EFFECT OF MILK PRESERVATION BY ACTIVATION OF LACTOPEROXIDASE SYSTEM ON THE QUALITY AND SHELF LIFE OF PASTEURISED AND FERMENTED MILK PRODUCTS

By

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ABSTRACT

The study was conducted to investigate the effect of milk preservation by the use of activated Lactoperoxidase system (LPS) on fresh, pasteurised and fermented milk quality. Fieldwork was done at Mwakaleli, Mbeya region, while the laboratory work was conducted at Sokoine University of Agriculture. After collection, milk LPS was activated by addition of sufficient amount of thiocyanate and hydrogen peroxide followed by milk shelf life determination under different storage conditions. The best LPS holding time before pasteurisation was found to be 3 hours followed by 6 hours, whereas the holding time above 6 hours performed poorly. The effect of LPS activation on fermented milk quality was also investigated. No significant ($P>0.05$) difference in pH between treated and untreated milk was observed on the milk that had a holding time of less than 9 hours, above this period milk recorded significantly ($P<0.05$) low pH. Likewise was the pH of the yoghurt with more or less similar trend. The analysed natural Thiocyanate content of the test milk was within normal range (4.09±1.1 ppm). This indicates the test milk could accommodate recommended external addition of thiocyanate for LPS activation without raising SCN^- content above the physiological limits. The LPS carry over effect on milk inocula could not be detected due to the LPS inactivation by pasteurisation (80^0 C/1min.). This explains the absence of any noticeable physical, chemical or organoleptic abnormality on milk and milk products final quality. In the field, activated LPS prolonged morning raw milk shelf life for more than 15 hours as compared to less than 12 hours registered by the untreated milk, whereas throughout the experiment under all storage conditions LPS treated pasteurised milk performed significantly ($P<0.001$) better than the control.

In order to solve the problem of raw milk short shelf life in the remote rural tropical environment a cheap and convenient charcoal cooler box was designed, constructed and tested with encouraging results in the storage of raw milk. The cooler performance was 8.94^{0} C and 5.45^{0} C below the environmental maximum and minimum temperatures respectively. When compared to the room temperature storage conditions, the cooler performed significantly ($P<0.001$) better than the room temperature. Therefore, under any tropical environmental condition accompanied with the absence refrigeration or cold room facilities, the application of both activated LPS and charcoal cooler box for raw milk storage is strongly recommended for the fresh milk shelf life extension to enable the marketing functions to operate.

DECLARATION

I, James Joseph Mwaikambo, do hereby declare to the Senate of Sokoine University of Agriculture that this dissertation is my original work and that it has never been submitted for a degree in any other University.

Signature.....

Date.....18th June 2021

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DEDICATION

This dissertation is dedicated to my beloved wife Rose, my son Lusako and my parents

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LIST OF ABBREVIATIONS

%	Percent
^{0}C	Degrees centigrade
APHA	American Public Health Association
C	Carbon
Ca	Calcium
cc	Cubic centimetres
CFC	Chlorofluorocarbons
cfu	Colony forming unit
CO_2	Carbon dioxide
cm	Centimetres
COB	Clot on boiling test
DNA	Deoxyribonucleic acid
FAO	Food and Agriculture Organisation
Fe	Iron
g	Gramme
H_2O_2	Hydrogen peroxide
HCFC`s	Hydro-Chlorofluorocarbons
HOSCN	Hypothiocynous acid
Hrs.	Hours
HTST	High temperature short time
IDF	International Dairy Federation
IgA	ImmunoglobulinA
ILRI	International Livestock Research Institute
K	Potassium
Kg	Kilogramme
Km	Kilometres
L	Litres
LA	Lactic acid

LP	Lactoperoxidase
LPS	Lactoperoxidase system
LSM	Least square mean
LTLT	Low temperature long time
m	Metres
m.a.s.l.	Metres above sea level
MALD	Ministry of Agriculture and Livestock Development
mg	Milligram
Mg	Magnesium
MIPS1	Milk peroxidase 1
MIPS2	Milk peroxidase 2
ml	Millilitres
mm	Millimetres
MOAC	Ministry of Agriculture and Co-operatives
N_2	Nitrogen
Na	Sodium
NAGase	N-Acetyl-β-D-glucosaminidase
NaOH	Sodium hydroxide
NaSCN	Sodium thiocyanate
nm	Nmoles
NORAD	Norwegian Agency for Development
O_2	Oxygen
OSCN	Thiocyanate oxide
P	Phosphorous
pH	Hydrogen ion concentration
ppm	Parts per million
RNA	Ribonucleic acid
S	Sulphur
SCN^-	Thiocyanate
se	Standard error
-SH	Sulfurhydril group

spp	Species
SUA	Sokoine University of Agriculture
TBS	Tanzania bureau of standards
TCA	Tri-chloroacetic acid
TPC	Total bacterial count
TZS	Tanzania Standard
US$	United States of America currency
UAC	Uyole Agricultural Centre
UHT	Ultra high temperature
VRB	Violet Red Bile Agar
WAR	World Animal Review

CHAPTER I

1.0 INTRODUCTION

Many tropical countries that are in the early stages of developing their dairy industry, the production and collection of milk in rural areas present a number of technical and organisation problems (FAO, 1972). In these countries, milk spoils easily during storage and transportation due to hot humid climate throughout the year and low hygiene standard. Cooling milk appears to be the most practical solution against spoilage, but usually facilities are not readily available. The challenging problems affecting the remote rural dairy producers are both the transportation of milk to the processing centre and appropriate milk preservation technology that enables milk to remain whole-some up to when it reaches the final consumer in towns and cities (FAO, 1988, Kurwijila, 1984). Usually milk production quantities per family are small and very much scattered in such a way that it is impractical or too expensive to refrigerate milk against spoilage. This problem is aggravated by the lack of appropriate facilities, management and energy to operate such systems (Van den Berg, 1988). Modern liquid milk processing technology cannot be assimilated by farmers because of technical, economical and practical reasons. In order to leap forward, an appropriate technology needs to be devised; this should be cheap, simple and should considerably increase milk shelf life at ambient temperature. (Kurwijila, 1990).

