# MANUFACTURE OF FLAVOURED PASTEURIZED MILK SACHETS WITH HIGHER SENSORY QUALITIES AND LONGER SHELF LIFE UNDER REFRIGERATED CONDITION

By

S.W.Udumalagala
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Department of Food Science & Technology
Faculty of Applied Sciences
Sabaragamuwa University of Sri Lanka
Buttala 91100
Sri Lanka.

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# DECLARATION

The work described in this thesis was carried out by me at the Department of Food Science & Technology, Faculty of Applied Sciences, Sabaragamuwa University of Sri Lanka, under the supervision of Mr. A.L.C.J. Liyanage, Lecturer, Department of Food Science & Technology, Faculty of Applied Sciences Sabaragamuwa University of Sri Lanka & Mr.G. Weerakoon, Consultant/Dairy technology, Rich Life Dairies (Pvt) Ltd, Pirivena Road, Molligoda Wadduwa. A report on this has not been submitted to any other university for another degree.

..Siddumalagala.. ..06/12/2007....

Signature Date

Certified by:

**Internal Supervisor**

Mr. A.L.C.J. Liyanage
Lecturer,
Department of Food Science & Technology,
Faculty of Applied Sciences,
Sabaragamuwa University of Sri Lanka,
Buttala.

....Signature
06/12/2007....Date

**External Supervisor**

Mr.G. Weerakoon
Consultant/Dairy Technology,
Rich Life Dairies (Pvt) Ltd,
Pirivena Road,
Molligoda,
Wadduwa.

.....Signature
2007:11:12.....Date

Dr. K.B.Palipane
Head,
Department of Food Science & Technology,
Faculty of Applied Sciences,
Sabaragamuwa University of Sri Lanka.
Buttala.

.....Signature
06-12-2007....Date

Head/Dept. of Food Sciences & Technology
Faculty of Applied Sciences
Sabaragamuwa University of Sri Lanka
BUTTALA

**AFFECTIONATELY DEDICATED**

**TO MY LOVING**

**PARENTS**

**AND**

**TEACHERS.**

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# ABSTRACT

Rich Life Dairies is a company which collects milk from local dairy farmers and processes them into various dairy products marketed under the brand Rich Life. Among other fluid milk based products, Rich Life flavoured pasteurized milk sachets have a prominent ranking. These milk sachets only have an estimated 4 days of shelf life which has created a lower demand for sachets in the market. The present study was focused on the identification of causative factors leading to the limited shelf life of pasteurized milk sachets and the application of necessary corrective measures to improve the shelf life of the product. The determination of shelf life of milk sachets with better sensory qualities under refrigerated condition (4°C) and available transportation conditions were also expected.

The quality of milk was assessed from the point of receiving raw milk which was subjected to routine platform tests. Every production step until the finished product is obtained was investigated to identify the factors which limit the shelf life of pasteurized milk sachets. Pasteurization efficiency was tested by using the phosphatase test and every sample was identified to be phosphatase negative. Shelf life of sachets under refrigerated conditions (4°C) was determined through microbiological examinations and using sensory analysis techniques during a period of one month.

According to the results, Flavoured Pasteurized milk sachets could be kept for 19 days at 4°C with no difference in taste from sachets freshly produced. But the odour was slightly deviated from that of the fresh ones after a lapse of 7days. The shelf life of milk sachets which were kept under the existing transportation conditions showed 15 days of shelf life. This taste and odour losses during the shelf life period could be minimized by using a strong flavour additive. The major causative factors that limit the shelf life are presumed to be poor microbial quality of raw milk, unsatisfactory condition during the transportation and poor storage in retail shops. Hence it can be concluded that the shelf life can further be extended by improving the quality of raw milk, storing the finished products under refrigeration immediately after packing and maintaining the chilled conditions during transportation and storage in retail shops.

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| | |
|---|---|
| °C | - Centigrade |
| min. | - minutes |
| Hrs | - Hours |
| s | - Second |
| g | - gram |
| ml | - mililiter |
| mm | - milimetre |
| SLSI | - Sri Lanka Standards Institute |
| SPC | - Standard plate count |
| CFU | - Colony forming units |
| E.coli | - Escherichia coli |
| BGLB | - Brilliant green lactose bile broth |
| MB | - Methylene blue |
| SNF | - Solids non fat |
| MPN | - Most probable number |
| UV | - Ultra violet |
| HTST | - High temperature short time |

# CHAPTER 01

## Introduction

Rich life dairies(Pvt) Ltd is a company engaged in manufacturing of a variety of dairy products including Flavoured pasteurized milk sachets, Sterilized milk, Tetra packs (Full cream, Low fat, Non fat),yoghurt, Ice cream, butter, ghee and fruit drinks marketed under the brand name "Cheers".

Flavoured pasteurized milk sachets have a higher demand among the younger generation specially school children as a refreshing and nourishing dairy product. The normal shelf life of pasteurized milk is 14 days. Shelf life dictates the total elapsed time allowed from production to consumption. But "Rich life" Pasteurized milk sachets have only 4 days of shelf life which is comparatively a limiting factor in market expansion. The problem has arisen when competing with other brands in the market and this has greatly affected in creating relatively a lower demand for their sachets in the market. Therefore the management has identified the need to manufacture flavoured pasteurized milk sachets with better sensory qualities and longer shelf life.

Shelf life of pasteurized milk is limited by the quality of incoming raw milk, care taken during processing, efficiency of pasteurization, the presence of any spoilage microflora of pasteurized milk, storage temperature and the treatment of the product while in distribution. Microbial activity is the prominent and most vital limitation factor of shelf life in pasteurized milk sachets. Therefore the identification of causative factors leading to the limited shelf life is essential to improve the quality of pasteurized milk sachets.

Manufacturing process of milk sachets contain the steps of pasteurization of raw milk, standardization, mixing the ingredients, homogenization, pasteurization the mixture, packaging and storage in under chilling condition. Flavoured pasteurized milk sachets contain cow milk, sugar, permitted flavour and permitted food color (E102, E122).It has 16% Brix, 2% fat, 7% moisture, 6.6 pH. Due to high nourishing qualities milk is an excellent growth medium for all of the common spoilage organisms, including molds and yeasts.

Most vegetative bacteria cells, yeasts and moulds in milk should be killed by pasteurization (Jay 1996). Efficiency of bacterial destruction varies according to the number and kinds of bacteria present before pasteurization. Pasteurization is the heat treatment most commonly applied to liquid milk. Pasteurization means a process of heating every particle of milk or milk product to at least 145°F (62.8°C) or to at least 161°F (71.7°C) and holding it continuously at these temperatures for at least 30 minutes or at least 15 seconds respectively. (Lampert 1970).Pasteurization itself has a negligible effect on nutritional value of milk. Loss of nutrients through pasteurization is insignificant compared to the safety it provides. Two general methods of Pasteurization in use are Low Temperature Long Time (LTLT or holding) system and the High Temperature Short Time (HTST).

After the product has been pasteurized, all precautions are to be taken in order to prevent the development of those organisms which have survived the heat treatment. Therefore the Pasteurized milk sachets should be kept at cold temperature to obtain the maximum shelf life (Ranasingha and Silva 2001).Once the hygienic conditions of production reaches a certain level, improvement of keeping quality of the pasteurized milk will be dependent upon the incidents of post pasteurization contamination. The post pasteurization contamination is a major cause for limiting the shelf life of pasteurized milk sachets.

Shelf life of the product depends on the care taken and the type of treatment administered during processing, as well as on the handing of the product while in distribution. Because of the poor condition encountered during transportation to long distances, the quality of milk sachets may be affected. When milk is not held at cold temperature (4-10°C) during transport, microorganisms which have survived during pasteurization grow rapidly and spoil the milk. Therefore it is needed to determine the shelf life of sachets under refrigerated condition as well as under transportation conditions.

The present study focuses on the identification and characterization of causative factors leading to the limited shelf life of pasteurized milk sachets and the application of necessary corrective actions to improve the storage life of the product.

### 1.1. Overall Objective

Identify the causative factors leading to the limited shelf life of flavoured pasteurized milk sachets and the application of necessary corrective measures to improve the shelf life of the product.

### 1.2. Specific Objectives:

1.2.1. To study the manufacturing process of Pasteurized milk sachets.

1.2.2. To assess quality of milk from the point of receiving raw milk through all the production steps until the finish product is obtained.

1.2.3. To determine the maximum shelf life of milk sachets under refrigerated conditions (0-4°C).

1.2.4. To assess the changes of sensory qualities of milk during transportation to retail shops and determine the shelf life of milk sachets under the available transportation conditions.

1.2.5. To determine the relationship between initial microbial counts of raw milk on the microbial counts of the pasteurized milk sachets.

# CHAPTER 2

# Literature Review

## 2.1 Milk as a food

Milk is defined in the Milk ordinance and code recommended by the United States Public Health Service as " the lacteal secretion practically free from colostrums, obtained by the complete milking of one or more healthy cows, which contains not less that 81/4% of milk solids-non-fat and not less than 31/4% of milk fat" (Johnson *et al.*,1987).

Milk is one of the few foodstuffs consumed in its natural state. It is not only the most important food during early childhood, but in one form or other continues in our normal diet throughout life. No other single substance can serve as a complete substitute for milk in the diet (Johnson *et al.*, 1987).

### 2.1.1 Composition of milk

Milk has a very complex composition. It consists of lipids, proteins, lactose, minerals (ash) and water. An average gross composition of cow's milk would be as follows. Water 87%, Fat 3.5% -3.7%, Lactose 4.9%, Proteins 3.5%, Ash 0.7% (Johnson *et al.*,1987).

#### 2.1.1.1 Water

Milk is a natural liquid food containing a high percentage of water (87%). Milk is actually a concentrated food designed to produce rapid growth in mammals and contains more solid material than many of our other common foods. Water is the medium in which all the other components of milk (total solids) are dissolved or suspended. A small percentage of the water in milk is hydrated to the lactose and salts, and some is bound in the proteins (Johnson *et al.*, 1987).

#### 2.1.1.2 Fat

Milk fat is the most variable of all milk components. The mixture of mixed triglycerides which makes up 98% to 99% of milk fat is peculiar to milk. It imparts smoothness and palatability to fat containing dairy products. The remaining 1 to 2% of milk fat is composed of phospholipids, sterols, carotinoids,

the fat-soluble vitamins A, D, E and K and some traces of free fatty acids (Johnson. *et al.*, 1987).

### 2.1.1.3 Lactose

The characteristic carbohydrate of milk is lactose (milk sugar).It is found only in the milk of mammals and in no other natural food stuff. Lactose is a disaccharide, that is, it can be hydrolyzed in to two other sugars, glucose (dextrose or corn sugar) and galactose. Being less sweet than other commercial sugars makes lactose useful in processing many foods. Lactose is a major contributor to the acceptability of milk as a beverage. Lactic acid bacteria utilize lactose as fermentation substrate and produce Lactic acid, which is the beginning of many fermented dairy products (Johnson *et al.*, 1987).

### 2.1.1.4 Proteins

The concentration of protein in milk varies from 3.0% to 4.0%.The percentage varies with the breed of the cow and in proportion to the amount of fat in the milk. There is a close relationship between the amount of fat and the amount of protein in milk .when higher the fat, higher the protein. The principal protein of milk, casein makes up 80% of the total, while whey protein makes up remain 20%. The whey protein fraction of cow's milk contains four main proteins: beta lactoglobulin, alpha-Lactalbumins, blood serum albumin, and immunoglobulins. The protein fraction of milk also includes the enzymes of milk (Johnson *et al.*, 1987).

### 2.1.1.5 Ash

The ash content of milk is an analytical value indicating the amount of noncombustible matter in milk. The ash content gives an idea of the total mineral content, but it can in no way indicate how the minerals were originally distributed in the milk (Johnson *et.al.*,1987).The average ash content of milk is 0.72%.The major mineral constituents of milk are Potassium, Calcium, Chlorine, Phosphorus, Sodium, Magnesium and Sulfur(Lampert, 1970).

### 2.1.1.6 Vitamins

Milk contains all the vitamins required by mammals. The fat soluble vitamins, A, D, E, and K, are found primarily in the milk fat and milk has limited amounts

of vitamin K. In some countries milk is fortified with vitamin A and vitamin D. The B vitamins and vitamin C are found with minor exceptions, exclusively in the nonfat portion of milk. The vitamin C (ascorbic acid) present in raw milk but it is very heat-labile and easily destroyed by pasteurization (Johnson *et.al.*, 1987).

#### 2.1.1.7 Enzymes

The enzymes of milk are present in quantitatively negligible amounts: Lipase, phosphatase, peroxidase, protease, reductase, Catalase, and diastase, are relatively unstable in heat. Enzymes are denatured and inactivated by high temperatures, they possess a pH of optimum activity, and they exhibit specificity for certain substrates.62.8°C for 30 minutes or 71.7°C for 15 seconds are sufficiently high for practical purposes to inactivate some of these enzymes. Lipase is the chief one which may cause processing problems in beverage milk. The complete destruction of phosphatase by pasteurization is the basis of the phosphates test used effectively to detect inadequate pasteurization (Hall and Trout, 1968).

## 2.2 Shelf life of pasteurized milk

Shelf life dictates the total elapsed time allowed from production to consumption. Shelf life of pasteurized milk is limited by various factors. Some of them are quality of incoming raw milk, care taken during processing, efficiency of pasteurization, and spoilage microflora of pasteurized milk, storage temperature and the treatment of the product while in distribution.

The spoilage microflora of pasteurized milk is of two types; post process contaminants which have entered the milk after heating and heat resistant bacteria which have survived heating (Varnam and Sutherland, 1996).

Shelf life is primarily dependent upon freedom from appreciable numbers of psychrophilic organisms in the pasteurized milk and upon adequate refrigeration at all points in the distribution.

### 2.2.1 Raw milk quality

The hygienic quality of raw milk used for processing in to liquid milks and other milk products, is influenced by a number of factors associated with milk

production on the farm. Those are health and cleanliness of the cow, the standard of milking practice and the hygienic condition of milking equipment. The milk for a large city comes from number of farms from thousands of cows. It is hauled to many local milk stations and then shipped to the plant. If the milk from that one source were mixed with milk from other sources, a greater and greater quantity of milk would become infected (Early, 1998).

The quality of milk can be categorized as physical hygiene, chemical hygiene and microbiological hygiene.

### 2.2.1.1 Physical hygiene

Physically milk is a rather diluted emulsion, colloidal dispersion and solution. Its physical properties are essentially those of water modified to some extent by the concentration and state of dispersion of the solid components (Johnson *et al.*, 1987). Acidity, specific gravity, viscosity and freezing point, are examples of physical hygiene

**Acidity and pH**

The natural acidity of milk is mainly due to the presence of casein, acid phosphates and citrates and to a lesser degree to albumin, globumin and carbondioxide. The acidity of a solution depends on the concentration of hydronium ions [H+] in it. The real acidity is formed due to production of mainly lactic acid by lactic acid bacteria ferment in milk. The average titratable acidity of normal fresh milk maybe expected to be from 0.14% to 0.17 % (Hall and Trout, 1968). The pH of cow's milk is commonly stated as falling between 6.5 and 6.7, with 6.6 the most usual value (Johnson *et al.*, 1987).

**Specific gravity**

The average specific gravity of milk at 15.5°C is 1.032.It varies from 1.028 to 1.039 due to the fluctuating constituents of the milk (e.g., water, fat and solid non fat).The specific gravity of a fluid varies with its temperature. The specific gravity of milk is lower by addition of water, cream or by increased temperature.

**Viscosity**

The viscosity of milk influenced largely by the colloidal constituents of milk; namely, calcium caseinate, albumen and globulin and dicalcium phosphate and

to a lesser extent by the clumping of the fat globules. The influence of heat on these constituents is reflected in a change in viscosity (Hall and Trout, 1968).The original viscosity of milk is reduced when milk is heated to temperatures commonly used in pasteurization. Aging, souring and the development of certain types of bacteria in milk are responsible for an increase in viscosity.

**Freezing Point**

The most constant physical property of milk is its freezing point. This point is dependent upon the concentration of salts and lactose in milk. Pasteurization affects the freezing point of milk only as far as it affects the concentration of the constituents in solution. Theoretically no significant effects should occur if the milk has been properly pasteurized (Hall and Trout, 1968).The freezing point of bovine milk is usually with in the range - 0.530 to -0.570°C. The freezing point of milk is the only reliable parameter to check milk for dilution with water.

