terms of bacteriological cleanness. Manual cleaning has now been replaced in most dairies by mechanized and in many cases automated cleaning. The technique is known as CIP, cleaning-in-place, which means that rinsing water and detergent solutions are circulated through tanks, piping and process lines with no need, as in manual cleaning, to dismantle the equipment (Alfa – Laval, 1999).

2.5.1 CIP

CIP can best be defined as circulation of cleaning fluids through machines and other items of equipment interconnected to form a cleaning circuit. The passage of the high velocity flow of liquids over the surfaces of the equipment generates a mechanical scouring effect which dislodges deposits of dirt. This however applies only to the flow in pipes, heat exchangers, pumps, valves, separators, etc. The usual technique for cleaning large tanks is to run down the walls by gravity. Here the mechanical scouring effect is often insufficient, though the effect can be improved to some extent by the use of specially designed spray nozzles. Tank cleaning requires the use of large volumes of detergent, which must be circulated rapidly.

The question of what type of equipment can be cleaned in the same circuit is determined with reference to the following factors;

- The product residue deposits must be of the same kind so that the same detergent and disinfectant solutions can be used.
- The surfaces of the equipment to be cleaned must be of the same materials or at least of materials compatible with the same detergent and disinfectant solutions.
- All components of the circuit must be available for cleaning at the same time.

Dairy CIP programs differ according to whether the circuit to be cleaned contains heated surfaces or not. There is a general distinction between

- CIP programs for circuits comprising piping, systems, tanks and other process equipment with no heated surfaces, and
- CIP programs for circuits including pasteurizers and other equipment with heated surfaces.

The main point of difference between the 2 types of system is that an acid circulation step must always be included in the second type to remove incrusted protein from the surfaces of heat treatment equipment. A CIP program for a pasteurizer circuit can thus for example consist of the following steps;

1. Rinsing with warm water for about 8 minutes;
2. Circulation of alkaline detergent solution for about 20 minutes at 75°C;
3. Displacement of alkaline detergent with water;
4. Circulation of (nitric) acid solution for about 15 minutes at 70°C;
5. Gradual cooling with cold water for about 8 minutes.

The pasteurizer is usually disinfected in the morning before production starts. This is done by circulating hot water at 90°C for about 8 minutes. Disinfection can also be inserted between steps 4 and 5 of the CIP program, being in that case preceded by about 6 minutes flushing with hot water at 75°C to displace the acid solution from step 4.(Alfa – Laval, 1999).

2.5.1.1 Verification of cleaning effect

Verification of the effect of cleaning must be regarded as an essential part of cleaning operations. It can take two forms; eyeball inspection and bacteriological control. Due to the advance of automation, process lines nowadays are seldom accessible for eyeball inspection, which must thus be replaced by close bacteriological control concentrated to a number of strategic points in the line. The results of CIP are generally checked by cultivating coliform bacteria, the criterion being a maximum of one coli bacterium per

cm^2 of the culture. If the count is higher than this, the result is unacceptable. These tests can be made in place on the working surfaces of the equipment after completion of the CIP program. This applies to tanks and piping systems, especially when excessively high bacteria counts have been detected in the products, Samples are often taken from the first products to pass through the line after cleaning.

All products must be checked for bacteriological quality in their packs in order to obtain full quality control of the manufacturing process. The complete quality control program, in addition to the coliform test, also includes determination of the total count of microorganisms and organoleptic control (tasting) (Alfa – Laval, 1999).

2.6 P3 – oxonia active

A slightly acid sanitizer based on a stabilized combination of peracetic acid and hydrogen peroxide. Rinses freely from equipment surfaces. A 0.2% solution contains 90 ppm (parts per million) peracetic acid. Food and Drug Authority approved for no rinse procedure. Methods of using are sanitation by spraying or circulation.

The specific conductivity of P3-oxonia active is usually insufficient for control via conductivity.

Dosage of P3-oxonia active can be volume proportional to the water flow for CIP systems and cyclic for continuous systems.

Application specifications for storage tanks, pipes, hoses;

Concentration: 0.5%

Temperature: Cellar temperature, 40°C maximum

Time: 30 minutes

P3-oxonia active is added via inoculation disinfection to the application solution of the spraying and distribution system for chain lubricants. The injection spot should be previously prepared with chain lubricants. Corrosion tests may be necessary.