In West Africa sub-region alone the World Bank estimates that, while in transit, 5million litres of milk are thrown away per year, because farmers are unable to process fresh milk

into other alternative dairy products (FAO, 1999). It has been estimated that about 21 million tons of milk equivalent to US$ 5,000 million representing almost 60% of the milk imports value by developing countries is spoiled or under valued due to lack of appropriate milk collection systems (Claesson, 1999a, Claesson, 1999b).

Despite the large cattle population in the country, estimated at 15.6 million by Tanzania MOAC (1996), Tanzania has a large and growing deficit in milk and milk products to warrant substantial dairy products import. Studies show that local milk production is increasing as a result of efforts by small-scale dairy producers whose income depend very much on the sales of dairy products (Kurwijila, *et al.*, 1996).

Though most of the local milk production originates from local Zebu cattle, with some potential for increasing milk production but so far little investments have been committed into this sector (Kurwijila, *et al.*, 1996). All the same the sector is a good source of animal protein, income and provides employment. The emphasis has always been on improvement and increase in local milk production without paying attention on the appropriate surplus milk handling and disposal system. Therefore for sustainable local dairy development it is imperative that all efforts committed on increased dairy production should be accompanied with sound dairy products marketing strategies, which is facilitated by the development of marketing infrastructures (Ashimogo *et al.*, 1990; Kurwijila, *et al.*, 1996, Kurwijila (1984)).

In the past, milk processing and marketing used to be done by centralised milk-

processing plants whose ability to collect and process milk has been declining (Kurwijila, *et al.*, 1996; Kurwijila, 1995, Assey, 1988). Due to this problem, the rural based small-scale dairy farmers do not have proper milk marketing outlets. The situation is worse during the rainy season when roads become impassable, cooling systems break down and during electricity failure. These circumstances together with high milk perishability and long distance force farmers to accept low milk price. The above-mentioned problems result into two situations in the dairy industry: the presence of surplus milk in the remote rural areas and a deficit of milk in the urban centres with consumers ready to pay high price per unit of the commodity (Kurwijila, *et al.*, 1996, Urio, 1984; Mchau, 1985; Kurwijila, 1987; Lohay, 1988). The application of refrigeration technology to retard/control bacterial growth in milk is not feasible for most remote rural areas due to technical and economic reasons (IDF, 1988). These areas cannot market their surplus milk to the urban centres due to distance, costs and poor transport infrastructure, as a result milk held at room temperature spoils due to long transit time to reach the market. Such marketing problems frustrate and limit rural small-scale dairy sector development and expansion. Therefore an increase in milk shelf life seems to be an appropriate solution to this problem, by allowing enough time for the marketing functions to operate.

A serious need therefore exist for alternative cheap technology other than refrigeration that can considerably increase raw milk shelf life. Among the chemical methods of milk preservation, indigenous antimicrobial system in milk activated Lactoperoxidase system (LPS) is advocated as appropriate for adoption in these areas (Claesson, 1999a,

Claesson, 1999b). Activated LPS can control bacterial spoilage of raw milk during collection, storage and transportation to dairy processing plants and distant markets. This LPS technique has bacteriostatic effect on raw milk that lasts for about 8-26 hours depending on the storage temperature *i.e.* 30^{O}C-15^{O}C respectively (IDF, 1988, FAO, 1999). In addition, both saprophytic and pathogenic milk micro-flora become more heat sensitive after the activation of LPS. The method can increase milk shelf life, quality and product value enabling rural producers to transport the milk to distant locations without having to invest in expensive cooling or refrigeration facilities.

It is imperative therefore for developing countries to take a lead on researching, developing and improving LPS technology to suit our own environment. This innovation has potential to increase the safety, shelf life, quality and product value of pasteurised milk and subsequent products (IDF, 1988, FAO, 1999). Activation of LPS in milk aims at the increase in milk off-take, rural family income and milk shelf life during protracted delivery to distant market and cooling or processing plants. The purpose of this study therefore was to investigate the effect of activated LPS on the quality and shelf life of pasteurised and fermented milk, with the following specific objectives;

i. To evaluate the effect of activated LPS on the pasteurised milk shelf life.

ii. To study the effect of activated LPS on the quality of fermented/cultured milk

iii. To assess the effect of activated LPS on the fresh milk quality under field condition.

iv. To assess the level of natural thiocyanate in milk (SCN^{-})

v. To assess performance of the charcoal cooler box on LPS

CHAPTER II

2.0 LITERATURE REVIEW

2.1. Milk spoilage

Milk spoilage is any change that renders milk unfit for human consumption. These changes may be caused by various factors including contamination by microbes, infestation by insects or degradation by indigenous enzymes (those present naturally in milk). In addition, physical and chemical changes such as oxidation of certain constituents of milk may promote spoilage. Milk begins to spoil soon after milking. The chemical reactions catalysed by enzymes, result in milk quality degradation e.g. development of off flavours, degradation of texture and loss of the nutrients. The typical micro-organisms that cause milk spoilage include bacteria: *Lactobacillus*, yeast: *Saccharomyces* and moulds: *Rhizopus* (Board, 1983; FAO, 1972. Vasavada and Cousin, 1993)

2.1.1 Milk hygiene

In any milk production programmes, it is a commercial necessity for the milk to reach the milk plant in a condition that is suitable for processing/manufacturing. Due to this, milk must attain a minimum standard of hygienic quality, which means milk must not contain excessive number of microbes and must not have undergone any marked souring or other changes (FAO, 1972). The hygienic quality of the milk at point of production is important for the milk final quality. Satisfactory hyperemic quality depends on minimising the risk of microbial contamination by ensuring thorough cleaning at all

times during milking or subsequent handling. In fact, milking should be done under the right environment, with clean healthy animals, equipment and milk handlers. Cooling milk cannot improve the initial hygienic quality of milk but can prolong its life by many hours through retarding microbial growth. It has been estimated that most of the economic benefits are obtained by cooling to 15^0C with clean milk or 10^0C with dirty milk. The added advantage of refrigeration to 4^0C is relatively small (FAO, 1972).