### 2.2.1.2 Chemical hygiene

The different components of milk, especially fat and protein may undergo chemical changes during storage. These changes are oxidation and lipolysis. The products of these reactions can cause off-flavouring in milk.

The oxidation of fat gives milk a metallic flavour. The presence of iron and copper salts accelerates the start of auto-oxidation and the development of metallic flavor, which is also caused by the presence of dissolved oxygen and exposure to light. To avoid the oxidation of fat and protein in milk, the most important issue is to control contact with oxygen and direct sunlight. When the milk is a waiting for transport, it must be protected from direct sun light.

The break down of fat into glycerol and free fatty acids is called lipolysis. Lipolysed fat has a rancid taste and smell. High storage temperatures encourage lipolysis, but the responsible lipase cannot act unless the fat globules have been damaged. In normal farming and dairying routines there are many opportunities for fat globules to be damaged.

### 2.2.1.3 Microbiological hygiene

Milk is an excellent growth medium for all of the common spoilage organisms, including molds and yeasts. Fresh non pasteurized milk generally contains varying numbers of micro organisms, depending on the care employed in milking, Cleaning and handling of milk utensils (Jay, 1996).

Bacteria which may be found in raw milk and which may be contribute to product deterioration include *lactic streptococci*, coliforms, psychrotrophic Gram negative rods and thermodurics, eg.*bacilli, brevibacteria, enterococci and micrococci*.Various pathogens found in milk are *Salmonella* sp. and *Campylobacter* spp., *Staphylococcus aureus*, *Bacillus cereus, Yersinia enterocolitica, Escherichia coli* 0157:H7 (enterohaemorrhagic E.coli) and *Listeria monocytogen* (Early, 1998).

Temperature is the greatest single factor affecting bacterial growth, reproduction and food deterioration. Based on growth temperatures micro organisms can be categorized as obligate psychrophile, psychrotroph, mesophile, thermophile and extreme thermophile.

Table 2.1Groups of micro – organisms based on growth temperatures

| Group | Minimum °C | Optimum °C | Maximum °C |
|---|---|---|---|
| Obligate psychrophile | -10 | 10 - 15 | 20 |
| Psychrotroph | -10 | 20 - 30 | 42 |
| Mesophile | 5 | 28 - 43 | 52 |
| Thermophile | 30 | 50 - 65 | 70 |
| Extreme thermophile | 65 | 80 - 90 | 100 |

Source: Garbett (1997).

Maximum temperature is the temperature above which bacteria will cease to develop, while optimum temperature is the temperature at which bacteria develop best.

Extensive growth of psychrotrophs prior to heat treatment can produce enough enzymes to give either flavour defects or physical stability problems during the subsequent storage of the heat treated product even though the organisms themselves have been destroyed by the heat treatment.

Bacteriological tests carried out to determine the quality of milk are Alcohol test, Clot on boiling test, Dye reduction tests, titratable acidity, pH, Standard plate count (SPC) and Coliform tests. The bacteria content of the raw milk is important to understand milk quality. The legal limit is stated in the Pasteurized milk ordinance as being 100000/ml in raw milk from the dairy farm. But when all aspects of the milk production process are done properly it is common for the SPC to be at 10000/ml or lower. The coliform count should always be less than 100/ml.

To reduce the chances of bacterial contamination of raw milk effective herdsmanship, good animal health care good hygienic management of milking equipment and proficient milking practice are necessary. To reduce the growth of contaminant bacteria and to slow the rate of spoilage, the temperature of milk should be rapidly lowered on production to 4°C, and ideally to 2°C, within 30 min. of milking (Early, 1998).

### 2.2.1.4 Adulterations

Some times variety of adulterants may be found in raw milk and this will reduce the quality of the raw milk. Those adulterants are extraneous water may be the result of poor practices of the farm, chemical compounds may be added accidentally or intentionally. (Salt, sugar, formalin, hydrogen peroxide, etc.). Therefore it is essential to carry out tests to assess the quality of raw milk, delivered to factories before processing (Early, 1998).

### 2.2.1.5 Organoleptic properties of milk

#### Color

The natural colour of milk varies from a bluish white to a brownish yellow, depending upon the amount of fat and solids-not-fat present. The white or milky appearance is due to the colloidal dispersion of the fat globules, calcium caseinate and calcium phosphate in the milk (Lampert, 1970).Pasteurization in itself has very little effect upon the color of milk.

#### Flavour (Odour, Taste)

Normal freshly drawn milk tastes slightly sweet to most people and has a characteristic although not pronounced odour. The pleasing flavour of milk may

be correlated with high lactose and a high chloride content (Varnam and Sutherland, 1996).Pasteurization itself has a negligible effect on the flavour of pasteurized milk (Early, 1998). Microflora of milk causes spoilage symptoms of off odour and off flavour, by the breakdown of amino acids used as their energy source.

## 2.3 Pasteurization

### 2.3.1 History of Pasteurization

Illness from contaminated milk and milk products have occurred world wide since cows have been milked. In the 1900s it was discovered that milk can transmit tuberculosis, brucellosis, diphtheria, scarlet fever and Q-fever to humans. Fortunately, the threat of these diseases and the incidence of outbreaks involving milk and milk products have been greatly reduced over the decades due to improved sanitary milk production practices and pasteurization. The public also had to be convinced that pasteurized milk was safer than raw milk.

The pasteurization process was originally developed by Pasteur between 1860 and 1864 to prevent the spoilage of wines. Although he is developed this technique, he was not responsible for applying it to milk. Jacobi, of New York, was one of the first (1875) to recognize the value of pasteurization in protecting the public health (Herrington, 1948).

Pasteurization was also considered profitable, because of its obvious advantages in reducing losses by spoilage and the process was rapidly adopted by dealers, where considerable time was required to transport milk from the farm to the consumer. Pasteurization spread from larger cities to the smaller ones as a result of public demand for a safer milk supply (Herrington, 1948).

### 2.3.2 Definition of Pasteurization

The International Dairy Federation (IDF) has defined Pasteurization as, a process applied to a product with the objective of minimizing possible health hazards arising from pathogenic micro organisms associated with milk by heat treatment which is consistent with minimal chemical, physical and organoleptic changes in the product (Early ,1998).

### 2.3.3 Definition of Pasteurized milk

According to Sri Lankan standard (SLS 181:1983) definition for Pasteurized milk is "The milk that has been heated to at least 63°C and held continuously at that temperature for at least 30minutes or heated to at least 71.5°C and held to that temperature continuously for at least 15 seconds or any other approved temperature time combination equivalent there to, that will serve to give a negative phosphatase test and cooled immediately to a temperature of 10°C or less and kept at that temperature".

### 2.3.4 Definition of Flavoured Pasteurized milk

The product prepared from milk, sugar and chocolate, cocoa, coffee or other permitted flavouring and with or with out permitted food colour, stabilizer and buffering agent (SLS 181:1983).

### 2.3.5 Importance of Pasteurization

Pasteurization involves a much milder heat treatment. Consumption of raw milk carries a disproportionately high risk of infection by milk borne pathogens (Varnam and Sutherland, 1996). Proper pasteurization should destroy all the pathogenic nonspore-producing bacteria. Fortunately the organisms responsible for the milk-borne diseases do not form spores, except anthrax.

Milk is pasteurized for two reasons:
(1) To make it keep longer and to improve its quality, and
(2) To protect the public health.
Proper pasteurization involves at least 3 steps.
(1) Every particle of the milk must be heated to the temperature selected.
(2)The milk must be held at that temperature long enough to destroy all pathogenic bacteria
(3)Milk must be cooled quickly to such a low temperature that any bacteria surviving the process will be unable to grow at an appreciable rate.

In terms of all bacteria, however, pasteurization efficiency varies from about 90 to 99 percent. The over-all efficiency depends upon the nature of the organisms present and upon their state of development (Herrington, 1948).Severe heat treatment gives milk an undesirable flavor. Therefore milk

treated in this way is palatable to most people. At present pasteurization is the most common means of heat processing milk for safety and in most industrialized countries pasteurized milk is the largest selling liquid milk product (Varnam and Sutherland, 1996).

### 2.3.6 Methods of pasteurization

Two general methods of pasteurization are in use. One is known as the "holding" or "low temperature long time" (LTLT) system and the other as the "continuous pasteurization" or "high temperature short time" (HTST) system.

#### 2.3.6.1 The Holding System (LTLT)

In the batch holding process the entire lot of milk is heated to a definite temperature for a given time62.8°C for 30 minutes. The higher the temperature used the shorter the holding period that can be employed. In this method the entire operation of heating, holding and cooling the product may be done in the same unit (Lampert, 1970).This condition has minimal effects on the organoleptic characteristics of the milk and there is no significant reduction in the cream line of milk.

#### 2.3.6.2 Continuous pasteurization System (HTST)

The principle of the continuous flow pasteurizer is an essential feature of high temperature short time pasteurization. This system consists of heating milk rapidly to a temperature of not less than 71.1°C for 15 seconds (Lampert, 1970).The heat treatment is accomplished using a plate heat exchanger.

This piece of equipment consists of a stack of corrugated stainless steel plates clamped together in a frame. In this method the bulk of the milk remains under adequate refrigeration, both before and after pasteurization, while a relatively small portion is being processed (Hall and Trout, 1968). In terms of actual processing time, perhaps not over a minute elapses from the time the 4°C raw milk leaves refrigerated storage until it is again cooled to 4°C (Early ,1998).

The chief advantage of H.T.S.T. pasteurization is its capacity to heat treat milk quickly and adequately while maintaining rigid bacteriological and quality control over both the raw and the finished product. The system is not well adapted to handle small quantities of several milk products is the main disadvantage in this system (Hall and Trout, 1968).

## 2.4 Manufacturing process of Pasteurized milk sachets

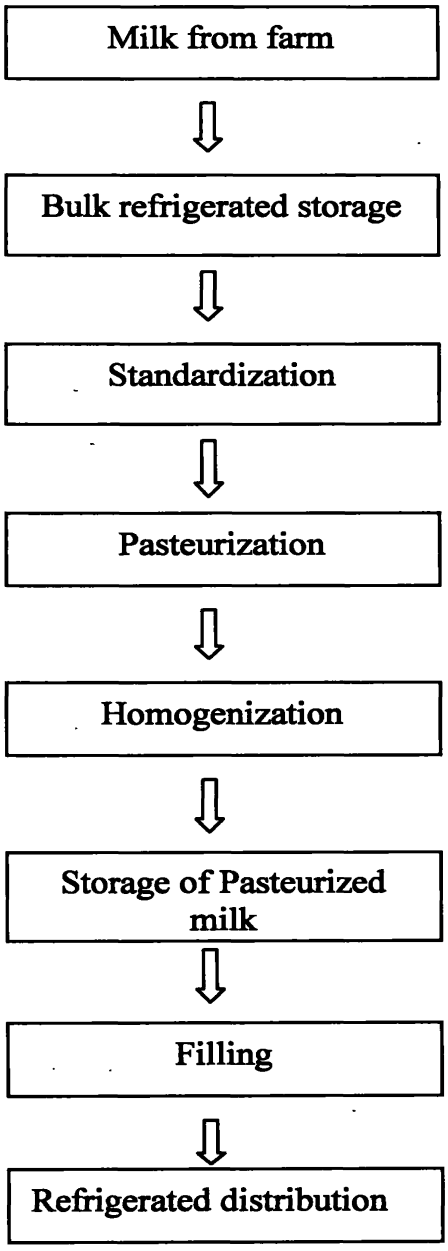


Fig. 2.1 The production of pasteurized milk

Source: Varnam and Sutherland (1996).

### 2.4.1 Milk reception and storage

There are number of testing carried out to determine the quality of incoming raw milk which delivered to factories (Early, 1998). Some of those platform tests are Alcohol test, Rezasurin test, Clot on boiling test, Tests for milk fat, non fat milk solids, antibiotics, added water, total solids, acidity and Organoleptic tests.

Milk is transferred from the road tanker to milk storage silos by centrifugal pumps, so damage to milk fat globules is minimized. Vacuum transfer is not possible as milk silos are often hundreds of thousands of litres capacity. The silos are filled from the bottom to avoid agitation and foaming. Before entering the silo, milk passes through a coarse filter which removes physical contaminants. Raw milk which has not been refrigerated must be processed as soon as possible after acceptance at the processing establishment. Raw milk must be processed within 36h from acceptance if the milk has been kept at not more than 6°C (Early, 1998).

### 2.4.2. Standardization

The fat and SNF percentages of milk varies, therefore those values have to be taken to a uniform standard. The values for full cream milk are, 3.25% fat, 8.25% SNF and for flavoured milk 2% fat, 8% SNF. Standardization is based on excess cream separation and addition of milk solids in dry form if SNF is less than the standard to produce a milk of desired fat content. Raw milk or pasteurized milk may be used for standardization and the method may involve batch processing or continuous processing.

### 2.4.3 Pasteurization

Pasteurized milk is heat treated under HTST conditions by means of indirect heat transfer, most commonly using a plate heat exchanger. The incoming milk at 4°C is pre heated in regeneration section of the pasteurizer for separation from milk which has achieved the pasteurization temperature. Separated skimmed milk and cream which are recombined to give standardized milk then passed to the homogenizer. Then homogenized milk passes into the pasteurization section to be raised to a temperature 72°C by steam heated hot water and held for at least 15s in the holding tube .This milk passes the flow diversion valve before

entering the regeneration section again. From the regeneration section pasteurized milk passes through the chilling sections and on to packing at a temperature of 4°C or less (Early, 1998).

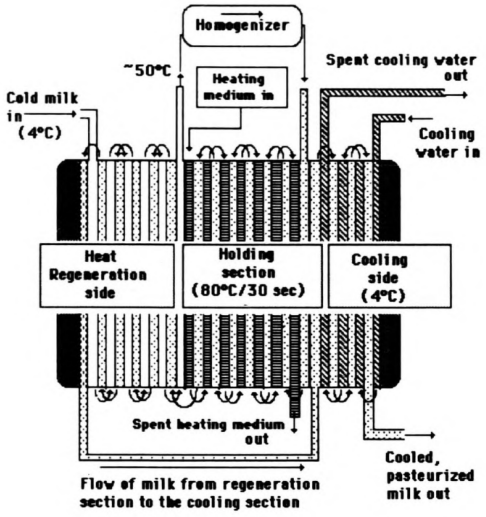


Fig. 2.2 Diagram of Plate Pasteurizer (-and Homogenizer)

Source: Hill (1947).

### 2.4.4 Homogenization

The process of making a stable emulsion of milk fat and milk serum by mechanical treatment. When milk is passed through the homogenizer, there is a reduction in the size of the fat globules. There is no cream line formation on milk after homogenization and also there is an increase in the viscosity of milk. The greatly increased number of fat globules and their much greater surface area makes homogenized milk very susceptible to the action of lipase, with consequent development of rancidity. To prevent this, homogenized milk must be made from pasteurized milk or the milk must be pasteurized promptly after it is homogenized (Lampert, 1970).

### 2.4.5 Refrigerated storage

In order to prevent the growth of surviving bacteria in pasteurized milk, it is essential that the product be cooled rapidly after the heating period. (Lampert.L.M, 1970).For the milk souring organisms, the thermodurics and the thermophiles which

may withstand pasteurization exposures do not grow rapidly enough with in a reasonable time to cause spoilage in the refrigerated milk (Hall and Trout ,1968).

### 2.4.6 Filling

Pasteurized milk has to be filled in to containers immediately after cooling to minimize contamination. The purpose of packaging is to contain, protect and preserve food and to inform consumers. Additional functions are to sell the product and provide convenience. Once packaged, the products are quickly conveyed to a cold storage warehouse. They are stored there for a short time and shipped to the supermarket on refrigerated trailers.