A 2.5% aqueous preparation was tolerated by human skin without reaction despite repeated application. At higher concentrations or prolonged skin contact, skin reactions must be expected. A 5% P3-oxonia active solution was sprayed in a proportion of 18 g/m^3 and was tolerated without reaction of the experimental animals.
Ecologically P3-oxonia active is particularly suitable because only small traces of acetic acid or its salts remain in the waste water after the reaction with organic material.

The product shows little acute toxicity (LD_{50} =3.40 (2.83 – 4.08)ml/kg). The product has little irritant effect on the skin. A 5% aqueous preparation, applied repeatedly to the skin of experimental animals (hairless mice), was tolerated without reaction, while higher concentrations led to skin reactions when applied repeatedly.

2.6.1 Compatibility of oxonia

P3-oxonia active is compatible with;

Metals	Aluminium, stainless steel, tinned iron, mild steel, copper and its alloys and galvanized iron show surface losses which remain within acceptable limits, but the stability of the sanitizing solution is impaired. Short term exposure is possible.
Plastics (in application solution)	Polyethylene, Polypropylene, rigid Poly Vinyl Chloride, higher concentrations and/or other plastic materials should be tested for their suitability at need.
Seals	In view of the wide range of different sealings, it is advisable to test their suitability at need.

2.6.2 Properties of oxonia

Concentrate;

Product strengths:	Particularly effective against all types of microorganisms, even in cold water.
Appearance:	colourless liquid.
Solubility:	at 20°C miscible with water in any proportion.
Density:	1.09 g/cm^3
Storage stability:	-20 to 35°C, minimum 1 year.
Viscosity (dynamic):	1.7 mPas (20°C)
Foam characteristics:	non foaming, suitable for CIP systems.
Flash point:	not applicable, do not heat above 40°C.

Application solution;

pH:	3.2 (1%, 20°C, deionized water)
Specific conductivity:	0.285 mS/cm (1%, 20°C, deionized water) (Brochure, Ecolab).

CHAPTER 3

Materials and methodology

3.1 Location

All experiments were conducted at the quality control laboratories of the Swiss Cheese Company (Pvt.) Limited, New Town, Mulleriyawa.

3.2 Materials

Sterilized glassware
Sample dippers
Ringer's solution
Plate count agar
Violet red bile agar
Incubator

3.3 Methodology

A thorough examination of the processing plant and its workers was done. Lab tests were done on samples of milk which were drawn using sterilized dippers wrapped in Al foil and poured into sterile McCarthy bottles immediately from the steps following pasteurization. The Samples were refrigerated. Total plate count (appendix 3) and coliform tests (appendix 4) were performed on each sample. Serial dilutions (appendix 2) were done up to 10^{-4} for the Total Plate Count and 10^{-2} for the coliform test. This was done daily.

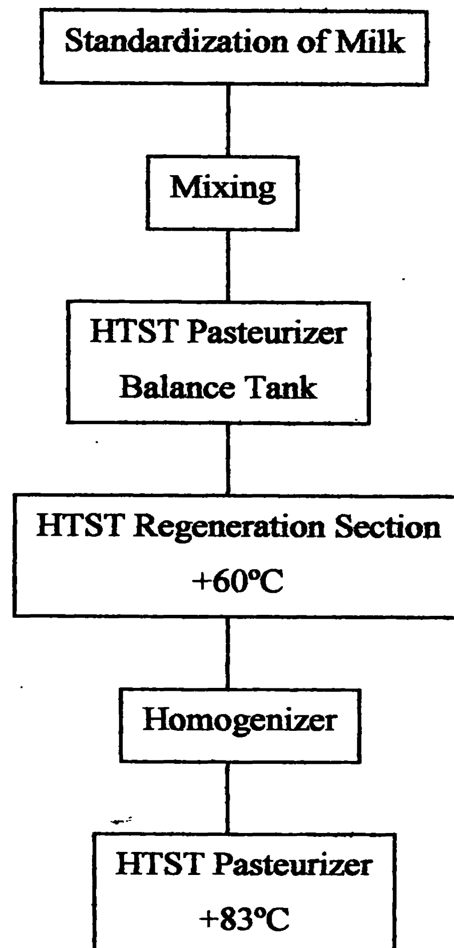
The same experiments were carried out on samples of milk drawn from the processing plant which was cleaned using the sanitizer oxonia (appendix 5). This too was done daily.