2.1.2 Microbial contamination

Bacteria and fungi (Yeast and Mould) are the principal causative microorganisms of milk spoilage and food borne illness. Microbes may contaminate milk at any time during milking, storage, processing, distribution, and handling or during preparation. Milk is one of the best media for bacterial growth hence its vulnerability to pathogens infection. The primary sources of contamination are soil, water, air, animal feeds, animal hides, animal intestine, plant surfaces, sewage and food processing machinery or utensils. Milk is also an excellent culture medium, which at its natural temperature 37^0C provide the optimal condition for microbial population growth, hence the need for protection and prevention against outside contamination (Rosenthal, 1991). Sanitation is a pre-requisite for milk handling. After milking, the milk should quickly be chilled to temperatures below 10^0C. This is important for inhibition of bacterial activity and for the preservation of milk quality. Commercially production of milk must always be done under conditions that will prevent contamination.

Therefore, in order to achieve high milk bacterial quality at farm level, it is important for

farmers to be aware of the source of contamination and understand how to control them. This includes mastitic cow, dirty teats (may contribute up to 100,000cfu/ml.) and contaminated equipments. Others are air, dust, water and poor handling (Harding, 1995).

2.1.2.1 Bacteria

Bacteria are unicellular organisms that have simple internal structures compared to cells of other organisms. The increase in number in a population is referred to as bacterial growth. This growth is a result of the cells binary fission. Under optimal growth bacteria may divide after every 20 minutes. Thus a single cell can produce up to 70 billion cells in 12 hours (Board, 1983). The factors that influence the growth of bacteria include nutrient availability, moisture, pH, oxygen levels and the presence or absence of the inhibitors (*e.g.* Antibiotics). The nutrient requirements of most bacteria are the chemical element such as C, O_2, N_2, P, S, Mg, K, Ca, Na, and Fe. Bacteria obtain these elements by utilising gases in the atmosphere and by metabolising certain food constituents such as carbohydrate and protein (Rosenthal, 1991).

The total bacteria count of cooled milk produced under good hygienic condition should be lower than 10,000 cfu/ml., over 3,000,000cfu/ml. leads to significant degradation of the fat, protein or lactose, forming off flavours and would reduce significantly the flexibility the processor has with respect to storage and use of milk (Harding, 1995). High numbers of bacteria produces enough enzymes to cause flavour defect. Though pasteurisation does eliminate the psychrophils but the enzymes produced are not affected. This problem is common on UHT milk, which is stored at ambient

temperatures for several months. The most common bacteria are the gram-negative rods (*Pseudomonas spp.*). Mesophilic bacteria are typified by Lactobacillus, which attack milk lactose sugars and convert it to lactic acid. Sporeforming bacteria (*Bacillus spp*)- Psychrotrophs do produce spores that survive pasteurisation. They may later grow and cause spoilage in pasteurised milk or finished products (Harding, 1995).

2.1.2.2 Temperature, pH and water activity

Temperature plays a significant role in the control of the growth of bacteria in milk. Bacteria may be classified into three groups based on their temperature requirement for optimal growth: Thermophiles (55^0C-65^0C), Mesophiles (20^0C-45^0C), and Psychrotrophs (10^0C-20^0C). Most bacteria grow in neutral environment (pH 7). (Harding, 1995, Henderson, 1991 and Rosenthal, 1991)

Bacteria also require a certain amount of available water for growth. The availability of water is expressed as water activity, which is defined as the ratio of water vapour in the food to that of pure water at specific temperature. The water activity of any food product is between 0 and 1, with 0 representing the absence of water and 1 representing pure water. Most bacteria cease growth at water activity below 0.91 though some hallophilic bacteria can grow in food with water activity lower than 0.75. Growth may be controlled by lowering the water activity either by addition of solutes such as sugar, glycerol and salt or by removing water through dehydration (Robinson, 1981; Harding, 1995).

Generally under tropical environment at room temperature, fresh milk shelf life lies

betwcen 4 and 8 hours and sometimes up to 12 hours depending on the initial quality of the milk and the prevailing environmental temperature. This shelf life of up to 12 hours for raw fresh milk can be achieved in the tropics and sub-tropics under conditions of low temperatures in the high altitudes. (Van den Berg 1988)

Usually the hot humid region extends approximately 5^0 North and 20^0 South of the equator. Thermal characteristic of this region is one of the factors that limit fresh milk shelf life. Stress caused by hot environment, solar radiation and high humidity accelerate bacterial activity since milk is critically affected by thermal stress.

In addition to the above reasoning, global warming is another nature's catastrophe posing a significant threat on the eco-system. Global warming is brought about by environmental pollution from industrial wastes which results in continued increase in atmospheric CO_2 content. The increase in this atmospheric CO_2 content result in depletion of the atmospheric ozone layer with a subsequent creation of holes that allows ultraviolet rays penetration resulting in global warming, this is referred to as green house effect (Kurwijila, 1991).