#### 2.4.6.1 Packaging materials for pasteurized milk

The glass bottle is the traditional packaging format for pasteurized milk. Cans, cartons and polyethylene are also used for packed Pasteurized milk. With regard to convenience, cartons and plastic containers also have an advantage over glass milk bottles. In comparison with bottled milk, pasteurized milk packed in laminated cartons and plastic containers can be expected to exhibit longer shelf lives under refrigeration. Due to direct exposure to light, milk in glass bottles will result chemical and sensory changes highly. The off flavour development and vitamin c loss due to fluorescent light progress more rapidly in polyethylene containers than in polyethylene-coated cartons (Early,1998).Even though the Polyethylene containers highly used due to its low cost, damages can be occurred during packaging and transportation.

The wastage of milk during packaging and transportation may differ considerably depending on the quality of the packaging material, maintenance of machines and management of the packaging division, storage and transport.

### 2.4.7 Post pasteurization contamination

This contamination may be from the environment, including equipment and personnel, or may even be from contamination of finished material with raw material. Pasteurization destroys the psychrophiles and Coliforms that may be in milk at the time of processing. So the post pasteurization contamination is the major cause for the presence of psychrophilic and Coliforms bacteria in pasteurized milk. The growth of psychrophilic bacteria in milk results in serious flavour defects such as bitter, fruity, putrid and rancid (Early ,1998).

So prevention of post pasteurization contamination and growth rate of psychrotrophic micro-organisms is needed. Therefore pasteurize milk should be packaged with minimum delay under conditions as well as cooled with out delay to minimize contaminations. Examination of pasteurized milk for the presence of coliforms is a useful routine method for assessing post pasteurization contamination (Early, 1998).Prevention of recontamination is not a discrete process such as pasteurization or homogenization, but is of major importance in production of pasteurized milk that is both safe and of satisfactory storage life (Varnam and Sutherland, 1996).

## 2.5 Kinetics of pasteurization

### 2.5.1 Temperature effect on enzyme destruction

Enzymes are proteins. They are denatured and inactivated by high temperatures. The different sensitivity to heat by some enzymes (in particular alkaline-phosphatase and lactoperoxidase) has permitted the development different methods to be used to verify the efficiency of thermal treatment of milk through pasteurization. Alkaline phosphatase and peroxidase are enzymes indigenous to milk. This phosphates enzyme gets destroyed when milk is heated to at least 71.1°C for 15 seconds. This inactivation temperature is slightly above that is required to destroy the most resistant disease organism likely to be found in milk, for example *Mycobacterium tuberculosis.* Therefore if the enzyme is inactivated, the disease bacteria must of necessity have been subjected to a temperature and time sufficient to destroy them. (Newlander and Atherton, 1964).

Peroxidase enzyme requires a temperature of 80°C to inactivate it and its absence in pasteurized milk is indicative of excessive heat treatment. Measurement of alkaline phosphatase and peroxidase activity are used to indicate that milk has been pasteurized, that the heat treatment employed has been appropriate and that the product is safe to drink. However storage at higher temperatures for extended times has shown the reactivation of alkaline phosphatase and cause misleading results (Early, 1998).

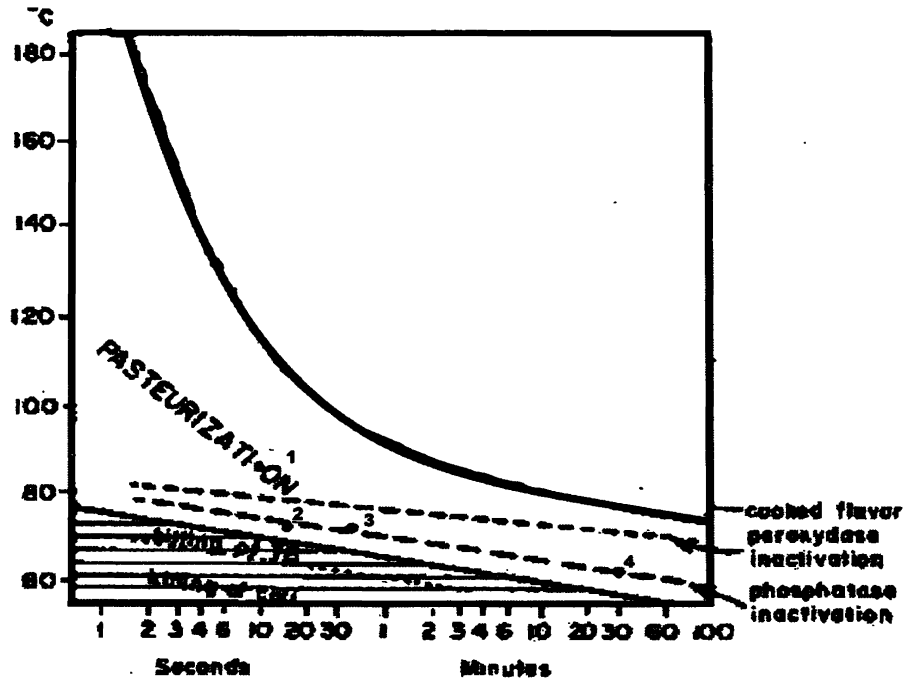


Fig. 2.3 Influence of heat treatment on milk characteristics

(1) Flash pasteurization (2) H.T.S.T.Process

(3) Short time pasteurization (4) Holder process

Source: Hall and Trout (1968).

### 2.5.2 Thermal destruction of microorganisms

Heat is lethal to microorganisms, but each species has its own particular heat tolerance. During a thermal destruction process, such as pasteurization, the rate of destruction is logarithmic, as is their rate of growth. Thus bacteria subjected to heat are killed at a rate that is proportional to the number of organisms present. The process is dependent both on the temperature of exposure and the time required at this temperature to accomplish to desired rate of destruction. Thermal Death Time (TDT) is the time necessary to kill a given number of organisms at a specified temperature. The extent of the pasteurization treatment required is determined by the heat resistance of the most heat-resistant enzyme or microorganism in the food.

The D value is a measure of the heat resistance of a microorganism. D value is the decimal reduction time, or the time required to destroy 90% of the organisms. The value is numerically equal to the number of minutes required for the survivor curve to traverse one log cycle (Fig.2.4)

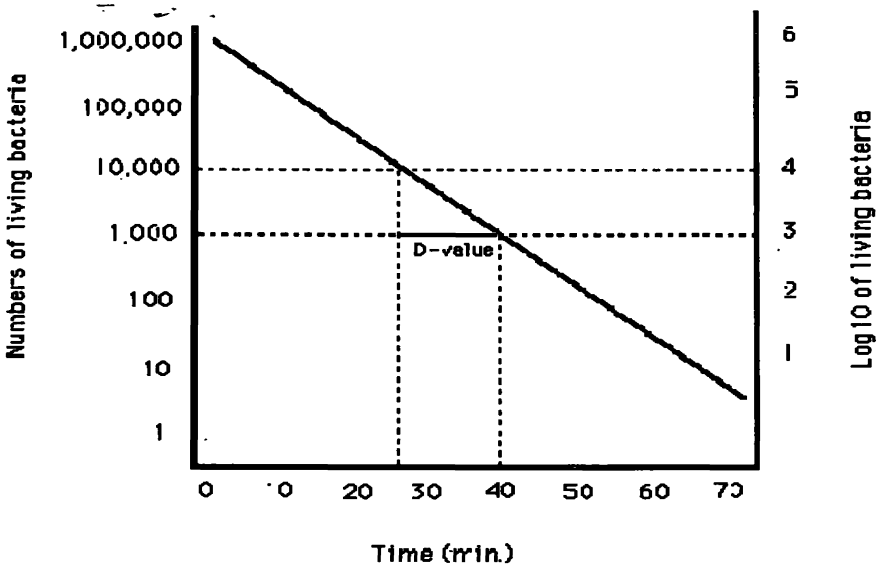


Fig.2.4 Survivor curve for a temperature of 72°C
Source: Hill (1947)

Table 2.2 Heat resistance of food poisoning organisms.

| **Organism** | **Heat resistance** |
|---|---|
| *Campylobacter jejuni* | $D_{55}$ = 1 minute |
| *Salmonella spp* | $D_{60}$ = 0.58 – 0.98 minutes |
| *Yersinia enterocolitica* | $D_{60}$ = 1-3 minutes |
| *Staphylococcus aureus* | $D_{60}$ = 2-15 min , $D_{71.7}$ = 4.1 seconds |
| *Vibrio parahaemolyticus* | $D_{60}$ = 5 min. |
| *Mycobacterium tuberculosis* | $D_{63}$ = 10 min |
| *Mycotoxigenic moulds* | $D_{65}$ = 0.5 – 3 min. |
| *Escherichia coli* | $D_{71.7}$ = 1 second |
| *Listeria monocytogenus* | $D_{71.7}$ = 3.3 seconds (most heat resistant strain)<br>$D_{71.7}$ = 0.6 – 2.0 seconds (other strains) |
| *Bacillus cereus* | $D_{100}$ = 2.8 minutes |

Source: Garbett (1997).

## 2.6 Detrimental effects of Pasteurization

Milk naturally contains healthy bacteria that inhibit the growth of undesirable and dangerous organisms. The pasteurization kills those friendly bacteria and with out these friendly bacteria, pasteurized milk is more susceptible to contamination. Pasteurization affects both vitamin C and $B_1$ (thiamin). In high temperatures vitamins C, $B_1$, A and D are destroyed sequentially. But vitamin A and D are not seriously influenced by pasteurization (Hill, 1947).

Pasteurization heat destroys the enzyme lipase which impairs fat metabolism and the ability to properly absorb fat soluble vitamin A and D. Pasteurization destroys

phosphatase enzyme which is essential for the absorption of Ca. This frequently leads to rickets, bad teeth and nervous troubles, for sufficient calcium content is vital to children and with the loss of phosphorus also associated with calcium, bone and brain formation suffer serious set backs. This also destroys 20% of the Iodine present in raw milk, causes constipation and generally takes from the milk its most vital qualities.

The nutrient losses induced by heating can be easily made up in a diet composed of a good variety from several other groups of food (Hall and Trout, 1968). Therefore these effects of pasteurization on the nutritional and physiological values of milk are negligible considering the safety benefits in regards to consumer health.

## 2.7 Testing methods for analyze milk quality

### 2.7.1 Alcohol test:

Non formation of flakes of curd shows negative results for alcohol test. This can be taken as a partial evidence of tolerance for thermal stability. But formation of large, medium or small flakes of curd appearing on the sides of the test tube indicates a positive test.

### 2.7.2 Clot on boiling test:

Milk of good quality does not coagulate if boiled and give negative results for Clot on boiling test. But if milk is heavily contaminated with lactic acid producing micro organisms it curdles, mainly due to denauration of protein as a result of microbial action.

### 2.7.3 Titrable acidity test:

In titrable acidity test the real acidity was determined by direct acid base titration. This acidity is developing due to the conversion of lactose sugar into lactic acid by lactic acid bacteria.

### 2.7.4 Dye reduction test:

The redox potential of milk is estimated by using dyes like rezasurin and Methylene blue as indicators. The microbes presence in milk take the oxygen of the milk and this removal of oxygen decreases the oxidation reduction potential of the milk. This declining potential can be measured by the color changes of the dye indicators with in a period of time.

The methylene blue test is of primary importance in assessing the bacterial content of a sample. The resazurin test, however, is more sensitive to high cell counts which are

frequently due to the presence of mastitis in herds and which indicate the presence of organisms likely to affect treatment while it assists in eliminating milk of poor keeping quality from the bulk supplies (Hill, 1947).

**2.7.5 Phosphatase Test:**

This test is based on the inactivation of the enzyme, phosphatase, which is present in all milks. The principal of the test is to determine the degree of inactivation of the enzyme by measuring the amount of phenol liberated from a di sodium phenyl substrate to which a small sample of milk has been added (Newlander and Atherton, 1964).

**2.7.6 Standard plate count:**

The SPC has long been the primary test for determining the bacterial quality of fresh raw or pasteurized milk. The SPC estimates the total numbers of aerobic type microorganisms. SPC can be used to check the microbiological quality of raw materials and final products, check the conditions of hygiene during the manufacturing process, determine whether a food has been subjected to temperature abuse during production, transport and storage, estimate the potential storage life of a product and determine levels of contamination in the processing environment (Garbutt, 1997). In conducting this procedure, careful consideration must be given to nutrients of plating medium, the temperature and time of incubation, and proper dilution of the sample to avoiding overcrowding of colonies on plates.

**2.7.7 Coliform test:**

The coliform bacteria count is used as an index of the level if sanitation and/or water quality employed in the handling and processing of milk products. Samples obtained at various points in the plant, submitted to this test, will enable the direct point of contamination to be determined (Hill, 1947). Coliforms are heat sensitive and are destroyed by pasteurization. Their presence in adequately heat treated products is therefore indicative of post heat process contamination (Early, 1998). A characteristic of the coliform organisms is that they form both acid and gas when they ferment milk sugar (Lampert, 1970). Coliform test contain 3 steps, presumptive test, Confirmatory test and completed test. If Escherichia coli is found, it may mean contamination of intestinal origin, possibly carried to the raw milk by unsanitary conditions among those handling the milk (Lampert, 1970).

# CHAPTER 3

## Materials and Methodology

### 3.1 Analysis of received raw milk

To ensure that the quality of the raw milk used for processing is up to accepted standards, received raw milk samples were checked for following evaluations. Alcohol precipitation, keeping quality, Clot on boiling, Organoleptic evaluation (Smell, Appearance, Taste), Fat, Specific Gravity, Solid non fat (SNF), Acidity and Adulteration. The samples were taken from the tanks of the milk bowser within half an hour of arrival at the plant.

### 3.1.1 Alcohol test

**Reagents**

Ethyl alcohol (72%, 75% and 68%)

**Materials**

Test tubes
1 ml graduated Pipettes

**Methodology**

1ml of 75% ethyl alcohol was taken in a test tube and 1 ml of milk was added to it. The contents were mixed by inverting the tube several times slowly and the formation of milk coagulates were observed. If the test is positive for 75% alcohol tests were carried out for 72% and 68% alcohol respectively.

Formation of flakes of curd on the sides of the test tube indicates a positive test.

The keeping quality of milk was tested directly by organoleptic tests or clot on boiling test and indirectly by test as Rezasurin test, Methylene blue dye reduction test and titrable acidity.

### 3.1.2 Resazurin test

**Reagents**

Resazurin solution

**Apparatus**

Sterile test tubes
Sterile rubber stoppers
Sterile 1ml and 10ml pipettes
Water bath (37°C)

**Methodology**

A well mixed 10ml milk sample was poured in to a sterile test tube, wetting only one side of the tube and 1ml of resazurin solution was added to it. Test tube was closed with the rubber stopper and the tube was inverted twice to ensure complete mixing. The test tube was placed in a water bath maintained at 37°C and the lid of the water bath was closed to prevent any falling of sunlight. After incubation for 10minutes the tube was removed from the water bath and examined using a Lovibond comparator. (Appendix III)

### 3.1.3 Clot on boiling test

**Apparatus**

10ml Graduated pipette
Boiling tube
Boiling water bath
Interval Timer

**Methodology**

5ml from the raw milk sample was pipetted in to a clean, dry test tube. The tube was heated in a boiling water bath for 5min.The tube was removed from the water bath, gently tilt it and the sides of the tube was observed.

### 3.1.4 Methylene blue reduction test

**Reagent**

Methylene blue solution

**Apparatus**

Sterilized Test tubes
Sterilized Rubber stoppers
Pipettes 1ml, 10ml
Water bath (37°C)

**Methodology**

The sample of milk was thoroughly mixed and 10ml of the milk was taken into a sterile test tube, leaving one side of the tube unwetted with milk.1ml of methylene blue solution was added to the milk The test tube was closed with a sterile rubber stopper and the tube was inverted gently twice. The tube was placed in a water bath maintained at 37°C.

Two control tubes were prepared using a mixture of 1ml of tap water and 10ml of milk, a mixture of 1ml of methylene blue solution and 10ml of milk having a fat content and colour similar to that of the milk being tested. Both control tubes

were closed with stoppers and immersed for 3minutes in boiling water. After the tubes were cooled and placed in the water bath.

All the tubes were examined after half an hour. The time has taken for complete decolourization of the sample was recorded. (The milk was regarded as decolourized when the whole column of milk is completely decolourized or decolourized up to range of 5mm of the surface) (Appendix V).

### 3.1.5 Organoleptic tests of milk

**Methodology**

Sample from the milk was taken and smell of the milk was checked immediately after the lid has been removed from the container. Then appearance was observed and the flavour of the milk was tasted. The lid was checked after removal and the milk container was checked as soon as it was emptied. All the observations were recorded.