3.3.1 Experiment 01 - Enumeration of microorganisms at different steps.

TPC and coliform tests were carried out on each sample obtained. These tests were done daily, 10 days prior to using oxonia.

3.3.2 Experiment 02 – Identification and prevention of sources of contamination.

Data of experiment 01 were used to identify steps in the process where recontamination occurred. The sanitizer oxonia was sprayed to the processing equipment as a preventive measure of contamination, and the effect of this sanitizer was evaluated by enumerating the microbial count daily via TPC and coliform tests. The results were statistically analyzed at 5% level of significance (appendix 6).



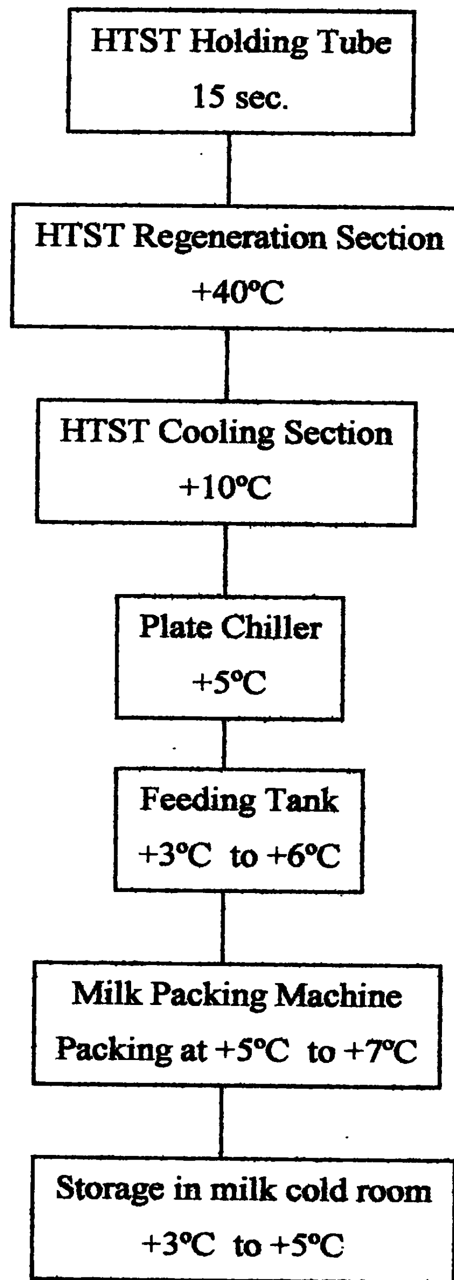


Figure 1 – Process Flow Diagram of Flavoured Pasteurized Milk

CHAPTER 4

Results and discussion

Raw milk contains a very high number of microorganisms. The microbial count of 1ml of raw milk is normally uncountable (Bylund, 1995). Heat processing is done to bring this count to a lower, safer level.

The process, starting from reception of milk at the company up to the processing stage of the HTST pasteurizer, flows raw milk Therefore these stages contain uncountable numbers of microorganisms and thus Total Plate Count and coliform tests were not done on samples of milk drawn from these stages. But the rest of the process, starting from the HTST pasteurizer to the final storing of packets in the cold room, contains heat treated or pasteurized milk. Therefore the microbial population of these processing steps are countable and thus TPC and coliform tests were carried out to enumerate the microbial number at each stage after pasteurization.

Milk going through the processing plant comes into contact with equipment at three places after the pasteurizer. They are the feeding tank, line and packing machine.

The first experiment was done to enumerate the microorganisms present in the processing plant. Total plate counts were done to enumerate all viable cells at each processing step while the coliform tests were done to enumerate only the bacteria that come under the group coliform.