2.1.2.3.The oxygen requirement

The oxygen requirements for optimal growth varies considerably for different bacteria, some require free oxygen supply (obligate aerobes) and others are poisoned by free oxygen (obligate anaerobes). Facultative anaerobes can grow in both conditions. In

addition to oxygen concentration, the oxygen reduction potential of the growth medium influences bacteria growth. The oxygen reduction potential is the relative measure of oxidising or reducing capacity of the growth medium. When bacteria contaminate food substrate it takes some time before they start growth. Lag phase is the period when bacteria are adjusting to the environment. Following the lag phase is the logarithmic phase in which population growth is accelerating in logarithmic fashion. As the population grows, the bacteria consume the available nutrients and produces waste products. When the nutrient supply is depleted, the growth rate enters the stationary phase in which the number of the viable cells remain constant due to the generation of new cells being equal to those dying off. (Rosenthal, 1991; Harding, 1995).

2.2. Milk preservation

Milk preservation is any method by which milk is kept from spoilage after milking. Preservation includes: drying, refrigeration, fermentation (reduction of pH), canning, pasteurisation, freezing, irradiation, and nutrients removal. Others are, control of oxygen and carbon dioxide concentration and addition of chemicals. Milk preservation for increased shelf life can be achieved through chemical and mechanical means. Natural preservation is through natural immune system inherent in milk, mechanically by the use of cooling systems *e.g.* Refrigerators or by use of heat (pasteurisation) and also the preservation can be attained through the use of chemical preservatives e.g. H_2O_2, CO_2, etc. (Vasavada and Cousin, 1993, Marsden 1972). Addition of CO_2 has been shown to lengthen the storage period of milk held at lower temperatures by three days since the bicarbonate ion produced is toxic to psychrotrophic bacteria. The CO_2 can be removed

under vacuum prior to processing.

In countries where cooling is difficult, glucose oxidase or xanthine oxidase may be added to activate LPS of milk and hence generate H_2O_2, which is bacteriostatic (Harding, 1995, Marsden, 1972). An ideal preservative for milk is any substance, which enables the physical properties and chemical composition of milk to remain unaffected by microbes or other spoilage, so that the milk retains its original wholesomeness and nutritional value. Milk preservatives must satisfy the following requirements.

- Must not react with any of the important constituent of the milk
- It must be easily eliminated before milk is used for human consumption or for industrial processing
- There should be no changes in taste or smell
- No toxic effect should remain in the milk after its elimination (FAO, 1972).

Among the largest list of preservatives suggested for use in milk *e.g.* antibiotics, bromine compounds of acetic acid, formaldehyde, quaternary ammonium compounds, mercaptopropionic acid, plant extracts, peroxides and oxygen under pressure. The least objectionable appears to be H_2O_2 and activated LPS. Most of the others entail a health hazard to consumer and few give satisfactory results (FAO, 1972, Marsden, 1972).

2.2.1 Natural preservation

Milk contains several non-immunological proteins that have antimicrobial properties. These non-immunological proteins form natural antimicrobial systems in milk. The

four most common antimicrobial proteins (non-specific factors) that have been studied are Lactoperoxidase, Lactoferrin, lysozyme and xanthine oxidase, plus a few phagocytes and possibly N-acetyl-ß-D-glucosaminidase. These proteins are involved in complex systems that cause microbes to become inactivated. Lactoperoxidase forms antimicrobial system with hydrogen peroxide and thiocyanate. Lactoferrin is an iron binding protein that binds both Fe^{3+} and carbonate ammonia. Lysozyme is a protein that can have direct or indirect enzymatic or nonenzymic effect on microorganisms. Xanthine oxidase is involved in a generation of H_2O_2, which can either be used for Lactoperoxidase system or as a direct antimicrobial agent (Reiter, 1978, Board, 1983; Vasavada and Cousin, 1993, Karen, *et al.*, 1996).

The germicidal action of natural milk is sporadically claimed as insurance for safety. This may also be achieved due to the presence in milk of agglutinins and precipitins, which cause the bacteria cells to clump together, bacteriolysins, which causes disruption of cells, and opsonins, which make bacteria less resistant. All the same, one should not use this argument in favour for consumption of non-pasteurised milk (Rosenthal 1991, Karen *et al.*, 1996).

Milks of different species contain different amounts of the various antimicrobial factors. Cow milk has high Lactoperoxidase, but low lactoferrin and lysozyme, while human breast milk has high lactoferrin and lysozyme, but low Lactoperoxidase. The ability to alter the activity of these anti-microbial factors in cows milk could have an impact on shelf life of raw milk and development of additional health and functional

foods based upon these factors (Karen *et al.*, 1996).

2.2.1.1 Lactoferrin

Lactoferrin is an iron binding glycoprotein that has antibacterial activity in milk. Lactoferrin is present in large quantities in mammalian secretions such as milk, tears, saliva, and seminal fluid, as well as in some white blood cells. Bovine milk contains 0.02-0.35 mg/ml of lactoferrin while colostrum, has 0.5 to 1.0 grams/litre. The highest lactoferrin concentration derived from cow mammary secretions range from 50 to 100 grams/litre. In human milk and colostrum, the reported concentrations of lactoferrin are 2 to 4 grams/litre and 6 to 8 grams/litre, respectively. In its natural state, lactoferrin is only partly saturated with iron (5 to 30%) (Lawrence 1970, Reiter, 1978, Robinson, 1981, IDF, 1988, Vasavada and Cousin, 1993).