### 3.1.6 Estimation of fat in milk

**Reagents**

Conc.Sulfuric acid
Amyl alcohol

**Apparatus**

Standard Gerber butyrometer
Standard lock stopper
Standard milk pipette calibrated to deliver 10.94ml water
Automatic measure to deliver 10ml of sulfuric acid
Automatic measure to deliver 1ml of amyl alcohol
Butyrometer rack
Centrifuge
Water bath for butyrometer (65°C)

**Methodology**

10 ml of Gerber sulphuric acid was added in to the butyrometer using the automatic dispenser.10.94 ml of well mixed milk sample was pipetted in to the tube using milk pipette. Finally 1 ml of fat free amyl alcohol was added in to the butyrometer using automatic measure. Do not wet the neck of the butyrometer with the acid, milk and alcohol. The neck of the butyrometer was closed firmly with a stopper without disturbing the contents. The butyrometer was shaken carefully to mix the contents thoroughly untill the curd particles are completely dissolved. The butyrometer was centrifuged immediately after the mixing and

after that it was placed (stopper downwards) in the water bath at 65°C for at least 3minutes.The fat percentage was read directly from the scale.

### 3.1.7 Estimation of specific gravity of milk by lactometer method

**Apparatus**

Calibrated lactometer (1.025-1.035 at 20°C)
Thermometer (-10 - 110°C)

**Methodology**

The milk sample was mixed at 20 °C well to avoid incorporation of air bubbles or any generation of froth. The milk was poured in to the cylinder slowly along the wall and the lactometer is lowered leaving ¼"of the stem above the level of the milk. The reading was taken on the upper edge of the meniscus when the lactometer was static. The temperature was detected and the lactometer reading is corrected as per temperature variations. (Appendix VI)

### 3.1.8 Calculation of Solid Non Fat

Fat content and Specific gravity in milk were obtained using above mentioned methods. Solid Non Fat (SNF) was calculated by using following formula.

SNF = (0.25D+0.22F +0.72)

F – Fat %
D = (Specific gravity -1) X 1000

### 3.1.9 Determination of Titratable Acidity and pH in Milk

**Reagents**

0.1N NaOH
1% phenolpthalein

**Apparatus**

Pipette
100ml Erlenmeyer flask
Burette
pH meter

**Methodology**

9ml of the well mixed sample of milk was pipetted in to a 100ml Erlenmeyer flask and 10 drops of 1% phenolphthalein solution was added in to it. The milk was titrated with 0.1N NaOH while agitating the sample continuously, until a permanent pale pink colour was observed. The quantity of NaOH used was read off and the amount of titratable acidity was calculated as a percentage of lactic acid.

$$\text{Titratable acidity} = \frac{(N)_{NaOH} \times (V)_{NaOH} \times \text{Molecular weight of lactic acid} / 1000 \times 100}{\text{Volume of sample}}$$

pH - pH of milk measured using the pH meter.

### 3.1.10 Tests for detection Adulteration on incoming raw milk

#### 3.1.10.1 Detection of sugar

**Reagents**

Conc.HCl
10% Resorcinol solution

**Apparatus**

Test tube
Graduated pipettes
Boiling water bath
Test tube holder

**Methodology**

7.5ml of suspected milk sample was transferred in to test tube. 0.5 ml of Conc.HCl and 1 ml of 10% resorcinol were added in to it respectively. Sample was mixed well and brought to boil in a water bath.

#### 3.1.10.2 Detection of salt

**Reagents**

$K_2CrO_4$ solution
$AgNO_3$ solution

**Methodology**

1 ml of milk was transferred to clean test tube. 2 drops of 10% K2CrO4 and 1 ml of AgNO3 solutions were added respectively. The mixture was shaked gently and the colour was observed.

#### 3.1.10.3 Detection of starch

**Reagents**

$I_2$ solution

**Apparatus**

Test tube
Pipettes

**Methodology**

10ml of milk was transferred in to a test tube. 0.5 ml of 1% $I_2$ solution was added in to it and the colour was observed.

## 3.2 Microbiological examinations

### 3.2.1 Preparation of decimal dilutions

**Reagents**

Ringer solution

**Apparatus**

Ringer's solution dilution tubes
Sterile Pipettes

**Methodology**

1ml of sample was measured using a pipette and transferred it to first dilution tube containing 9 ml of the sterile ringer solution .It was well mixed by shaking 25 times to obtain $10^{-1}$ dilution. 1 ml of the mixed first dilution was measured and transferred to the second dilution tube to obtain $10^{-2}$ dilution. Further 10-fold dilutions were prepared in the same way.

### 3.2.2 Enumeration of microbes using Standard plate count method

**Reagents**

Standard plate count agar

**Apparatus**

Sterile Petri dishes
Sterile pipettes (1ml)
Incubator at 30°C
Colony counter

**Methodology**

Two sterile Petri dishes were taken and using sterile pipettes, 1ml of the test sample was transferred in to each petri dish. This procedure was repeated with the dilutions, using fresh sterile pipettes and sterile Petri dishes for each dilution. 15ml of the plate count medium at 45 °C was poured in to each Petri dish. The inoculum with the medium was mixed carefully and allowed to solidify placing the petri dishes on a clean horizontal surface. The dishes were inverted and incubated at 37°C for 48 hours. After the specified period of incubation, the colonies were counted in each dish using the colony counting equipment. A control plate was prepared with 15ml of the medium, to check its sterility.

### 3.2.3 Enumeration of Yeast and moulds count

**Reagents**

Potato dextrose agar
10% tartaric acid

**Apparatus**

Sterile 1ml pipettes
Sterile Petri dishes

**Methodology**

Two sterile Petri dishes were taken and using sterile pipettes 1ml of the test sample was transferred in to each petri dish. This procedure was repeated with the dilutions, using fresh sterile pipettes and sterile Petri dishes for each dilution. Sterile potato dextrose agar medium was acidified with 10% tartaric acid solution immediately before pouring on plates.15ml of the potato dextrose agar medium at 45°C was poured in to each petri dish. The inoculum with the medium was mixed carefully and allowed to solidify placing the Petri dishes on a clean horizontal surface. The dishes were inverted and incubated at 25°C. The yeast and moulds colonies on each plate were count after 5days of incubation. A control plate was prepared with 15ml of the medium, to check its sterility.

### 3.2.4 Detection and Enumeration of Coliforms and Escherichia coli

#### 3.2.4.1 MPN method

The most probable number (MPN) method makes use of a statistical technique for estimating the most probable number of bacteria per specific unit of material under test.

3 serial dilutions are used and 3 tubes of medium are inoculated from each.

#### 3.2.4.2 Examination for presumptive coliforms

**Reagents**

MacConkey broth
Ringers solution

**Apparatus**

Sterile 1ml pipettes
Sterile petri dishes
36°C incubator
Durham tubes

**Methodology**

The sample was mixed thoroughly and serial decimal dilutions of the sample was prepared following the dilution technique mentioned in 3.2.1.1ml of each of the dilutions or where appropriate the original sample was pipetted in to each of 3 separate tubes of 10ml quantities of single strength MacConkey broth. The tubes were incubated at 36°C for 48 hours. The tubes showing colour changes and gas productions after 48hours were recorded.

### 3.2.4.3 Conformatory test for coliforms

**Reagents**

Brilliant green lactose bile broth (BGLB) solution

**Apparatus**

Test tubes
36°C incubator
Durham tubes

**Methodology**

A portion from each of the positive tubes from presumptive coliform test was inoculated into tubes containing 10ml quantities of BGB broth. The tubes were incubated at 36°C for 48 hours. The BGB tubes showing gas production and turbidity were recorded.

### 3.2.4.4 Examination for E.coli

**Reagents**

Trypton water
Kovacs reagent

**Apparatus**

Test tubes
44°C incubator

**Methodology**

A portion from each of the positive tubes from presumptive coliform test was inoculated into tubes containing 10ml quantities of Trypton water. The tubes were incubated at 44°C for 48 hours. After 48 hours tryptone water tubes were examined for indole test and observations were recorded.

**Indole test**

5 drops of Kovacs reagent was added in to trypton water tubes and tubes were shaked well. Tubes were allowed standing for 10minutes and the results were recorded.

### 3.3 Microbial testing for raw milk

#### 3.3.1 Enumeration of the initial microbial count (Standard plate count method)

Initial microbial count in raw milk was assessed using Toatal plate count method as mentioned in 3.2.2.

The number of micro organisms (N) per milliliter of the product was calculated using the following equation.

$$N = \frac{\sum C}{(n_1+0.1n_2)\, d}$$

$\sum C$ - sum of colonies counted on all the dishes retained
$n_1$ - number of dishes retained in the first dilution
$n_2$ - number of dishes retained in the second dilution
d - Dilution factor corresponding to the first dilution

#### 3.3.2. Enumeration of Yeast and moulds

Yeast and moulds counts in raw milk were assessed as mentioned in 3.2.3.

#### 3.3.3. Detection and Enumeration of Coliforms

The presumptive test and the confirmation test for coliforms were carried out as mentioned in 3.2.4.2. and 3.2.4.3. respectively.

#### 3.3.4. Detection and Enumeration of E coli

Examination of E coli was carried out using the positive tubes from 3.2.4.2. as mentioned in 3.2.4.4.

### 3.4. Preliminary study on the manufacturing process of flavoured pasteurized milk sachets

### 3.5. Assessing the quality of milk after Pasteurization

#### 3.5.1 Phosphates test

**Reagents**

Buffer solution
Buffer substrate

**Apparatus**

Clean sterile tubes
Graduated pipettes 10ml, 1ml
Water bath 37°C
Stopper
Incubator 37°C

**Methodology**

5ml of buffer substrate solution was taken in to a clean sterile test tube. Tube was closed with a rubber stopper and was placed in 37°C water bath for 5minutes. 1ml of the well mixed sample was added in to it and mixed well. Tube was incubated at 37°C for 30minutes. The control was prepared with previously well boiled milk and given the same treatment. The two tubes were compared in a comparator using a phosphatase disc. (Appendix IV)

### 3.5.2 Methylene blue reduction test

This test was carried out as mentioned in 3.1.4.The control tube was prepared by immersing stoppered test tube containing 1ml of tap water and 10ml of mixed milk having a fat content and colour similar to that of the milk being tested ,in boiling water for 3 minutes.

### 3.5.3 Enumeration of microbes (Standard plate count)

The microbial count in pasteurized raw milk was assessed using Total plate count method as mentioned in 3.2.2.

### 3.5.4. Enumeration of yeast and moulds

Yeast and moulds counts in pasteurized raw milk were assessed as mentioned in 3.2.3.

## 3.6. Assessing the quality of flavoured Pasteurize milk sachets

### 3.6.1. Enumeration of microbes (Standard plate count)

Final microbial count was assessed in pasteurize milk sachets after all the treatments were given, using standard plate count method as mentioned in 3.2.2.

### 3.6.2. Enumeration of yeast and moulds

Yeast and moulds counts in pasteurize milk sachets were assessed as mentioned in 3.2.3.

### 3.6.3. Detection and Enumeration of Coliforms

Presumptive test and confirmation test for coliforms were carried out as mentioned in 3.2.4.2. and 3.2.4.3. respectively.

### 3.6.4. Detection and Enumeration of E coli

Examination of E coli was carried out using the positive tubes from 3.2.4.2. as mentioned in 3.2.4.4.

### 3.6.5. Determination of Titrable Acidity

Titrable acidity was determined as mentioned in 3.1.8.

### 3.6.6. Assess the temperature changes after filling

**Apparatus**

Thermometer (-10 - 110°C)

**Methodology**

Temperature changes in filling, after packaging to master bags, after transferring to freezer were measured. After kept in the freezer, the time was measured needed to reach 4°C.

### 3.6.7. Determination the relationship between microbial count in raw milk and finished product

This relationship was assessed using Paired T test

## 3.7. Determination the maximum shelf life and changes of sensory quality of Pasteurized milk sachets under refrigerated condition (4°C)

12 Pasteurized milk sachets, produced in the same day same time were kept in refrigerator under 4°C just after packing. Maximum shelf life and changes of sensory quality were determined using the following tests, which were proceed after two days of storage and were continued till sachets get spoiled.

### 3.7.1. Enumeration of microbes (Standard plate count)

The microbial count in Pasteurized milk sachets was assessed using Total plate count method as mentioned in 3.2.2.

### 3.7.2. Enumeration of yeast and moulds

Yeast and moulds counts in raw milk were assessed as mentioned in 3.2.3.

### 3.7.3. Determination titratable Acidity

Titratable acidity was determined as mentioned in 3.1.8.

### 3.7.4. Evaluation of Organoleptic characteristics of pasteurized milk sachets

#### 3.7.4.1. Preparation of sensory evaluation paper

The sensory evaluation paper was prepared emphasizing the important organoleptic characteristics. A nine point Hedonic scale was employed along with descriptive terminology, which would aid the panelists in expressing reaction. (Appendix I)

Table 3.1 Preference and corresponding numerical scores in nine point hedonic scale.

| Preference | Numerical scale |
|---|---|
| Like extremely | 1 |
| Like very much | 2 |
| Like moderately | 3 |
| Like slightly | 4 |
| Neither like nor dislike | 5 |
| Dislike slightly | 6 |
| Dislike moderately | 7 |
| Dislike very much | 8 |
| Dislike extremely | 9 |

### 3.7.4.2. Sensory Analysis procedure

**Materials**

Sensory evaluation paper

Coded samples

Glass of portable water

**Methodology**

Sensory evaluation was done with the participation of same 15 untrained panelists of Rich life dairies limited. Each panelist was provided with 2 coded samples of pasteurized milk with a evaluation paper and they were asked to give scores according to his/her preference for mouth feel, flavour and overall acceptability for each sample using nine point hedonic scale. Glass of water were provided for each panelist for mouth rinsing after tasting each sample and suitable environment was provided for them to do their evaluation unbiasely. This sensory evaluation was carried out once in every two days during a month.

### 3.7.4.3. Statistical Analysis

All the responses obtained from the sensory evaluation for flavour, mouth feel and overall acceptability in milk sachets were analyzed using Friedman test. In this analysis each panelist considered as a block.

**Alcohol test**

Alcohol test was carried out as mentioned in 3.1.1. before sample taken for sensory evaluation in each day.

### 3.8. Assessment of the quality changes of milk during transportation and determine the maximum shelf life under the transportation condition

**Apparatus**

Regiform box
Digital thermometer (305 CIE)
Freezer

**Methodology**

12 sachets samples produced in the same day same time were kept in a freezer for *two hours. After two hours sachet samples were placed in a regiform box and kept in normal temperature for two hours. Temperature changes were measured using digital thermometer. Next those sachet samples were kept in a refrigerator under 4°C and Organoleptic characteristics were evaluated as mentioned in 3.6.4.3 after every 2days.

(*large numbers of sachets were delivered to Kandana and which needs 2 hours for the transportation)

### 3.9. Assessment of the suitability of raw material used for processing

#### 3.9.1. Sugar

#### 3.9.1.1 Assessment of the percentage of impurities

**Apparatus**

Filter paper
Oven

**Methodology**

10g of sugar was dissolved in 100ml distilled water. Filter paper was kept in an oven (40°C for 1 hour) and weight was measured. ($W_1$)

The solvent was filtered completely using the filter paper. Filter paper was dried again using the same procedure and the weight was measured. ($W_2$)

**Calculation**

$$\% \text{ of impurities} = \frac{(W2-W1) \times 100}{10}$$

### 3.9.1.2 Assessment of the percentage of moisture

**Apparatus**

Oven

**Methodology**

The weight of dish was measured ($W_1$).20g of sugar was measured ($W_2$) and dried for 3 hours at 105°C.The weight was measured again. ($W_3$)

**Calculation**

$$\% \text{ of moisture} = \frac{(W_2-W_1) - (W_3-W_1)}{20} \times 100$$

### 3.9.1.3 Assessment of the microbial quality

10g of sugar was measured and added to sterilize 90ml ringer solution. The mixture was shacked well. 1ml of the mixture was measured using a sterilized pipette and decimal dilutions were prepared as mentioned in 3.2.1.