Results obtained from tests of experiment 01 are as follows;

Coliform test			TPC		
10^{-1}	10^{-2}	Avg.	10^{-3}	10^{-4}	Avg.
148	26	2040	51	6	55,000
153	17	2380	44	3	37,000
189	25	2195	58	7	64,000
174	19	1820	26	4	33,000
98	9	940	44	6	52,000
141	19	1655	24	5	37,000
102	17	1360	124	2	72,000
166	16	1630	34	3	32,000
153	19	1715	35	3	32,500
184	21	1970	33	4	36,500

Table 4.1.1 – TPC and coliform tests results of storage tank before using oxonia.

The Sri Lanka Standards Institution gives specifications for pasteurized, homogenized milk (appendix 1). They are that the Total Plate Count should not exceed 30,000 colonies per ml and that the coliform colony count should be 0 for every ml. But the results of table 4.1.1 shows that the average coliform count per ml is very high. The coliform count has gone above 1,000 on most of the days.

The TPC is also higher than 30,000 every day. Therefore the storage tank is a source of Microbial contamination, especially coliform contamination, through cross contamination.

Coliform test			TPC		
10^{-1}	10^{-2}	Avg.	10^{-3}	10^{-4}	Avg.
16	0	80	23	3	26,500
17	1	135	27	2	23,500
15	2	175	21	3	25,500
11	2	155	23	1	16,500
8	1	90	11	0	5,500
6	0	30	13	1	11,500
12	0	60	11	0	5,500
7	0	35	6	0	3,000
10	0	50	6	1	8,000
10	0	50	6	1	8,000

Table 4.1.2 – TPC and coliform test results of the milkline before using oxonia.

The milk line shows presence of coliforms, in certain days as high as 175 colonies per ml. But the TPC values are less than 30,000. Therefore the line is a source of coliform contamination, but the TPC values are within a safe range and are up to Sri Lanka Standards (appendix 1).

Results of table 4.1.3 below show the presence of coliforms. The colony count per ml has gone up to 365 in certain days indicating heavy coliform cross contamination.

The TPC of the packing machine is higher than 30,000 and is not in a safe level regarding SLS (appendix 1).

Coliform test			TPC		
10^{-1}	10^{-2}	Avg.	10^{-3}	10^{-4}	Avg.
26	1	180	38	5	44,000
17	2	185	46	3	38,000
20	0	100	76	1	43,000
5	0	25	47	3	38,500
10	0	50	53	5	51,500
3	1	65	28	4	34,000
22	2	210	28	4	34,000
43	3	365	67	5	58,500
6	2	130	29	7	49,500
8	0	40	36	3	33,000

Table 4.1.3 - TPC and coliform test results of packing machine before using oxonia.

The thorough examination of the plant and its workers along with the above test results revealed the sources of contamination to be parts of the plant itself. The feeding tank, line and packing machine had microorganisms present in them which caused recontamination of milk after pasteurization through cross contamination.

Experiment 02 was done by spraying the sanitizer oxonia on the surfaces of the sources of contamination, which were the feeding tank, line and packing machine, as a preventive measure.

Test results of experiment 02 are as follows;

Coliform test			TPC		
10^{-1}	10^{-2}	Avg.	10^{-3}	10^{-4}	Avg.
1	0	<10	8	1	9,000
0	0	<10	6	0	3,000
5	0	25	7	2	13,500
3	0	15	2	1	6,000
0	0	<10	4	1	7,000
1	0	<10	1	0	<1,000
0	0	<10	12	2	16,500
0	0	<10	1	0	<1,000
1	0	<10	1	1	5,500
2	0	10	12	0	6,000

Table 4.1.4 - TPC and coliform test results of storage tank after using oxonia.

The coliform count which was very high before the application of oxonia has gone down to less than 10 colonies per plate, which means that there are no coliform colonies at all in 10^{-2} dilution and a maximum of a single colony in 10^{-1} dilution.

The Total Plate Count has gone less than 30,000 colonies per ml, which is the SLS specification (appendix 1). On certain days it has gone less than 1,000 which indicate that there are no microbial colonies at all in 10^{-4} dilution while there is only one colony in 10^{-3} dilution.