Lactoferrin is a glycoprotein with a molecular weight of 76500 that has two metal binding sites that bind ferric ions and bicarbonate ions. Lactoferrin inhibits only bacteria with high iron requirement, such as Coliform but has no effect to those requiring low amount of iron. The bacteriostatic effect of lactoferrin is temporary because some Gram-negative bacteria can adapt to low iron and synthesize iron chelaters. Lactoferrin works by damaging the outer membrane of Gram-negative bacteria resulting in permeability problems (Robinson 1981, Vasavada and Cousin, 1993).

Lactoferrin has many proposed biological functions, including antibacterial/ anti inflammatory activities, defence against gastro-intestinal infections, participation in

local secretory immune systems in synergy with some immunoglobulins and other protective proteins, provision of an iron-binding antioxidant protein in tissues, and possibly promotion of growth of animal cells such as lymphocytes and intestinal cells. A role for milk lactoferrin in iron absorption by the intestine has long been postulated, but remains unproven.

Most microorganisms need iron for growth and lactoferrin has the potential to inhibit the growth of bacteria, and even kill them by depriving them of iron. The effectiveness of the antibacterial activity of lactoferrin depends on the iron requirement of the organism, the availability of exogenous iron, and the concentration and degree of iron saturation of lactoferrin. It has been shown that 'natural' lactoferrin is bacteriostatic against a wide range of microorganisms, including gram-negative bacteria with high iron requirements (coliforms, which are major mastitis pathogens), and also against some gram-positive organisms such as *Staphylococcus aureus* (also a major mastitis pathogen), *Bacillus species*, and *Listeria monocytogenes*. Lactic acid bacteria in the stomach and intestine have low iron requirements and are generally not affected. There is also evidence that on certain Streptococcal mutants and *Vibrio cholerae*, lactoferrin can exert a direct, bactericidal effect that is independent of iron-deprivation (Karen *et al.*, 1996).

2.2.1.2 Lysozyme

Lysozyme is an enzyme present in the milk of some species, especially human milk. There are two types of lysozyme. One type is found in the hen egg-white and is

known as chicken-type or c-lysozyme. The other type is found in the goose egg-white and is known as goose type or g-lysozyme. Human and equine lysozymes are considered to be the c-lysozyme type. However, cow milk may contain both c- and g-lysozymes because both types are found in various other body fluids and in the stomach tissue of the cow. Lysozyme kills bacteria by disrupting the glycosidic bond between the two components of peptidoglycan, a constituent of the bacterial cell wall. Lysozyme can act in concert with IgA, Lactoperoxidase and ascorbate to lyse bacteria. Lysozyme is a small basic protein that has molecular weight of 15,000. Bovine milk contains 13 μg of lysozyme/100 ml. Lysozyme has three functions:

- A direct enzymatic effect that degrades the bacterial cells peptidoglycans and polysaccharides of Gram-negative bacteria.
- An indirect enzymatic effect seen when peptidoglycans cleave to yield muramyl dipeptide and an immunostimulating effect is produced.
- The positively charged lysozyme can neutralize the negatively charged groups on the bacteria cell membrane.

Lysozyme has found its greatest use in inactivating the vegetative cells and germinating spores of Bacillus and Clostridium. Lysozyme hydrolyzes the peptidoglycans in Clostridium and other Gram-positive bacteria (Vasavada and Cousin, 1993).

Lysozyme activity is nearly undetectable in cow's milk, but very high in human's milk (0.12 grams/litre). The concentration of lysozyme is highest in human colostrum and pre-colostral milk. The limited lysozyme activity in cow's milk increases due to

mastitis and high somatic cell counts. Heating cow milk at 75^0C for 15 minutes destroys 25 percent of the activity of this enzyme. However, human milk lysozyme is more heat stable than cow milk lysozyme

Lysozyme possesses antibacterial activity against a number of bacteria. This enzyme usually functions in association with lactoferrin or immunoglobulin A. Lysozyme is effective against *Escherichia coli* in concert with immunoglobulin A. It causes lysis of some species of salmonellae in association with ascorbate and peroxide, both of which are present in low concentrations in milk. Microwave irradiation can decrease the activity of lysozyme against *Escherichia coli*. In addition, lysozyme can limit the migration of neutrophils into damaged tissue and might function as an anti-inflammatory agent (Karen *et al.*, 1996).

2.2.1.3 Xanthine oxidase

This is an enzyme that is associated with the fat globule membrane in bovine milk. This enzyme contains iron and molybdenum and catabolises purines producing uric acid, superoxide and H_2O_2; however in milk there are few free purines and the xanthine oxidase reacts with acetyldehydes produced by lactic acid bacteria to produce the hydrogen peroxide. The H_2O_2 is bactericidal by itself or can be used to activate the LPS. Xanthine oxidase can act synergistically with Lactoperoxidase and -SCN to complete the LPS. The interaction between lysozyme and lactoferrin, xanthine oxidase and Lactoperoxidase can further enhance the antibacterial nature of these systems (Vasavada and Cousin, 1993).

2.2.1.4. N-Acetyl-ß-D-Glucosaminedase

N-Acetyl-ß-D-glucosaminedase (NAGase) is an enzyme whose activity has been implicated as an indicator of tissue damage during mastitis. It is a lysosomal enzyme that is secreted in large quantities in the mammary gland during involution and inflammation. The NAGase enzyme has also been found in other bovine secretions, such as uterine fluids. The specific function of NAGase in the mammary gland is not known, however, recent research has suggested that NAGase may exhibit some antimicrobial activity.

During lactation, cow milk normally has low NAGase activity. Similarly, NAGase is low in mammary secretions in the early dry period, coinciding with the period of highest incidence of new intramammary infection. By the mid-dry period, however, NAGase activity is at its highest in mammary secretions, concurrent with the lowest incidence of new intramammary infection. Therefore, the high levels of NAGase activity, along with elevated lactoferrin concentrations, in the mammary gland during the mid-dry period may contribute to increased antibacterial activity found in mammary secretions at that time.