**Enumeration of microbial population (Standard plate count method)**

The test was carried out as mentioned in 3.2.2. using the above prepared mixture.

**Enumeration of Yeast and moulds**

The test was carried out as mentioned in 3.2.3. using the above prepared mixture.

**Detection and Enumeration of Coliforms**

Presumptive test and confirmation test for coliforms were carried out as mentioned in 3.2.4.2. and 3.2.4.3. respectively.

**Detection and Enumeration of E coli**

Examination of E coli was carried out using the positive tubes from 3.2.4.2. as mentioned in 3.2.4.4.

### 3.9.2. Tap water

#### 3.9.2.1. Enumeration of microbial population (Standard plate count method)

The decimal dilutions were prepared as mentioned in 3.2.1. and the test was carried out as mentioned in 3.2.2.

#### 3.9.2.2. Detection and Enumeration of Coliforms

Presumptive test and confirmation test for coliforms were carried out as mentioned in 3.2.4.2. and 3.2.4.3. respectively.

**3.9.2.3. Detection and Enumeration of E coli**

Examination of E coli was carried out using the positive tubes from 3.2.4.2. as mentioned in 3.2.4.4.

**3.10. Assessment of the suitability of packaging material used for processing**

Few packets were taken with out filling the milk and with out sterilize the packaging material using ultra violet radiation. Interior wall of the packets were washed with adding ringer solution and shaked thoroughly. Same procedure was carried out for the packaging material sterilized using ultra violet radiation. The decimal dilutions were prepared as mentioned in 3.2.1.

**3.10.1. Enumeration of microbial population (Standard plate count)**

The test was carried out as mentioned in 3.2.2. using the test sample and above prepared dilutions.

**3.10.2 Detection and Enumeration of Coliforms**

The presumptive test and the confirmation test for coliforms were carried out as mentioned in 3.2.4.2. and 3.2.4.3. respectively.

**3.10.3. Detection and Enumeration of E coli**

Examination of E coli was carried out using the positive tubes from 3.2.4.2. as mentioned in 3.2.4.4.

**3.11. Assessment of the cleanliness in processing line**

**3.11.1. Balance tank**

**3.11.1.1. Enumeration of microbial population (Standard plate count method)**

The test was carried out as mentioned in 3.2.2. using the test sample and dilutions.

**3.11.1.2. Detection and Enumeration of Coliforms**

The presumptive test and the confirmation test for coliforms were carried out as mentioned in 3.2.4.2. and 3.2.4.3. respectively.

**3.11.1.3. Detection and Enumeration of E coli**

Examination of E coli was carried out using the positive tubes from 3.2.4.2. as mentioned in 3.2.4.4.

### 3.11.2. Swab Test

**Apparatus**

14 inch stainless steel wire
6 inch unmedicated ribbon gauze
Test tube

**Methodology**

Ribbon gauze was wrapped round the lower end of a looped stainless steel wire to form a swab 2 inches in length. The swab was put in a test tube containing 25ml sterile ringer solution and test tube with the content was autoclaved.

The swab was removed from its container and pressed against the side of the tube to remove all excess solution. It was rubbed heavily over the area which examined. After the swab was returned to its tube of solution it is twirled rapidly six times in the solution to mix the sample. Then it was removed, after the excess liquid was pressed out by pressure against the side of the tube. After the mixing was completed 1ml of the sample was measured using a sterilized pipette and decimal dilutions were prepared as mentioned in 3.2.1 for microbial examinations.

### 3.11.3. Filling out

#### 3.11.3.1 Enumeration of microbial population (Standard plate count method)

Swab was obtained from filling point of the machine as mentioned above and the test was carried out as mentioned in 3.2.2. using the test sample and dilutions.

#### 3.11.3.2. Detection and Enumeration of Coliforms

Using the swab was obtained from filling point the presumptive test and the confirmation test for coliforms were carried out as mentioned in 3.2.4.2. and 3.2.4.3. respectively.

#### 3.11.3.3. Detection and Enumeration of E coli

Examination of E coli was carried out using the positive tubes from 3.2.4.2. as mentioned in 3.2.4.4.

### 3.11.4. Sealing machine

#### 3.11.4.1 Enumeration of microbial population (Standard plate count method)

Swab was obtained from the machine as mentioned in 3.10.2.The test for enumerating total colony count was carried out as mentioned in 3.2.2.using the test sample and dilutions were prepared using the swabs

**3.11.4.2. Detection and Enumeration of Coliforms**

Using the swab which was obtained from the machine, the presumptive test and the confirmation test for coliforms were carried out as mentioned in 3.2.4.2. and 3.2.4.3. respectively.

**3.11.4.3. Detection and Enumeration of E coli**

Examination of E coli was carried out using the positive tubes from 3.2.4.2. as mentioned in 3.2.4.4.

**3.12. Assessment of personnel hygiene**

The swabs were taken from employees hands and the test was carried out as mentioned in 3.10.2.

**3.12.1 Enumeration of microbial population (Standard plate count method)**

The test was carried out as mentioned in 3.2.2. using the test sample and dilutions were prepared from the swabs.

**3.12.2. Detection and Enumeration of Coliforms**

Using the swabs obtained from employees hands the presumptive test and the confirmation test for coliforms were carried out as mentioned in 3.2.4.2. and 3.2.4.3. respectively.

**3.12.3. Detection and Enumeration of E coli**

Examination of E coli was carried out using the positive tubes from 3.2.4.2. as mentioned in 3.2.4.4.

**3.13. Enumeration of microbial population in the environment**

**Reagents**

Total plate count agar
Potato dextrose agar

**Methodology**

15ml of the plate count medium and potato dextrose medium at 45 °C were poured in to each Petri dish separately. Petri dishes were placed near the sealing machine, balance tank and mixing unit. The lids were removed and kept for 15 minutes exposed to the environment. The lids were put back and incubated the inverted exposed dishes with standard plate count agar and Potato dextrose agar at 30°C and 25 °C for 48 hours and for 5days respectively.

# CHAPTER 04

## Results and Discussion

### 4.1 Analysis of received raw milk

Milk is the major raw material used by the dairy industry. Therefore it is essential that the milk should be examined as received at the plant in order to avoid processing of a liquid which is likely to endanger the entire final product from the point of quality.

The Alcohol test, Clot on boiling, Organoleptic tests, titrable acidity test, dye reduction test, Tests for determine fat ,specific gravity, Solid Non Fat and Tests for adulterations were carried out as platform (rejection) test..

Table.4.1. Quality of raw milk

| Parameter | observations |
|---|---|
| Acidity | 0.165 |
| pH | 6.6 |
| LR | 26.9 |
| SNF | 8.28 |
| Fat | 3.91 |
| SPC | $1.415 \times 10^6$ |
| Yeast and moulds | <1 |
| MB | 4 hours |
| Rezasurin | 5 |
| Clot on Boiling | Negative |
| Alcohol test | Negative |
| Adulterations | Negative |

**4.1.1 Organoleptic test:** Received milk should have good organoleptic quality in order to improve the organoleptic quality of final product. The parameters checked resulted with the following observations.

Taste : slightly sweetish

Appearance: Yellowish white

Smell : Typical milk smell

**4.1.2 Alcohol test:** There was no formation of large, medium or small flakes of curd and thus milk composition showed negative for the alcohol test. This can be taken as a partial evidence of tolerance for thermal stability.

**4.1.3 Clot on boiling test:** When boiled, milk gave negative results for Clot on boiling test indicating that the quality of milk is good.

**4.1.4 Dye reduction test:** At the completion of the rezasurin test the colour indicated by the disc reading of 6 showed the milk is of good quality.

The milk needed 4 hours to decolourize with the methylene blue dye up to within 5 mm from the surface. This time is within the limits of more than 2 hrs and less than 6 hrs considered as acceptable raw milk quality.

**4.1.5 Titratable acidity:** The average titrable acidity of normal fresh milk could be expected to vary from 0.14 to 0.17%, calculated as the equivalent of lactic acid. The freshly drawn milk from the udder of the cow is acidic in nature having pH 6.6. (Hall and Trout, 1968). The acidity 0.165 obtained from the titration is conforming to the above mentioned range and pH value is 6.6. Hence there is no significant formation of acidity due to conversion of lactose in to lactic acid by microbial action.

**4.1.6 SNF:** According to the standards, fat and SNF values should be at least 2.5% and 8% respectively. The fat and SNF values obtained from the experiment exceed the minimum standard values. Hence the incoming milk is of good quality.

**4.1.7 SPC:** For milk to be classed as top quality, the bacteria count (Colony Forming Units/CFU), should normally be less than 100 000 per ml. In some countries, 10 000 per ml can be reached easily. But results reveal initially the raw milk contain high microbial population ($1.415 \times 10^6$ CFU/ml), owing to the contaminations at farms and during transportation or handling. (Appendix XII)

These findings of platform tests given above reveal that the raw milk is in good quality. But having high number of SPC will affect the shelf life of pasteurized milk sachets. Because the enzymes produced by these micro organisms before the heat treatment shall remain during the pasteurization and cause problems during the storage.

The milk freshly drawn from the cow is at a temperature of around 38°C.To reduce the growth of contaminant bacteria and slow the rate of spoilage, the temperature of milk should be rapidly lowered on production to 4°C with in 30 min. of milking

(Early, 1998). Hence it is essential to aware the farmers about the milking practices and bring the milk to collection centers just after milking.

Psychrotrophic activity can have implications for the quality and processability of raw milk held for prolonged periods at low temperatures (Early, 1998). Therefore holding of raw milk in collection centres for long time before processing should be minimized.

## 4.2 Manufacturing process of flavoured pasteurized milk sachets.

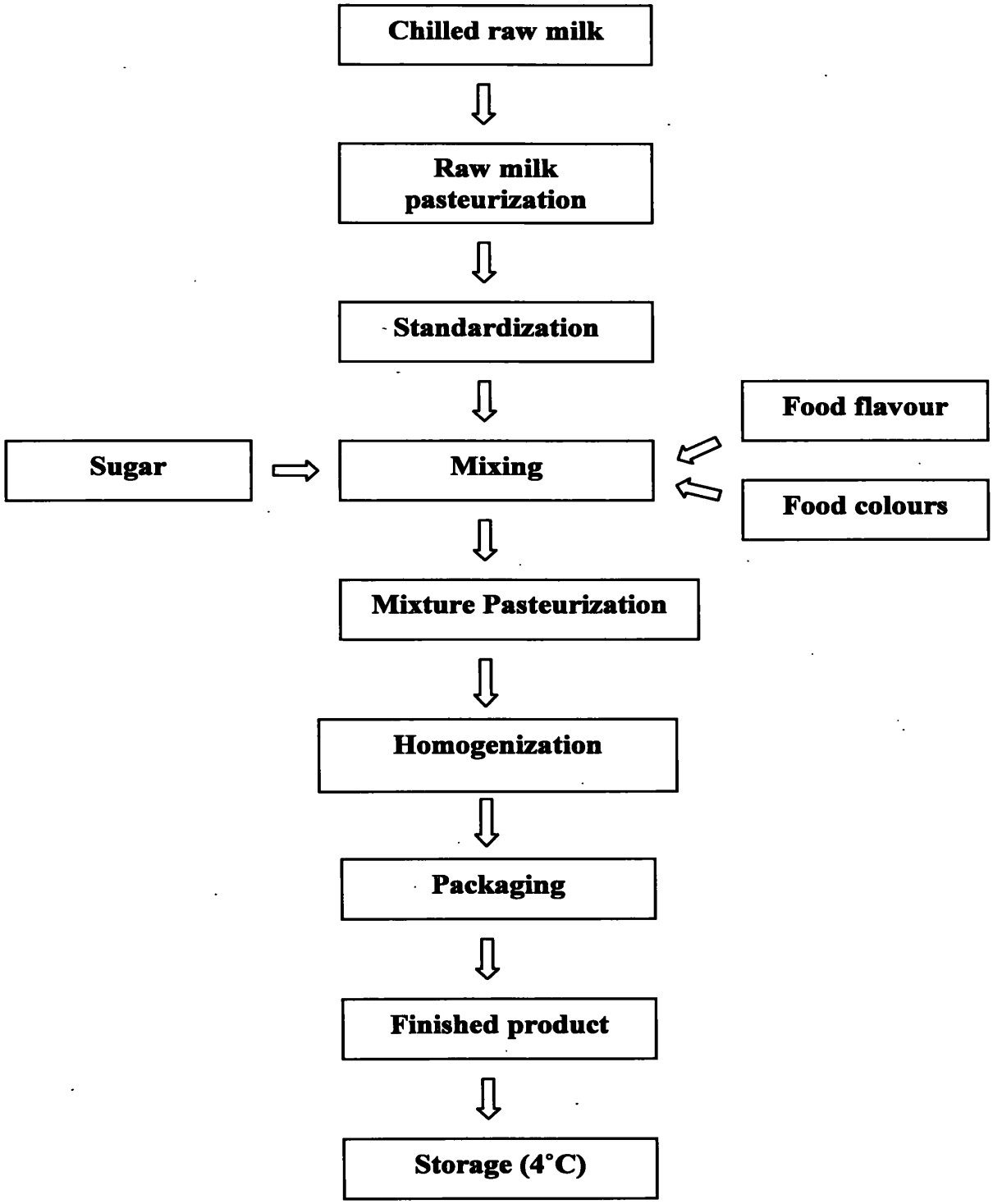


Fig. 4.1 manufacturing process of flavoured pasteurized milk sachets.

It is essential to understand the effectiveness of each and every step in production process in order to identify the causative factors leading to the limited shelf life of milk sachets. (Ranasinghe and Silva, 2000)

### 4.3 Determination of Pasteurized milk quality

Table 4.2 Quality of Pasteurized milk

| Parameter | Observations |
|---|---|
| Phosphatase test | Negative |
| MB | 6 hours |
| SPC | 239 |
| Yeast and moulds | <1 |
| Fat | 3.3 |

**4.3.1 Phosphatase test:** At the end of 30 min. period of incubation, there was no colour change occurring and the disc reading was given as zero. Therefore negative phosphatase test immediately after heat treatment indicates the milk has been pasteurized properly.

**4.3.2 SPC:** When milk is pasteurized (72°C for 15 min.) the standard plate count decreased appreciably (239 CFU/ml) due to the effect of heat treatment, where probably most of the psychrophiles and the mesophiles are destroyed.

Once the pasteurized milk is chilled the microbial number was further reduced compared to that of fresh milk by the reduction of multiplication of thermophilic bacteria.

**4.3.3 MB test:** In methylene blue test 6 1/2 hours was taken to decolourize the methylene blue dye up to within 5mm from the surface. This time is within the limits of more than 6 hrs and less than 8 hrs and hence the pasteurized milk quality is considered as good. Pasteurized milk takes more time than raw milk to decolourize the methylene blue dye due to the reduction of microbial counts present.

The fat content was standardized to 3.3 in pasteurized milk by the separation of cream. There was no yeast and moulds count in both raw and pasteurized milk. According to the results obtained it shows that the milk is pasteurized properly.

But this pasteurized milk is held for a long time in vats until it is taken in to the production and hence creates possibilities for post pasteurization contaminations.

Therefore alcohol test, COB test and rezasurin test are necessary to be carried out to the milk before taking it in to produce flavoured milk sachets.

### 4.4. Final product analysis of Flavoured pasteurized milk

This analysis was carried out to ensure the milk sachets are up to quality standards before released to the market.

Table 4.3 Quality of flavoured pasteurized milk sachets

| Parameter | Observations |
|---|---|
| Taste | normal |
| Fat | 2 % |
| Acidity | 0.115 |
| Alcohol (75%) | negative |
| SPC | 20 |
| Yeast and moulds | <1 |
| Coliform | abscent |
| E coli | abscent |

Due to the addition of ingredients to the pasteurized milk, initial acidity is now reduced to 0.115.

After the mixture was pasteurized (at 95°C), the standard plate count decreased further (20 CFU/ml) showing absence of both the coliforms and E. coli. Yeasts and moulds were also nil. Therefore the final flavoured pasteurized milk sachets are in conformity with the SLS standards. (Appendix II).This proves the quality of the finish product is good.

(This also showed negative for the alcohol test which was carried out using 75% alcohol.)

#### 4.4.1 Determination the temperature changes after filling

There was a significance temperature variation occurring after filling the milk sachets until transferring to storage.