Coliform test			TPC		
10^{-1}	10^{-2}	Avg.	10^{-3}	10^{-4}	Avg.
0	0	<10	0	0	<1,000
0	0	<10	3	0	1,500
1	0	<10	0	0	<1,000
1	0	<10	0	0	<1,000
0	0	<10	4	0	2,000
0	0	<10	0	0	<1,000
0	0	<10	5	0	2,500
0	0	<10	0	0	<1,000
0	0	<10	1	0	<1,000
0	0	<10	0	0	<1,000

Table 4.1.5 - TPC and coliform test results of pipeline after using oxonia.

The coliform count of the line has gone down to less than 10 in every sample. Coliform colonies can be seen only at certain instances, which is very rare. This proves the action of oxonia against coliform cross contamination to be very good.

The Total Plate Count has gone less than 1,000 in most of the samples. This shows that oxonia is very effective in controlling microbial cross contamination during processing.

Results of the packing machine too show that oxonia is effective against microbial cross contamination and especially coliform contamination. The coliform colony count has been reduced to less than 10 in every sample tested, while the Total Plate Count has been reduced to meet SLS specifications (appendix 1).

Coliform test			TPC		
10^{-1}	10^{-2}	Avg.	10^{-3}	10^{-4}	Avg.
0	0	<10	9	1	9,500
1	0	<10	4	0	2,000
0	0	<10	2	0	1,000
0	0	<10	1	0	<1,000
0	0	<10	2	1	6,000
0	0	<10	7	3	18,500
1	0	<10	10	1	10,000
0	0	<10	7	3	18,500
0	0	<10	14	2	17,000
1	0	<10	18	3	24,000

Table 4.1.6 - TPC and coliform test results of packing machine after using oxonia.

Oxonia is a coliurless liquid of 2-10% per acetic acid and 20-60% hydrogen peroxide. Hydrogen peroxide, if even trace amounts left in food, is toxic. Therefore oxonia was sprayed on the processing plant immediately before CIP cleaning. Thus, once the plant is cleansed with CIP, any residual hydrogen peroxide, left by oxonia, would be completely removed by the action of acid, base and steam employed by the CIP system. Therefore thorough cleansing by CIP after oxonia application should be maintained so as not to leave even traces of hydrogen peroxide behind, and to ensure food safety.

CHAPTER 5

Conclusion and recommendations

The statistical analysis (appendix 6) of the average values of TPC and coliform tests before and after using oxonia proved oxonia to be a very effective sanitizer at 5% level of significance.

Oxonia application has brought down the TPC result less than 30,000 and the coliform test result closer to 0, thus improving the standard of pasteurized milk up to SLS specifications (appendix 1).

Any remaining coliforms or other microorganisms can be further eliminated by improving worker hygiene, applying GMP (Good Manufacturing Practices), sanitizer usage and HACCP (Hazard Analysis and Critical Control Point) implementation.

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Requirements for milk

Item	Characteristic	Requirements: Raw milk Type 1 Cow	Raw milk Type 1 Buffalo	Type 2 Pasteurized unhomogenized	Type 3 Pasteurized homogeni-zed	Type 4 Sterilized	Type 5 Standardized pasteurized *	Type 6 Standardized sterilized	Type 7 Toned pasteurized *	Type 8 Toned sterilized	Type 9 Skimmed pasteurized	Type 10 Skimmed sterilized	Type 11 Flavoured pasteurized *	Type 12 Flavoured sterilized	Type 13 Ultra heat-treated	Methods of test (Ref. to Appendices)
	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)	(13)	(14)	(15)	(15)
i	Milk fat per cent by mass, min.	3.5	7	3.5	3.5	3.5	3.25	3.25	2.0	2.0	0.5 (max)	0.5 (max)	2	2	3.25	A
ii	Milk solids other than milk fat (per cent by mass, min.)	8.5	9	8.5	8.5	8.5	8.25	8.25	8.5	8.5	8.5	8.5	7.2	7.2	8.25	B & C
iii	Creaming index, max.	-	-	-	20	20	-	20	-	-	-	-	-	-	20	D
iv	Phosphatase activity	-	-	to satisfy the test		-	to satisfy the test	-	to satisfy the test	-	to satisfy the test	-	to satisfy the test	-	-	E
v	Turbidity test	-	-	-	-	to satisfy the test	-	to satisfy the test	-	to satisfy the test	-	to satisfy the test	-	to satisfy the test	to satisfy the test	F
vi	Methylene blue reduction test	-	-	to satisfy the test		-	to satisfy the test	-	to satisfy the test	-	to satisfy the test	-	to satisfy the test	-	-	G
vii	Colony count per ml not more than*	-	-	10000	30000	-	30000	-	10000	-	10000	-	10000	-	-	
viii	Coliform**	-	-	-	absent in 1 ml	-	absent in 1 ml	-	absent in 1 ml	-	absent in 1 ml	-	absent in 1 ml	-	-	