There is a relationship between the presence of pathogens in the udder and NAGase levels in milk. Marked increases in NAGase activity resulting from the presence of major mastitis pathogens have been observed. Since NAGase has been found in uterine fluids, it has been suggested that NAGase may have a role in the bactericidal function of the uterus as well. NAGase does inhibit microbial activity of *Actinomyces*

pyrogenes, *Staphylococcus aureus*, *Streptococcus agalactiae*, and *Pseudomonas aeroginosa* while the *Escherichia coli* and *Enterobacter aerogenes* are not inhibited (Vasavada and Cousin, 1993).

2.2.1.5 Milk cells

Generally the leukocytes in normal milk (no mastitis) are macrophages. These cells probably retain some of their phagocytic abilities in milk. However, a role of these cells in milk keeping quality has not been completely described.

2.2.1.6 The Lactoperoxidase antibacterial system (LPS)

All life forms are involved in the struggle for existence. Over the millennia natural selection has ensured all living organisms have developed intricate mechanisms against major threats from other organisms *e.g.* struggle against microbial proliferation i.e. the antimicrobial defence mechanisms. Mammalian system depend on both antibodies and their co-factors, together with a number of non specific systems plus specific system that rely on enzymes or metal binding proteins. In the area of non-specific mammalian anti-microbial mechanisms, the principle contributors are the Peroxidase enzymes. The most widely studied examples have been Lactoperoxidase from bovine milk and human salivary Peroxidase as well as gastric juice of mammals. The system is essentially latent and is switched on by the H_2O_2 usually provided by microbial growth (Vasavada and Cousin, 1993, Calzyme, 1997).

However, Lactoperoxidase has no antibacterial effect when stands on its own but is involved in catalyzing the oxidation of thiocyanate by hydrogen peroxide to produce an antibacterial compound. The Lactoperoxidase system (LPS) is made active after the presence of three important components LP, H_2O_2 and SCN^-. A balanced ratio of H_2O_2 to SCN^- ought to be achieved for the optimal activity of the LP system (Henderson, 1971, Korhonen, 1980; Board 1983; Vasavada and Cousin, 1993; FAO, 1999, Nicholette *et al.*, 1999). Thiocyanate in milk is derived from blood in which its concentration is about 10 times higher than in milk. The SCN^- content is highly dependent on feeding regime as many plants contain SCN^- precursors (Korhonen, 1980). Lactoperoxidase is the most abundant enzyme in cow's milk its concentration varies over a wide range from almost nil up to about 50 mg/l. This concentration is affected by breed, age, lactation stage, nutrition and health condition (Korhonen, 1980). LPS (LP/Thiocyanate/ Hydrogen peroxide) is a natural indigenous antibacterial system in milk to safeguard the new-born. It is active during the first few hours (2-3 hours) after milking. In bovine colostrum, the Lactoperoxidase content is very low, but increases rapidly after 4 to 5 days postpartum. The level of Lactoperoxidase activity in human milk is about 20 fold lower than that in bovine milk (Karen *et al.*, 1996). The enzyme is present in bovine and buffalo in relatively high concentration. Lactoperoxidase enzyme catalyses the reaction;

$$H_2O_2 + SCN^- \rightarrow OSCN^- + H_2O$$

Both H_2O_2 and SCN^- are essential for antimicrobial activity. Lactoperoxidase is

present on bovine milk, in the whey protein at concentration ranging from 10-30 mg/ml depending on the cow and its breed (Hui, 1993). Lactoperoxidase is a basic glycoprotein with a molecular weight of about 77,000 and iron (Fe^{3+}) heme group. It has its highest activity at pH 4 to 7, which would be in the range of fresh milk (Vasavada and Cousin, 1993). There is little H_2O_2 in milk but can be produced by lactic acid bacteria that contaminate milk. However if free oxygen is present in the milk, H_2O_2 can be produced by reactions with xanthine oxidase, copper sulfhydryl oxidase and ascorbic acid. Because H_2O_2 is not very stable it can be reduced by catalase or bound to enzymes, such as Lactoperoxidase.

Thiocyanate is present in bovine milk in up to 15 ppm especially in milk with high somatic cell count. SCN^- is common anion that is present in many animal tissues (mammary glands, salivary glands, nasal secretions, stomach, kidneys *etc.*) and animal secretions (cerebral fluid, saliva, lymph fluid plasma, semen, cervical/uterus mucus, tears and gastric juice *etc.*) (Björck, 1990). The type of feed especially clove and feed-containing glucosides affects the concentration of SCN^-. Saliva is rich in thiocyanate, as levels of between 50-300 ppm have been documented under it, likewise is the gastric juice with 40-50 ppm. (Lawrence, 1970, IDF, 1988). The health of the cows do affect milk SCN^- level because cows with disease such as mastitis contain more leukocytes and hence obtain increased thiocyanate concentration from the blood.

The mode of bacterial inhibition by Lactoperoxidase involves a change of the cytoplasmic membrane because $OSCN^-$ binds to the free -SH groups of key enzymes

causing the pH gradient to drop and potassium and amino acids to leak from the cell. This prevents the uptake of carbohydrates, amino acids and other nutrients because their transport mechanisms are inhibited. Further activities of the cell involved in protein DNA and RNA synthesis are disrupted. Gram-negative bacteria are more readily killed and lysed by the LPS than Gram positive. This could be due to the difference in cell composition and thickness. Some Gram-positive Streptococci are resistant to the hypothiocyanate (Vasavada and Cousin, 1993).