Table 4.4 Temperature changes after filling

| Stages | Temperature |
|---|---|
| Filling | 17°C |
| After packing in to master bag | 19°C |
| Transferring to storage | 27°C |

After the storage under their chilled condition these milk sachets took 6 hours to reach to 4°C. Psychrotrophic growth is optimum in temperature between 20°C-30°C (Garbett, 1997).Growth of microbes that may have with standed during pasteurization exposures can reoccurr within a reasonable time to cause spoilage in the refrigerated milk. Therefore keeping under this condition for long time will affect the shelf life of finished milk sachets. The finished products should be stored under refrigerated condition immediately after packing.

### 4.5 Determination the relationship between microbial counts of raw milk and finished product

Table 4.6 Initial microbial counts in raw milk and microbial counts in finished product

| Experiment No. | Initial count (CFU/ml) | Final count (CFU/ml) |
|---|---|---|
| 1 | $12.8 \times 10^5$ | 35 |
| 2 | $7.54 \times 10^5$ | 12 |
| 3 | $7.50 \times 10^5$ | 9 |
| 4 | $8.09 \times 10^5$ | 14 |
| 5 | $9.36 \times 10^5$ | 20 |
| 6 | $10.7 \times 10^5$ | 24 |
| 7 | $7.09 \times 10^5$ | 9 |
| 8 | $10.7 \times 10^5$ | 18 |
| 9 | $7.90 \times 10^5$ | 11 |
| 10 | $5.13 \times 10^5$ | 6 |

The Initial microbial number of the milk with the microbial number of finished product was determined by using a paired t test. According to the data analysis, there was a significant difference between the two samples at a significance level of 0.05 ($p < 0.05$). Hence there is an effect of initial microbial count of raw milk on the microbial number of finish product. The microbial count of the finished product varies according to the initial count of the raw milk. Therefore microbial quality of the raw milk directly affects the shelf life of the pasteurized milk sachets.

## 4.6 Assessment of the suitability of raw materials and sanitary conditions of the production process

### 4.6.1 Determination of Standard plate counts at different stages of processing

The microbial population in milk varied from stage to stage of processing and significant differences in the total count was observed with the various stages of processing.

Table 4.4 Standard plate count of different sources

| Source | Standard plate count (CFU/ml) |
|---|---|
| Received raw milk | $1.415 \times 10^6$ |
| Pasteurized milk | 239 |
| Sugar | 17 |
| Tap water | 108 |
| Balance tank | 334 |
| Filling point | 110 |
| Sealing machine | 8 |
| Packaging material | |
| UV treated | 1 |
| UV untreated | 18 |
| Flavoured pasteurized milk | 20 |
| Hand count | 118 |
| Air count | 10 |

Standard plate counts were obtained from average values of 4 experiments.

Initially the raw milk contained a significantly high microbial population ($1.415 \times 10^6$). But when milk was pasteurized (at 72°C for 15min) the standard plate count decreased appreciably (239) due to the effect of heat treatment, where probably most of the psychrophiles and the mesophiles could have destroyed. According to the SLS standards the count per ml should be less than 30 000. (Appendix II). Therefore the pasteurized milk is in good quality in these terms.

But the standard plate count of milk sample obtained from balance tank showed an increased value (334), due to addition of sugar, flavours and essence during the mixing.

The average standard plate count of tap water samples taken at different occasions averaged to 108 CFU/ml which is closer to the specified standards of 100 CFU/ml water.

Swab samples were obtained from hands of workers, filling point and packaging machine resulting colony counts of 118,105 and 8 respectively. But these counts were not higher than the standard values. Air count was low for standard plate count (10 CFU) and also for yeast and moulds (1 colony).

### 4.6.2 Detection of of coliform and E. coli

Milk and swabs taken from various stage of processing showed different results for coliforms and E coli. The counts were obtained according to the MPN method (Appendix VII) and average MPN values of four experiments (Appendix XI) are as follows.

**Table 4.5** Detection of coliform and E.coli in different sources

| Source | coliform | E coli |
|---|---|---|
| Raw milk | 0.5 | 0.3 |
| Pasteurized milk | 0.3 | 0.3 |
| Tap water | 0.3 | 0.3 |
| sugar | 0.3 | 0.3 |
| Balance tank | 0.6 | 0.3 |
| Fill out | 0.3 | 0.3 |
| Hand count | 0.8 | 0.3 |
| Packaging material | 0.3 | 0.3 |
| Pasteurized flavoured milk | 0.3 | 0.3 |

Raw milk proved the presence of coli due to contaminations in farms during transportation and handling. Presence of coliform in balance tank is probably due to the contamination in the production flow line itself.

Swabs from both hands of two labourers working in the sealing machine showed the presence of coliforms. Therefore employees entering the processing area should

wash their hands with disinfectant before commencing work. Results also revealed the presence of coliforms in balance tank. Hence more attention should be payed about the CIP process.

None of the source showing presence of E coli confirms the good hygienic quality of the sources.

### 4.6.3 Determination the suitability of sugar for the production

Table 4.6 Quality of the sugar

| Characteristics | Results |
|---|---|
| Appearance | White fine crystals |
| Moisture | 0.44 |
| Impurities | 0.91 |
| Yeast and moulds | nil |

According to the specifications moisture and impurities of sugar should be less than 0.1%. But the results of moisture and impurities revealed smaller variations with the specifications. But the microbial quality is satisfying with the specifications. Hence there is no major effect from the quality of sugar for the shelf life limitation of pasteurized milk sachets.

## 4.7 Determination the maximum shelf life of pasteurized milk sachets under refrigerated condition

### 4.7.1 Sensory evaluation

The responses of the 15 penalists for the organoleptic characteristics obtained within an interval of two days during a one month period were analyzed using Friedman statistical test. According to the statistical analysis results of overall acceptability there is no significant difference between the samples at a significance level of 0.05 ($p > 0.05$) until 19 days. Statistical analysis also revealed that there is no significant difference in mouth feel between the samples at a significance level of 0.05 ($p > 0.05$) until 17 days. However the statistical analysis revealed that there is a significant difference in flavour between the samples at a significance level of 0.05 ($p < 0.05$) after 7 days. Hence there was a slight deviation in mouth feel and flavour from the freshly produced sachets after 17 and 7 days respectively. But these variations can be minimized by using strong flavour additives. Therefore flavoured

pasteurized milk sachets can be kept for an estimated 19 day period under refrigerated condition with qualities similar to freshly produced sachets.

### 4.7.2 Microbial studies

Total plate count was obtained on each day parallel to the sensory evaluation. (Appendix XIII). The obtained standard plate counts during one month period were lower than SLS specification for microbial limits in flavoured pasteurized milk sachets and therefore it is safe for the consumption.

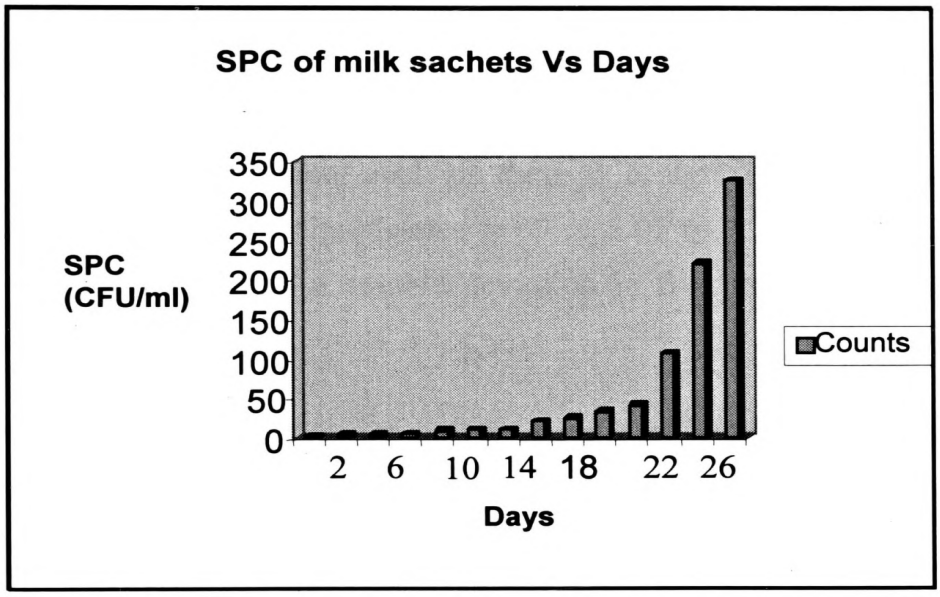


Fig. 4.2 Changes in Standard plate count of milk sachets stored under Refrigeration condition (4°C)

Shelf life of pasteurized fluid milk could be defined as, "the period of time that a product can be kept under refrigeration conditions with its acceptable quality." The term "acceptable quality" means that the product's mouth feel, flavour and appearance are satisfactory to the consumer and that the milk is safe to drink.

According to results obtained from both sensory evaluation and microbial studies, the shelf life of pasteurized milk sachets can be prolonged till 19 days by keeping under refrigeration condition (4°C).

## 4.8 Determination the Shelf life of pasteurized milk sachets under the transportation condition

### 4.8.1 Sensory evaluation

The responses for the organoleptic characteristics obtained from the 15 penalists were statistically analyzed using the Friedman test (MINITAB statistical package). Statistical analysis revealed that there is no significant difference in overall acceptability and in mouth feel between the samples at a significance level of 0.05 ($p>0.05$) until 15 days. These results ensure that under the transportation conditions, the flavoured pasteurized milk sachets exceed the shelf life till 15 days similar to the sachets in fresh condition.

However, according to the statistical analysis there is a significant difference in flavour between the samples at a significance level of 0.05 ($p <0.05$) after 5 days. Hence this reveals that there was a notable deviation in flavour from that of the freshly produced ones after 5 days.

The finished pasteurized milk sachets should be stored for a very short time and then transported to the market on refrigerated trailers. In Rich life dairies currently there are no insulated trailers to distribute the sachets to retail shops. Therefore the temperature increases gradually during transportation to long distances. The micro organisms which survived in pasteurization will grow rapidly in such favourable conditions resulting in the breakdown of milk components and subsequent conversion to compounds detected as off-flavors. This was the cause for the reduction of overall acceptability of milk sachets after 15 days. Therefore it is essential to maintain the chilled conditions during transportation as well as during storage in retail shops.

# CHAPTER 5

## Conclusions and Recommendations

According to the findings, it can be concluded that there is an effect arising from the initial microbial count of raw milk on the microbial count of pasteurized milk sachets.

The results of sensory evaluation and microbial tests revealed that flavoured pasteurized milk can be kept for a period of 19 days under refrigerated condition (around 4°C) with qualities similar to freshly produced sachets.

According to the results obtained from various stages of production process, it can be concluded that the major causative factors which limit the shelf life of milk sachets are poor microbial quality of raw milk, keeping sachets in room temperature for a long time without immediate storage after packaging, unsatisfactory conditions found during the transportation process and the poor storage conditions in retail shops.

Even with the adverse transportation conditions, the pasteurized milk sachets stored under refrigerated condition (4°C) could be kept for a period of 15 days with qualities similar to the sachets found in fresh condition.

Therefore it can ultimately be concluded the that shelf life of flavoured pasteurized milk sachets can be extended by storing the finished products under refrigerated conditions immediately after packing and maintaining the cold chain (i.e. chilled conditions) during transportation and during storage in retail shops.

The management should pay more attention to maintain the cold chain through out the process until the consumers receives the product. And also it is essential to conduct awareness programmes for farmers regarding clean milk production practices.

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# Appendix I

## Sensory Evaluation Data

Name: ..... Date: .....

Product Description: .....

You are kindly requested to assess each food sample presented, with reference to under mentioned sensory attributes according to your preference, considering the following scale.

9 - Like Extremely
8 - Like Very Much
7 - Like Moderately
6 - Like Slightly
5 - Neither Like nor Dislike
4 - Dislike Slightly
3 - Dislike Moderately
2 - Dislike Very Much
1 - Dislike Extremely

| Sample No. | Code | Mouth feel | Flavour | Overall acceptability |
|---|---|---|---|---|
| 1 | 678 | | | |
| 2 | 526 | | | |

Comments:

.....
.....
.....
.....
.....

Thank you.

# Appendix II

**Sri Lanka Standards requirements for milk**

| Characteristic | Cow milk | Pasteurized Unhomogenized | Standardized pasteurized | Pasteurized homogenized | Flavoured Pasteurized |
|---|---|---|---|---|---|
| Milk fat % by mass | 3.5 | 3.5 | 3.25 | 3.5 | 2 |
| Milk solids other than milk fat(MSNF) | 8.5 | 8.5 | 8.25 | 8.5 | 7.2 |
| Creaming index (max) | - | - | - | 20 | - |
| Phosphatase activity | - | To satisfy the test | To satisfy the test | To satisfy the test | To satisfy the test |
| Turbidity test | - | - | - | - | - |
| Methylene blue reduction test | - | To satisfy the test | To satisfy the test | To satisfy the test | To satisfy the test |
| Colony count per ml (not more than) | - | 30 000 | 30 000 | 30 000 | 30 000 |
| Coliform | - | - | - | absent | absent |

Source: (SLS 181:1983)

# Appendix III

**Grading of milk by 10 min. Rezasurin test**

| Disc reading | Conclusion |
|---|---|
| 6 to 4 | Satisfactory |
| 3 ½ to 1 | Doubtful |
| ½ to 0 | Unsatisfactory |

Source: Davis (2002)

# Appendix IV

**Grading of pasteurized milk by phosphatase test**

| Disc reading after incubation | Interpretation |
|---|---|
| 0 | Properly pasteurized |
| 6 | Doubtful |
| 10 or over | Under pasteurized |

# Appendix V

**Grading of milk by Methylene Blue Reduction Test**

| Grade of milk | Time for decolorization |
|---|---|
| Good | More than 8 hours |
| Fair to good | More than 6 hours & less than 8 hours |
| Passable | More than 2 hours & less than 6 hours |
| Bad | Less than 2 hours |

Source: Lampert (1970)

# Appendix VI

**Corrections to lactometer reading temperature**

| °C | correction |
|---|---|
| 30 | +0.0029 |
| 29.5 | + 0.0028 |
| 29 | +0.0026 |
| 28.5 | +0.0024 |
| 28 | +0.0023 |
| 27.5 | +0.0021 |
| 27 | +0020 |
| 26.5 | +0.0018 |
| 26 | +0.0017 |
| 25.5 | +0.0015 |
| 25 | +0.0014 |
| 24.5 | +0.0012 |
| 24 | +0.0011 |
| 23.5 | +0.0009 |
| 23 | +0.0008 |
| 22.5 | +0.0007 |
| 22 | +0.0005 |
| 21.5 | +0.0004 |
| 21 | +0.0003 |
| 20.5 | +0.0001 |
| 20 | +0.0000 |
| 19.5 | -0.0001 |
| 19 | -0.0003 |
| 18.5 | -0.0004 |
| 18 | -0.0005 |

# Appendix VII

**Most Probable Number Table**

| Number of positive tubes for the three Dilutions factor retained | | | MPN | Confidence limits | | | |
|---|---|---|---|---|---|---|---|
| | | | | 99% | | 95% | |
| 0 | 0 | 0 | 0.3 | | | | |
| 0 | 1 | 0 | 0.3 | 0.1 | 2.3 | 0.1 | 1.7 |
| 1 | 0 | 0 | 0.4 | 0.1 | 2.8 | 0.1 | 2.1 |
| 1 | 0 | 1 | 0.7 | 0.1 | 3.5 | 0.2 | 2.7 |
| 1 | 1 | 0 | 0.7 | 0.1 | 3.6 | 0.2 | 2.8 |
| 1 | 2 | 0 | 1.1 | 0.2 | 4.4 | 0.4 | 3.5 |
| 2 | 0 | 0 | 0.9 | 0.1 | 5.0 | 0.2 | 3.8 |
| 2 | 0 | 1 | 1.4 | 0.3 | 6.2 | 0.5 | 4.8 |
| 2 | 1 | 0 | 1.5 | 0.3 | 6.5 | 0.5 | 5.0 |
| 2 | 1 | 1 | 2.0 | 0.5 | 7.7 | 0.8 | 6.1 |
| 2 | 2 | 0 | 2.1 | 0.5 | 0.8 | 0.8 | 6.3 |
| 3 | 0 | 0 | 2.3 | 0.4 | 17.7 | 0.7 | 12.9 |
| 3 | 0 | 1 | 4 | 1 | 25 | 1 | 18 |
| 3 | 1 | 1 | 4 | 1 | 29 | 2 | 21 |
| 3 | 1 | 1 | 7 | 2 | 37 | 2 | 28 |
| 3 | 2 | 0 | 9 | 2 | 52 | 3 | 39 |
| 3 | 2 | 1 | 15 | 3 | 66 | 5 | 51 |
| 3 | 2 | 2 | 21 | 5 | 82 | 8 | 64 |
| 3 | 3 | 0 | 20 | 10 | 190 | 10 | 140 |
| 3 | 3 | 1 | 50 | 10 | 320 | 20 | 240 |
| 3 | 3 | 2 | 110 | 20 | 640 | 30 | 480 |
| 3 | 3 | 3 | >110 | | | | |