* SLS 516 Part

** SLS 516 Part

APPENDIX 2

Dilution technique

1. A sterile blow-out pipette is held vertically, introducing the tip not more than 1 inch below the surface of the sample, and suck up and down 10 times to about 1ml mark of the pipette.
2. Using the same pipette prepare 10 dilution, withdrawing 1ml of the well mixed sample (pipette tip touching against the wall of the sample bottle to remove excess liquid adhering to the outside of the pipette), and transfer to the first test tube of 9ml sterile Ringer's solution (pipette tip touching the side of the test tube ½ to 1 inch above the diluent level), gently blowing out the remaining drops after 3 sec. Discard the pipette.
3. With a fresh sterile pipette, mix the 10 dilution test tube as in no. 1. Prepare 10 dilution by withdrawing 1ml of the well mixed 10 dilution, and transfer to the second tube of 9ml sterile Ringer's solution as in no. 2. Discard the pipette.
4. Taking fresh sterile pipettes and Ringer's solution test tubes each time, further dilutions as required are prepared similarly.

APPENDIX 3

TPC method

1. Thoroughly mix the sample and prepare 2 dilutions following the dilution technique. (Appendix 2)
2. Inoculate 3 sterile Petri dishes with 1 ml per dish of each prepared dilutions. Inoculate another sterile Petri dish with 1 ml sterile Ringer's solution as "control".
3. Pour molten plate count agar at 45°C to each inoculated dish and mix well.
4. Allow the agar to set, and incubate the inoculated dishes and "control" inverted at 30°C for 48 hours.
5. Count the growing colonies on a colony illuminator, selecting the dishes with 30 – 300 colonies.
6. Take the avg. count of all the bacterial colonies on the dishes counted, multiply with the dilution inoculated, and report as "number of total bacterial colonies per ml".

APPENDIX 4

Coliform colony count method

1. Mix the sample thoroughly and prepare 2 dilutions following the dilution technique.
2. Inoculate 3 sterile Petri dishes with 1 ml per dish of each dilution. Inoculate a sterile Petri dish with 1 ml sterile Ringer's solution as "control".
3. Pour molten violet red bile agar at 45°C so as to restrict surface growth.
4. Incubate the inoculated dishes and "control" inverted at 32°C for 48 hours.
5. Count the colonies on a colony illuminator, selecting the dishes with 30 – 300 colonies.
6. Take the avg. count of all the coliform colonies on the dishes counted, multiply with the dilution inoculated, and report as "number of coliform colonies per ml".

APPENDIX 5

Bacterial & fungicidal effect of P3 – Oxonia Active.

Sterilization time in minutes								
Test organisms	Organism conc./ml inoculum	5 °C			20 °C		
		0.2 %+	0.5 %+		0.2 %+	0.5 %+	
Gram-positive bacteria							
Streptococcus faecalis WS 1761 DLG	5.0×10^6	2.5	1		1	1	
Lactobacillus brevis DSM 20054	6.8×10^6	1	1		1	1	
Lactobacillus lindneri K 4160	6.0×10^6	2.5	1		1	1	
Pediococcus damnosus DSM 20289	5.0×10^6	2.5	1		1	1	
Leuconostoc oenos DSM 20252	4.9×10^6	1	1		1	1	
Gram-negative bacteria							
Escherichia coli ATCC 11229	5.4×10^6	1	1		1	1	
Serratia marcescens DSM 1636	5.1×10^6	5	1		2.5	1	
Pectinatus cerevisiphilus DSM 20467	4.2×10^6	1	1		1	1	
		0.5 %+	0.75 %+	1 %+	0.5 %+	0.75 %+	1 %+
Yeasts							
Saccharomyces cerevisiae ATCC 9763	5.0×10^6	10	5	2.5	1	1	1
Saccharomyces diastaticus K 5033	3.0×10^6	2.5	2.5	1	2.5	1	1
Hansenula anomala K 5411	4.6×10^6	20	10	2.5	2.5	1	1
Zyosaccharomyces bailii DSM 70410	3.0×10^5	20	10	5	5	2.5	1
Moulds							
Byssochlamys fulva DSM 1808	3.0×10^5	240	60	40	120	10	1
Penicillium expansum K 7630	1.0×10^5	20	5	5	1	1	1