The Lactoperoxidase system occurs naturally in several environments. In calves, the intestinal flora is colonized by lactobacilli that produces hydrogen peroxide, which activates the LPS. This can prevent undesirable bacteria such as *E. coil* from establishing in the intestinal mucosa. The LPS is also active in the mouth of the humans, this helps prevent acid production in dental plaque that reduces dental caries. LPS inhibits many bacteria that cause masitis in cow. LPS has no toxic effect on the host cell *i.e.* Mammalian cells as well as HeLa cells and Chinese hamster ovary cells (Vasavada and Cousin, 1993).

Practical application of LPS is advocated in the preservation of both refrigerated and non-refrigerated milk by destroying the bacterial pathogens to extend the shelf life of milk and cultured dairy products. So far LPS has been used successfully to extend the shelf life of refrigerated raw milk. For instance it has been possible to slow down the growth of *Pseudomonas fluorescens* by 200 hours at 4^0C and 20 hours at 30^0C by activation of the LPS. The principle holds true for a mixed population of common

psychotropic bacteria. At 4^0C it took longer than 6 days for the multiplication of this mixed flora after the activation of LPS (Vasavada and Cousin, 1993, Robinson 1981). The system keeps psychotropic and coliform counts constant or decreased in the milk. The safety of the milk in relation to the food borne pathogens can be increased by the use of LPS in combination with heat and other preservation methods.

2.2.2 Milk preservation by activation of the Lactoperoxidase system

The Lactoperoxidase concentration required is between 1-2 mg/L but much less than this is usually found in milk. The main factor limiting the LPS system in milk is the quantities of SCN^- and H_2O_2 (Björck, 1978). The antibacterial effect of LPS is proportional to the SCN^- concentration present, while the maximum effect is obtained at equivalent concentration on molar basis of SCN^- and H_2O_2. Addition of equivalent amount of these compounds results in enhanced bacterial effect (Björck *et el,* 1975). The effective LPS antibacterial effect is achieved when the level of SCN^- has been increased by 10 – 15 ppm followed by corresponding quantities of H_2O_2 (Lambert, 1993). Hydrogen peroxide is not normally detected in milk and its source in-vivo is not quite clear but the newly drawn milk has been found to contain some few traces of it. Hydrogen peroxide may possibly be derived from metabolism of the mammary tissue and leukocytes (FAO 1999). The source in-vitro of H_2O_2 can be direct addition of dilute solution of H_2O_2, alkali percarbonate or peroxides (*e.g.* earth alkali peroxide or carbamide peroxide). They all release H_2O_2 when in contact with water (Korhonen, 1980). Enzymes used for H_2O_2 production are glucose oxidase and Xanthine oxidase the last being the most important *in-vivo* source of H_2O_2 since milk contains a large amount

of this enzyme (Björck *et al*, 1975). Xanthine oxidase may react with some substrates e.g. Hypoxanthine to produce H_2O_2 (FAO, 1999). The combination of three of these factors; Lactoperoxidase, Thiocyanate and H_2O_2 provide the mechanism of in-vivo bacteriostatic properties of the newly drawn milk which lasts for about 1 to 2 hours

2.2.2.1 Factors affecting the Lactoperoxidase system (LPS)

LPS limiting substances include various Peroxidase inhibitors, Catalase and reducing agents having free Sulfhydryl (-SH) groups. Peroxidase inhibitors include high concentration of H_2O_2 (300 – 500 ppm), which inactivate the Lactoperoxidase in milk, and the antibacterial effect is taken by H_2O_2. Catalase and Lactoperoxidase do compete for H_2O_2 and at low concentration reduces the antibacterial effect of Lactoperoxidase. However, at normal physiological level of Lactoperoxidase and Catalase in milk, no interference by Catalase has been observed. This suggests that Lactoperoxidase has high affinity for H_2O_2 than Catalase, which explains why the system is effective against Catalase secreting bacteria. Reducing agents such as cystein and glutathione have been known to reduce antibacterial activity of LPS. Milk protein contains very little –SH groups, those present are located at β-lactoglobulin and do not react with oxidisable substances in untreated milk. Interference by reducing agents is therefore likely to be of minor importance in the raw milk (Korhonen, 1980).

2.2.2.2 Mechanism of the Lactoperoxidase System

In the activated Lactoperoxidase system (LPS) the active agent is the intermediate

oxidation product of SCN^-, which is OSCN- The compound is formed and decomposed in the following reaction.

$$SCN^- + H_2O_2 \xrightarrow{\Delta LP} OSCN^- + H_2O$$

$$4OSCN^- + H_2O_2 \longrightarrow 3SCN^- + SO_4^{2-} + CO_2 + NH^{4+}$$

By this reaction Thiocyanate is converted to Hypothiocynous acid (HOSCN). At the pH of the milk HOSCN is dissociated and exist mainly in the form of $OSCN^-$. The $OSCN^-$ produced in the above reaction is an antibacterial compound. which interferes with the metabolism of the bacteria. It oxidizes the bacterial surfaces (membrane proteins), and certain vital bacterial metabolic enzymes, which contain –SH groups. This reaction results in the immediate inhibition of the respiration. Depending on the nature of the bacteria, it may lead to the temporary growth inhibition or death of the cell due to the blockage of metabolism and ability to multiply. LPS improves storage stability of raw milk by delaying the growth of Pschrotrophs. At low cell concentration LPS is bactericidal against several gram-negative bacteria, including *E. coli, Pseudomonus aeruginosa* and *S. typhimurium* and show both bacteriostatic and bactericidal activity towards gram-positive bacteria. Most of the $OSCN^-$ not reacted with the bacteria is decomposed back to SCN- within a few hours and H_2O_2 is fully consumed in the course of reaction. The loss of antibacterial properties is accompanied with the SO_4^{2+}, NH^{+3} and CO_2 produced (Korhonen, 1980; Board, 1983; and FAO, 1999). The anti bacterial oxidation of thiocyanate is not stable at neutral pH any surplus of these is decomposed spontaneously back to thiocyanate. The velocity of this reaction is temperature

dependent i.e. more rapid at higher temperatures (CAC/GL-13 1991).