Source: (SLS 516:1982)

# Appendix VIII

**Presumptive coliform count in different sources**

| Source | 1st experiment | | | 2nd experiment | | | 3rd experiment | | | 4th experiment | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Raw milk** | 2 | 1 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 1 | 1 | 1 |
| **Pasteurized milk** | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| **Tap water** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| **Sugar** | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| **Balance tank** | 1 | 1 | 0 | 2 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 |
| **Fill out** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| **Hand count** | 2 | 2 | 1 | 2 | 1 | 1 | 1 | 1 | 1 | 2 | 1 | 0 |
| **Packaging material** | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 |
| **Pasteurized flavoured milk** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 |

# Appendix IX

**Confirmatory test for coliforms**

| Source | 1st experiment | 2nd experiment | 3rd experiment | 4th experiment |
|---|---|---|---|---|
| **Raw milk** | 0 0 0 | 0 0 1 | 1 1 0 | 1 1 0 |
| **Pasteurized milk** | - | 0 0 0 | - | - |
| **Tap water** | - | - | - | - |
| **Sugar** | - | 0 0 0 | - | - |
| **Balance tank** | 0 1 0 | 1 0 1 | 1 1 0 | 1 1 0 |
| **Fill out** | - | - | - | - |
| **Hand count** | 1 1 0 | 2 1 0 | 1 0 1 | 1 1 0 |
| **Packaging material** | - | - | 0 0 0 | - |
| **Pasteurized flavoured milk** | - | - | 0 0 0 | - |

(Confirmatory test was carried out only for the positive tubes from the presumptive test)

0 - not detected (3 tube M.P.N)

1, 2, 3 – detected (3 tube M.P.N)

# Appendix X

**Detection of E coli by Indole test**

| Source | 1st experiment | 2nd experiment | 3rd experiment | 4th experiment |
|---|---|---|---|---|
| **Raw milk** | 0 0 0 | - | 0 0 0 | 0 0 0 |
| **Pasteurized milk** | - | 0 0 0 | - | - |
| **Tap water** | - | - | - | - |
| **Sugar** | - | 0 0 0 | - | - |
| **Balance tank** | 0 0 0 | 0 0 0 | 0 0 0 | 0 0 0 |
| **Fill out** | - | - | - | - |
| **Hand count** | 0 0 0 | 0 0 0 | 0 0 0 | 0 0 0 |
| **Packaging material** | - | - | 0 0 0 | - |
| **Pasteurized flavoured milk** | - | - | 0 0 0 | - |

(The test was carried out only for the positive tubes from the presumptive test)

0 - not detected (3 tube M.P.N)

# Appendix XI

**Detection of coliform and E.coli in different sources**

| Source | 1st experiment | | 2nd experiment | | 3rd experiment | | 4th experiment | |
|---|---|---|---|---|---|---|---|---|
| | Coliform | E.coli | Coliform | E.coli | Coliform | E.coli | Coliform | E.coli |
| Raw milk | 0.3 | 0.3 | 0.3 | 0.3 | 0.7 | 0.3 | 0.7 | 0.3 |
| Pasteurized milk | 0.3 | 0.3 | 0.3 | 0.3 | 0.3 | 0.3 | 0.3 | 0.3 |
| Tap water | 0.3 | 0.3 | 0.4 | 0.3 | 0.3 | 0.3 | 0.3 | 0.3 |
| Sugar | 0.3 | 0.3 | 0.3 | 0.3 | 0.3 | 0.3 | 0.3 | 0.3 |
| Balance tank | 0.3 | 0.3 | 0.7 | 0.3 | 0.7 | 0.3 | 0.7 | 0.3 |
| Fill out | 0.3 | 0.3 | 0.3 | 0.3 | 0.3 | 0.3 | 0.3 | 0.3 |
| Hand count | 0.7 | 0.3 | 1.5 | 0.3 | 0.7 | 0.3 | 0.7 | 0.3 |
| Packaging material | 0.3 | 0.3 | 0.3 | 0.3 | 0.3 | 0.3 | 0.3 | 0.3 |
| Pasteurized flavoured milk | 0.3 | 0.3 | 0.3 | 0.3 | 0.3 | 0.3 | 0.3 | 0.3 |

The counts were obtained according to the MPN method (Appendix VII)

# Appendix XII

**Initial microbial counts in raw milk**

| Experiment No. | Dilution factor | Initial microbial count (cfu/ml) |
|---|---|---|
| 1 | $10^{-4}$ | 248 |
| | $10^{-5}$ | 34 |
| 2 | $10^{-4}$ | 152 |
| | $10^{-5}$ | 14 |
| 3 | $10^{-4}$ | 135 |
| | $10^{-5}$ | 30 |
| 4 | $10^{-4}$ | 160 |
| | $10^{-5}$ | 18 |
| 5 | $10^{-4}$ | 188 |
| | $10^{-5}$ | 18 |
| 6 | $10^{-4}$ | 204 |
| | $10^{-5}$ | 32 |
| 7 | $10^{-4}$ | 124 |
| | $10^{-5}$ | 32 |
| 8 | $10^{-4}$ | 192 |
| | $10^{-5}$ | 45 |
| 9 | $10^{-4}$ | 155 |
| | $10^{-5}$ | 19 |
| 10 | $10^{-4}$ | 96 |
| | $10^{-5}$ | 17 |

# Appendix XIII

**Changes in standard plate count of flavoured pasteurized milk sachets, stored under refrigerated condition (4°C)**

| Days | Standard plate count | | |
|---|---|---|---|
| | $10^0$ | $10^1$ | $10^2$ |
| 16 | 8 | Nil | Nil |
| 18 | 11 | Nil | Nil |
| 20 | 12 | Nil | Nil |
| 22 | 16 | Nil | Nil |
| 25 | 27 | Nil | Nil |
| 27 | 30 | Nil | Nil |
| 29 | 34 | Nil | Nil |
| 2 | 53 | 1 | Nil |
| 4 | 65 | 1 | Nil |
| 6 | 89 | 1 | Nil |
| 9 | 102 | 2 | Nil |
| 11 | 201 | 2 | 1 |
| 13 | 410 | 5 | 2 |
| 16 | > 410 | 9 | 2 |

# Appendix XIV

**Statistical Analysis data for determining the relationship between the initial microbial number of the raw milk and the final microbial count of the finished product**

**Paired T-Test and CI: Initial, Final**

Paired T for Initial - Final

| | N | Mean | StDev | SE Mean |
|---|---|---|---|---|
| Initial | 10 | 1415900 | 651746 | 206100 |
| Final | 10 | 25 | 26 | 8 |
| Difference | 10 | 1415875 | 651726 | 206094 |

95% CI for mean difference: (949659, 1882092)
T-Test of mean difference = 0 (vs not = 0): T-Value = 6.87 P-Value = 0.000

H0: Initial microbial number of the raw milk affects not significantly on the final microbial count of the finished product.
H1: Initial microbial quality affects significantly on the final microbial count of the finished product.

P value < 0.05
Hence, reject H0
Therefore the initial microbial number of the raw milk affects the final microbial count of the finished product.

# Appendix XV

**Statistical Analysis of sensory data for the determination of shelf life of flavoured pasteurized milk sachets under refrigerated condition**

**Friedman Test on mouth feel**

Friedman Test: responses (C3) versus No. of Days (C2) blocked by No. of penalists (C1)

S = 0.07 DF = 1 P = 0.796
S = 0.33 DF = 1 P = 0.564 (adjusted for ties)

| C2 | N | Est Median | Sum of Ranks |
|---|---|---|---|
| 1 | 15 | 2.0000 | 22.0 |
| 2 | 15 | 2.0000 | 23.0 |

Grand median = 2.0000

Friedman Test: responses (C3) versus No. of Days (C2) blocked by No. of penalists (C1)

S = 0.10 DF = 2 P = 0.951
S = 0.50 DF = 2 P = 0.779 (adjusted for ties)

| C2 | N | Est Median | Sum of Ranks |
|---|---|---|---|
| 1 | 15 | 2.0000 | 29.0 |
| 2 | 15 | 2.0000 | 30.5 |
| 3 | 15 | 2.0000 | 30.5 |

Grand median = 2.0000

Friedman Test: responses (C3) versus No. of Days (C2) blocked by No. of penalists (C1)

S = 0.76 DF = 3 P = 0.859
S = 2.71 DF = 3 P = 0.438 (adjusted for ties)

| C2 | N | Est Median | Sum of Ranks |
|---|---|---|---|
| 1 | 15 | 2.0000 | 35.0 |
| 2 | 15 | 2.0000 | 37.0 |
| 3 | 15 | 2.0000 | 37.0 |
| 4 | 15 | 2.0000 | 41.0 |

Grand median = 2.0000

Friedman Test: responses (C3) versus No. of Days (C2) blocked by No. of penalists (C1)

S = 0.87 DF = 4 P = 0.929
S = 3.06 DF = 4 P = 0.548 (adjusted for ties)

| C2 | N | Est Median | Sum of Ranks |
|---|---|---|---|
| 1 | 15 | 2.0000 | 41.5 |
| 2 | 15 | 2.0000 | 44.0 |
| 3 | 15 | 2.0000 | 44.0 |
| 4 | 15 | 2.0000 | 49.0 |
| 5 | 15 | 2.0000 | 46.5 |

Grand median = 2.0000

Friedman Test: responses (C3) versus No. of Days (C2) blocked by No. of penalists (C1)

S = 1.26 DF = 5 P = 0.939
S = 4.23 DF = 5 P = 0.517 (adjusted for ties)

| C2 | N | Est Median | Sum of Ranks |
|---|---|---|---|
| 1 | 15 | 2.0000 | 47.5 |
| 2 | 15 | 2.0000 | 50.5 |
| 3 | 15 | 2.0000 | 50.5 |
| 4 | 15 | 2.0000 | 56.5 |
| 5 | 15 | 2.0000 | 53.5 |
| 6 | 15 | 2.0000 | 56.5 |

Grand median = 2.0000

Friedman Test: responses (C3) versus No. of Days (C2) blocked by No. of penalists (C1)

S = 2.10 DF = 6 P = 0.910
S = 6.00 DF = 6 P = 0.423 (adjusted for ties)

| C2 | N | Est Median | Sum of Ranks |
|---|---|---|---|
| 1 | 15 | 2.0000 | 53.0 |
| 2 | 15 | 2.0000 | 56.5 |
| 3 | 15 | 2.0000 | 56.5 |
| 4 | 15 | 2.0000 | 63.5 |
| 5 | 15 | 2.0000 | 60.0 |
| 6 | 15 | 2.0000 | 63.5 |
| 7 | 15 | 2.0000 | 67.0 |

Grand median = 2.0000

Friedman Test: responses (C3) versus No. of Days (C2) blocked by No. of penalists (C1)

S = 6.02 DF = 7 P = 0.537
S = 14.48 DF = 7 P = 0.043 (adjusted for ties)

| C2 | N | Est Median | Sum of Ranks |
|---|---|---|---|
| 1 | 15 | 2.0000 | 57.0 |
| 2 | 15 | 1.8750 | 61.0 |
| 3 | 15 | 2.0000 | 61.0 |
| 4 | 15 | 2.0000 | 69.0 |
| 5 | 15 | 2.0000 | 65.0 |
| 6 | 15 | 2.0000 | 69.0 |
| 7 | 15 | 2.0000 | 73.0 |
| 8 | 15 | 2.1250 | 85.0 |

Grand median = 2.0000

Friedman Test: responses (C3) versus No. of Days (C2) blocked by No. of penalists (C1)

S = 37.43 DF = 8 P = 0.000
S = 63.74 DF = 8 P = 0.000 (adjusted for ties)

| C2 | N | Est Median | Sum of Ranks |
|---|---|---|---|
| 1 | 15 | 1.889 | 57.0 |
| 2 | 15 | 1.778 | 61.5 |
| 3 | 15 | 1.889 | 61.5 |
| 4 | 15 | 2.000 | 69.0 |
| 5 | 15 | 2.000 | 65.5 |
| 6 | 15 | 2.000 | 69.0 |
| 7 | 15 | 2.000 | 74.0 |
| 8 | 15 | 2.111 | 85.5 |
| 9 | 15 | 3.333 | 132.0 |

Grand median = 2.111

Same p value (p=0.000) resulted after this step.

# Appendix XVI

**Friedman Test on Flavour**

Friedman Test: responses (C3) versus No. of Days (C2) blocked by No. of penalists (C1)

S = 0.07 DF = 1 P = 0.796
S = 1.00 DF = 1 P = 0.317 (adjusted for ties)

| C2 | N | Est Median | Sum of Ranks |
|---|---|---|---|
| 1 | 15 | 2.0000 | 22.0 |
| 2 | 15 | 2.0000 | 23.0 |

Grand median = 2.0000

Friedman Test: responses (C3) versus No. of Days (C2) blocked by No. of penalists (C1)

S = 0.30 DF = 2 P = 0.861
S = 2.00 DF = 2 P = 0.368 (adjusted for ties)

| C2 | N | Est Median | Sum of Ranks |
|---|---|---|---|
| 1 | 15 | 2.0000 | 28.5 |
| 2 | 15 | 2.0000 | 30.0 |
| 3 | 15 | 2.0000 | 31.5 |

Grand median = 2.0000

Friedman Test: responses (C3) versus No. of Days (C2) blocked by No. of penalists (C1)

S = 6.20 DF = 3 P = 0.102
S = 17.22 DF = 3 P = 0.001 (adjusted for ties)

| C2 | N | Est Median | Sum of Ranks |
|---|---|---|---|
| 1 | 15 | 1.8750 | 32.0 |
| 2 | 15 | 2.1250 | 34.0 |
| 3 | 15 | 2.1250 | 36.0 |
| 4 | 15 | 2.3750 | 48.0 |

Grand median = 2.1250

Friedman Test: response (C3) versus No. of Days (C2) blocked by No. of penalists (C1)

S = 10.13 DF = 4 P = 0.038
S = 19.61 DF = 4 P = 0.001 (adjusted for ties)

| C2 | N | Est Median | Sum of Ranks |
|---|---|---|---|
| 1 | 15 | 1.8000 | 35.5 |
| 2 | 15 | 2.0000 | 38.0 |
| 3 | 15 | 2.0000 | 40.5 |
| 4 | 15 | 2.4000 | 55.5 |
| 5 | 15 | 2.8000 | 55.5 |

Grand median = 2.2000

Friedman Test: response (C3) versus No. of Days (C2) blocked by No. of penalists (C1)

S = 31.32 DF = 5 P = 0.000
S = 45.43 DF = 5 P = 0.000 (adjusted for ties)

| C2 | N | Est Median | Sum of Ranks |
|---|---|---|---|
| 1 | 15 | 1.9167 | 35.5 |
| 2 | 15 | 2.0833 | 38.5 |
| 3 | 15 | 2.0833 | 41.0 |
| 4 | 15 | 2.4167 | 58.5 |
| 5 | 15 | 2.7500 | 58.0 |
| 6 | 15 | 3.2500 | 83.5 |

Grand median = 2.4167

Same p value (p=0.000) resulted after this step.