+ Concentration of oxonia.

APPENDIX 6

Statistical analysis on effect of oxonia as a sanitizer

A. Coliform count analysis

Coliform count for the storage tank;

Paired T-Test and CI: Before, After

Paired T for Before - After

	N	Mean	StDev	SE Mean
Before	10	1771	416	132
After	10	7	8	3
Difference	10	1764	413	131

95% CI for mean difference: (1469, 2059)
T-Test of mean difference = 0 (vs not = 0): T-Value = 13.51 P-Value = 0.000

Since p value is less than 0.05, H_0 is rejected at 5% level of significance.

Coliform count for the milk line;

Paired T-Test and CI: Before, After

Paired T for Before - After

	N	Mean	StDev	SE Mean
Before	10	86.0	51.8	16.4
After	10	1.0	2.1	0.7
Difference	10	85.0	50.1	15.8

95% CI for mean difference: (49.2, 120.8)
T-Test of mean difference = 0 (vs not = 0): T-Value = 5.36 P-Value = 0.000

Since p value is less than 0.05, H_0 is rejected at 5% level of significance.

Coliform count for the packing machine;

Paired T-Test and CI: Before, After

Paired T for Before - After

	N	Mean	StDev	SE Mean
Before	10	135.0	104.1	32.9
After	10	1.5	2.4	0.8
Difference	10	133.5	104.0	32.9

95% CI for mean difference: (59.1, 207.9)
T-Test of mean difference = 0 (vs not = 0): T-Value = 4.06 P-Value = 0.003

Since p value is less than 0.05, H_0 is rejected at 5% level of significance.

B. TPC analysis

TPC for the storage tank;

Paired T-Test and CI: Before, After

Paired T for Before - After

	N	Mean	StDev	SE Mean
Before	10	45100	14562	4605
After	10	6700	5067	1602
Difference	10	38400	10276	3250

95% CI for mean difference: (31049, 45751)
T-Test of mean difference = 0 (vs not = 0): T-Value = 11.82 P-Value = 0.000

Since p value is less than 0.05, H_0 is rejected at 5% level of significance.

TPC for the milk line;

Paired T-Test and CI: Before, After

Paired T for Before - After

	N	Mean	StDev	SE Mean
Before	10	13350	8972	2837
After	10	650	973	308
Difference	10	12700	9301	2941

95% CI for mean difference: (6046, 19354)
T-Test of mean difference = 0 (vs not = 0): T-Value = 4.32 P-Value = 0.002

Since p value is less than 0.05, H_0 is rejected at 5% level of significance.

TPC for the packing machine;

Paired T-Test and CI: Before, After

Paired T for Before - After

	N	Mean	StDev	SE Mean
Before	10	42400	8560	2707
After	10	10700	8407	2659
Difference	10	31700	11875	3755

95% CI for mean difference: (23205, 40195)
T-Test of mean difference = 0 (vs not = 0): T-Value = 8.44 P-Value = 0.000

Since p value is less than 0.05, H_0 is rejected at 5% level of significance.

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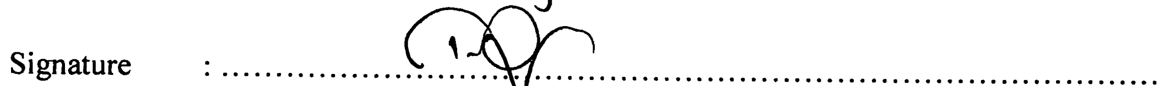
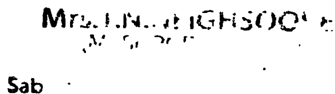
Signature : ....



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