# Appendix XVII

**Friedman Test on Overall acceptability**

Friedman Test: responses (C3) versus No. of Days (C2) blocked by No. of penalists (C1)

S = 0.00 DF = 1 P = 1.000

| C2 | N | Est Median | Sum of Ranks |
|---|---|---|---|
| 1 | 15 | 2.0000 | 22.5 |
| 2 | 15 | 2.0000 | 22.5 |

Grand median = 2.0000

Friedman Test: responses (C3) versus No. of Days (C2) blocked by No. of penalists (C1)

S = 0.00 DF = 2 P = 1.000

| C2 | N | Est Median | Sum of Ranks |
|---|---|---|---|
| 1 | 15 | 2.0000 | 30.0 |
| 2 | 15 | 2.0000 | 30.0 |
| 3 | 15 | 2.0000 | 30.0 |

Grand median = 2.0000

Friedman Test: C3 versus C2 blocked by C1

S = 0.48 DF = 3 P = 0.923
S = 6.00 DF = 3 P = 0.112 (adjusted for ties)

| C2 | N | Est Median | Sum of Ranks |
|---|---|---|---|
| 1 | 15 | 2.0000 | 36.5 |
| 2 | 15 | 2.0000 | 36.5 |
| 3 | 15 | 2.0000 | 36.5 |
| 4 | 15 | 2.0000 | 40.5 |

Grand median = 2.0000

Friedman Test: C3 versus C2 blocked by C1

S = 0.80 DF = 4 P = 0.938
S = 6.00 DF = 4 P = 0.199 (adjusted for ties)

| C2 | N | Est Median | Sum of Ranks |
|---|---|---|---|
| 1 | 15 | 2.0000 | 43.0 |
| 2 | 15 | 2.0000 | 43.0 |
| 3 | 15 | 2.0000 | 43.0 |
| 4 | 15 | 2.0000 | 48.0 |
| 5 | 15 | 2.0000 | 48.0 |

Grand median = 2.0000

Friedman Test: responses (C3) versus No. of Days (C2) blocked by No. of penalists (C1)

S = 2.29 DF = 5 P = 0.808
S = 10.00 DF = 5 P = 0.075 (adjusted for ties)

| C2 | N | Est Median | Sum of Ranks |
|---|---|---|---|
| 1 | 15 | 2.0000 | 48.5 |
| 2 | 15 | 2.0000 | 48.5 |
| 3 | 15 | 2.0000 | 48.5 |
| 4 | 15 | 2.0000 | 54.5 |
| 5 | 15 | 2.0000 | 54.5 |
| 6 | 15 | 2.0000 | 60.5 |

Grand median = 2.0000

Friedman Test: responses (C3) versus No. of Days (C2) blocked by No. of penalists (C1)

S = 3.40 DF = 6 P = 0.757
S = 12.00 DF = 6 P = 0.062 (adjusted for ties)

| C2 | N | Est Median | Sum of Ranks |
|---|---|---|---|
| 1 | 15 | 2.0000 | 54.0 |
| 2 | 15 | 2.0000 | 54.0 |
| 3 | 15 | 2.0000 | 54.0 |
| 4 | 15 | 2.0000 | 61.0 |
| 5 | 15 | 2.0000 | 61.0 |
| 6 | 15 | 2.0000 | 68.0 |
| 7 | 15 | 2.0000 | 68.0 |

Grand median = 2.0000

Friedman Test: responses (C3) versus No. of Days (C2) blocked by No. of penalists (C1)

S = 4.27 DF = 7 P = 0.749
S = 13.18 DF = 7 P = 0.068 (adjusted for ties)

| C2 | N | Est Median | Sum of Ranks |
|---|---|---|---|
| 1 | 15 | 2.0000 | 59.5 |
| 2 | 15 | 2.0000 | 59.5 |
| 3 | 15 | 2.0000 | 59.5 |
| 4 | 15 | 2.0000 | 67.5 |
| 5 | 15 | 2.0000 | 67.5 |
| 6 | 15 | 2.0000 | 75.5 |
| 7 | 15 | 2.0000 | 75.5 |
| 8 | 15 | 2.0000 | 75.5 |

Grand median = 2.0000

Friedman Test: responses (C3) versus No. of Days (C2) blocked by No. of penalists (C1)

S = 8.32 DF = 8 P = 0.403
S = 21.06 DF = 8 P = 0.007 (adjusted for ties)

| C2 | N | Est Median | Sum of Ranks |
|---|---|---|---|
| 1 | 15 | 2.0000 | 63.5 |
| 2 | 15 | 2.0000 | 63.5 |
| 3 | 15 | 2.0000 | 63.5 |
| 4 | 15 | 2.0000 | 72.5 |
| 5 | 15 | 2.0000 | 72.5 |
| 6 | 15 | 2.0000 | 81.5 |
| 7 | 15 | 2.0000 | 81.5 |
| 8 | 15 | 2.0000 | 81.5 |
| 9 | 15 | 2.0000 | 95.0 |

Grand median = 2.0000

Friedman Test: responses (C3) versus No. of Days (C2) blocked by No. of penalists (C1)

S = 15.75 DF = 9 P = 0.072
S = 33.69 DF = 9 P = 0.000 (adjusted for ties)

| C2 | N | Est Median | Sum of Ranks |
|---|---|---|---|
| 1 | 15 | 2.0000 | 66.5 |
| 2 | 15 | 2.0000 | 66.5 |
| 3 | 15 | 2.0000 | 66.5 |
| 4 | 15 | 2.0000 | 76.5 |
| 5 | 15 | 2.1000 | 76.5 |
| 6 | 15 | 2.1000 | 86.5 |
| 7 | 15 | 2.0000 | 86.0 |
| 8 | 15 | 2.1000 | 86.0 |
| 9 | 15 | 2.1000 | 100.5 |
| 10 | 15 | 2.6000 | 113.5 |

Grand median = 2.1000
Same p value (p=0.000) resulted after this step.

# Appendix XVIII

**Statistical Analysis of sensory data for determining the shelf life of Flavoured pasteurized milk sachets under transportation condition**

**Friedman Test on mouth feel**

Friedman Test: responses (C3) versus No. of Days (C2) blocked by No. of penalists (C1)

S = 0.00 DF = 1 P = 1.000
S = 0.00 DF = 1 P = 1.000 (adjusted for ties)

| C2 | N | Est Median | Sum of Ranks |
|---|---|---|---|
| 1 | 15 | 2.0000 | 22.5 |
| 2 | 15 | 2.0000 | 22.5 |

Grand median = 2.0000

Friedman Test: responses (C3) versus No. of Days (C2) blocked by No. of penalists (C1)

S = 0.40 DF = 2 P = 0.819
S = 2.00 DF = 2 P = 0.368 (adjusted for ties)

| C2 | N | Est Median | Sum of Ranks |
|---|---|---|---|
| 1 | 15 | 2.0000 | 29.0 |
| 2 | 15 | 2.0000 | 29.0 |
| 3 | 15 | 2.0000 | 32.0 |

Grand median = 2.0000

Friedman Test: responses (C3) versus No. of Days (C2) blocked by No. of penalists (C1)

S = 0.64 DF = 3 P = 0.887
S = 2.18 DF = 3 P = 0.536 (adjusted for ties)

| C2 | N | Est Median | Sum of Ranks |
|---|---|---|---|
| 1 | 15 | 2.0000 | 35.5 |
| 2 | 15 | 2.0000 | 35.5 |
| 3 | 15 | 2.0000 | 39.5 |
| 4 | 15 | 2.0000 | 39.5 |

Grand median = 2.0000

Friedman Test: responses (C3) versus No. of Days (C2) blocked by No. of penalists (C1)

S = 2.80 DF = 4 P = 0.592
S = 7.30 DF = 4 P = 0.121 (adjusted for ties)

| C2 | N | Est Median | Sum of Ranks |
|---|---|---|---|
| 1 | 15 | 2.0000 | 40.5 |
| 2 | 15 | 2.0000 | 40.5 |
| 3 | 15 | 2.0000 | 45.5 |
| 4 | 15 | 2.0000 | 45.5 |
| 5 | 15 | 2.0000 | 53.0 |

Grand median = 2.0000

Friedman Test: responses (C3) versus No. of Days (C2) blocked by No. of penalists (C1)

S = 3.57 DF = 5 P = 0.613
S = 8.56 DF = 5 P = 0.128 (adjusted for ties)

| C2 | N | Est Median | Sum of Ranks |
|---|---|---|---|
| 1 | 15 | 2.0000 | 46.0 |
| 2 | 15 | 2.0000 | 46.0 |
| 3 | 15 | 2.0000 | 52.0 |
| 4 | 15 | 2.0000 | 52.0 |
| 5 | 15 | 2.0000 | 61.0 |
| 6 | 15 | 2.0000 | 58.0 |

Grand median = 2.0000

Friedman Test: responses (C3) versus No. of Days (C2) blocked by No. of penalists (C1)

S = 12.85 DF = 6 P = 0.045
S = 23.36 DF = 6 P = 0.001 (adjusted for ties)

| C2 | N | Est Median | Sum of Ranks |
|---|---|---|---|
| 1 | 15 | 2.0000 | 48.5 |
| 2 | 15 | 2.0000 | 48.5 |
| 3 | 15 | 2.0000 | 55.5 |
| 4 | 15 | 2.0000 | 55.5 |
| 5 | 15 | 2.1429 | 66.0 |
| 6 | 15 | 2.1429 | 62.5 |
| 7 | 15 | 2.7143 | 83.5 |

Grand median = 2.1429

Friedman Test: responses (C3) versus No. of Days (C2) blocked by No. of penalists (C1)

S = 34.74 DF = 7 P = 0.000
S = 51.81 DF = 7 P = 0.000 (adjusted for ties)

| C2 | N | Est Median | Sum of Ranks |
|---|---|---|---|
| 1 | 15 | 2.0000 | 49.0 |
| 2 | 15 | 2.0000 | 49.0 |
| 3 | 15 | 2.0000 | 56.5 |
| 4 | 15 | 2.0000 | 56.5 |
| 5 | 15 | 2.1250 | 68.0 |
| 6 | 15 | 2.1250 | 63.5 |
| 7 | 15 | 2.7500 | 88.0 |
| 8 | 15 | 3.0000 | 109.5 |

Grand median = 2.2500

Same p value (p=0.000) resulted after this step.

# Appendix XIX

**Friedman Test on flavour**

Friedman Test: responses (C3) versus No. of Days (C2) blocked by No. of penalists (C1)

S = 1.67 DF = 1 P = 0.197
S = 3.57 DF = 1 P = 0.059 (adjusted for ties)

| C2 | N | Est Median | Sum of Ranks |
|---|---|---|---|
| 1 | 15 | 2.0000 | 20.0 |
| 2 | 15 | 2.0000 | 25.0 |

Grand median = 2.0000

Friedman Test: responses (C3) versus No. of Days (C2) blocked by No. of penalists (C1)

S = 3.90 DF = 2 P = 0.142
S = 6.88 DF = 2 P = 0.032 (adjusted for ties)

| C2 | N | Est Median | Sum of Ranks |
|---|---|---|---|
| 1 | 15 | 1.6667 | 24.0 |
| 2 | 15 | 2.0000 | 31.5 |
| 3 | 15 | 2.3333 | 34.5 |

Grand median = 2.0000

Friedman Test: responses (C3) versus No. of Days (C2) blocked by No. of penalists (C1)

S = 21.58 DF = 3 P = 0.000
S = 27.67 DF = 3 P = 0.000 (adjusted for ties)

| C2 | N | Est Median | Sum of Ranks |
|---|---|---|---|
| 1 | 15 | 2.0000 | 25.0 |
| 2 | 15 | 2.0000 | 33.5 |
| 3 | 15 | 2.0000 | 35.0 |
| 4 | 15 | 3.0000 | 56.5 |

Grand median = 2.2500

Same p value (p=0.000) resulted after this step.

# Appendix XX

**Friedman Test on Overall acceptability**

Friedman Test: responses (C3) versus No. of Days (C2) blocked by No. of penalists (C1)

S = 0.00 DF = 1 P = 1.000
S = 0.00 DF = 1 P = 1.000 (adjusted for ties)

| C2 | N | Est Median | Sum of Ranks |
|---|---|---|---|
| 1 | 15 | 2.0000 | 22.5 |
| 2 | 15 | 2.0000 | 22.5 |

Grand median = 2.0000

Friedman Test: responses (C3) versus No. of Days (C2) blocked by No. of penalists (C1)

S = 0.40 DF = 2 P = 0.819
S = 2.00 DF = 2 P = 0.368 (adjusted for ties)

| C2 | N | Est Median | Sum of Ranks |
|---|---|---|---|
| 1 | 15 | 2.0000 | 29.0 |
| 2 | 15 | 2.0000 | 29.0 |
| 3 | 15 | 2.0000 | 32.0 |

Grand median = 2.0000

Friedman Test: responses (C3) versus No. of Days (C2) blocked by No. of penalists (C1)

S = 0.64 DF = 3 P = 0.887
S = 2.18 DF = 3 P = 0.536 (adjusted for ties)

| C2 | N | Est Median | Sum of Ranks |
|---|---|---|---|
| 1 | 15 | 2.0000 | 35.5 |
| 2 | 15 | 2.0000 | 35.5 |
| 3 | 15 | 2.0000 | 39.5 |
| 4 | 15 | 2.0000 | 39.5 |

Grand median = 2.0000

Friedman Test: responses (C3) versus No. of Days (C2) blocked by No. of penalists (C1)

S = 2.80 DF = 4 P = 0.592
S = 7.30 DF = 4 P = 0.121 (adjusted for ties)

| C2 | N | Est Median | Sum of Ranks |
|---|---|---|---|
| 1 | 15 | 2.0000 | 40.5 |
| 2 | 15 | 2.0000 | 40.5 |
| 3 | 15 | 2.0000 | 45.5 |
| 4 | 15 | 2.0000 | 45.5 |
| 5 | 15 | 2.0000 | 53.0 |

Grand median = 2.0000

Friedman Test: responses (C3) versus No. of Days (C2) blocked by No. of penalists (C1)

S = 3.57 DF = 5 P = 0.613
S = 8.56 DF = 5 P = 0.128 (adjusted for ties)

| C2 | N | Est Median | Sum of Ranks |
|---|---|---|---|
| 1 | 15 | 2.0000 | 46.0 |
| 2 | 15 | 2.0000 | 46.0 |
| 3 | 15 | 2.0000 | 52.0 |
| 4 | 15 | 2.0000 | 52.0 |
| 5 | 15 | 2.0000 | 61.0 |
| 6 | 15 | 2.0000 | 58.0 |

Grand median = 2.0000

Friedman Test: responses (C3) versus No. of Days (C2) blocked by No. of penalists (C1)

S = 12.85 DF = 6 P = 0.045
S = 23.36 DF = 6 P = 0.001 (adjusted for ties)

| C2 | N | Est Median | Sum of Ranks |
|---|---|---|---|
| 1 | 15 | 2.0000 | 48.5 |
| 2 | 15 | 2.0000 | 48.5 |
| 3 | 15 | 2.0000 | 55.5 |
| 4 | 15 | 2.0000 | 55.5 |
| 5 | 15 | 2.1429 | 66.0 |
| 6 | 15 | 2.1429 | 62.5 |
| 7 | 15 | 2.7143 | 83.5 |

Grand median = 2.1429

Friedman Test: responses (C3) versus No. of Days (C2) blocked by No. of penalists (C1)

S = 34.74 DF = 7 P = 0.000
S = 51.81 DF = 7 P = 0.000 (adjusted for ties)

| C2 | N | Est Median | Sum of Ranks |
|---|---|---|---|
| 1 | 15 | 2.0000 | 49.0 |
| 2 | 15 | 2.0000 | 49.0 |
| 3 | 15 | 2.0000 | 56.5 |
| 4 | 15 | 2.0000 | 56.5 |
| 5 | 15 | 2.1250 | 68.0 |
| 6 | 15 | 2.1250 | 63.5 |
| 7 | 15 | 2.7500 | 88.0 |
| 8 | 15 | 3.0000 | 109.5 |

Grand median = 2.2500

Same p value (p=0.000) resulted after this step.

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Mrs.T.N.NEIGHSOOREI
(MSSc.PGD.ASLA,BA)
Liprarian
Sabaragamuwa University of Sri Lanka
P.O.Box 02 Belihuloya,Sri Lanka
Tele.: 094 45 2280045
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