2.2.2.3 The antibacterial spectrum of the Lactoperoxidase system

The LPS is considered to be non-specific in antibacterial activity but considerable differences are found between different bacteria important in milk keeping quality. For instance, gram-negative bacteria are more sensitive than gram positive, where-as gram-negative bacteria get killed but the gram positive are temporality inhibited. Most Lactobacilli and Streptococci are only temporarily inhibited and not killed. Among *Streptococcus Spp.* only *S. pyogenes* is known to be killed by activated LPS (Korhonen, 1980 and 1999). Also it has been found that LPS is strongly bactericidal for *Pseudomonas Spp* and other Psychrotrophic bacteria, as these organisms are known to cause spoilage in refrigerated milk (Björck, 1978). Using this method it is not possible to disguise poor quality milk, which originally contained a high bacterial population because the LPS is mostly bacteriostatic in nature.

The product involves changes in cytoplasmic membrane because Hypocyanate ($OSCN^-$) binds to the free –SH groups of key enzymes causing the pH gradient to drop and Potassium and amino acid to leak from the cell. This prevents the up take of carbohydrates, amino acids and other nutrients because the transport mechanisms are inhibited.

Gram-negative bacteria are easily killed and lysed by Lactoperoxidase system than Gram-positive bacteria due to difference in both cell composition and thickness. Against

a mixed raw milk flora dominated by Mesophilic bacteria the effect is bacteriostatic but the system is bactericidal against gram-negative *E. coli*.

Table 1: Antibacterial activities of LPS in milk or in Synthetic Medium against different bacteria

Micro-organisms	Nature of the effect
Starter Cultures	
Streptococcus cremoris	Bacteriostatic
Thermophilic lactobacilli	Bacteriostatic
Mastitis Organism	
Streptococcus agalactiae	Bacteriostatic
Streptococcus uberis	Bacteriostatic
Staphylococcus aurens	Bactericidal
Escherichia coli	Bactericidal
Pathogenic and enteropathogenic organisms	
Salmonella typhosa & S. paratyphi	Bactericidal
Salmonella typhimurium	Bactericidal
Escherichia coli	Bactericidal
Pseudomonas aeruginosa	(Bactericidal)*
Streptococcus pylogenes	Bactericidal
Milk Spoilage organism	
Pseudomonas fluorescence	Bactericidal
Gram negative strains	Bactericidal
Raw milk flora	Bactericidal /Bacteriostatic

* The system is bacteriostatic against a mixed raw milk flora dominated by mesophilic bacteria but the system is bactericidal against gram-negative *E. coli*. Several investigations have revealed that the most important milk borne bacteria e.g. *Salmonella, E. Coli* strains *Listeria*

Korhonen (1980)

2.2.2.4 Toxicology of Lactoperoxidase system

Activated LPS is obtained through adjustment of the original thiocyanate content of the

milk. This results in elevated residual content of this compound in the milk. Despite the fact that the thiocyanate content residual is much lower than that found in the saliva and many vegetable foods, but careful clinical studies have been carried out both in normal milk consumers in Sweden and on goitrous students in West Sudan which is, an endemic goitre region. Neither of these studies showed any negative effect on thyroid functioning after the activation of the LPS in milk (Claesson, 1999a, Claesson, 1999b). Clinical studies have clearly demonstrated that milk treated according to this method will not cause any interference of the iodine uptake of the thyroid grand, neither in persons with normal iodine status nor in cases of iodine deficient (CAC/GL-13, 1991).

The facts available indicate that antibacterial agent produced by the LPS is completely destroyed by heating the milk at 60^0C for 15 minutes (Björck *et al,* 1975). Pasteurisation therefore ensures the complete removal and elimination of the antibacterial compounds (residue concentration of the active oxidative product *i.e.* $OSCN^-$) before the milk is further processed or distributed to consumers. H_2O_2 is completely utilised in the reaction though some residues of SCN^- are noticed. However the level required 10 – 15 ppm is within the ranges normally found in milk and that the level added are lower compared to that in human saliva 50 – 300 ppm, or in human gastric juices 40 – 50 ppm, (Korhonen, 1980).

The potential risks on human health associated with SCN^- level seem to be not significant. But as a prerequisite for the utilisation of LPS in milk preservation, there should be a strict, appropriate and reliable method that ensures exact and uniform dosage

of LPS compound in milk. The method should be utilised only when refrigeration of the milk is not feasible, in areas that currently lack adequate infrastructure for collection of liquid milk (CAC/GL-13, 1991).

It has also been discovered that activation of the LPS in the milk can reduce the content of aflatoxin M1 that often is very high after feeding coconut and groundnut products. This is extremely important for milk to the children since aflatoxin M1 is the most carcinogenic substance known (Hui, 1993; Claesson, 1999b).

2.2.2.5 Utilization of activated LPS method

This method should be utilised in situations where for technical, economical and practical reasons do not allow the use of cooling facilities for maintaining quality of the raw milk. The use of LPS in areas that currently lack adequate infrastructures for collection of liquid milk would ensure the production of milk as safe and wholesome food that would otherwise be impossible (IDF, 1988, CAC/GL -13, 1991, Claesson, 1999b). The cost of treatment of raw milk is 1-3% of the farm gate price or U$ 0.007 (Claesson, 1999a). The proper LPS active resident time at room temperature is in between 3 and 6 hours. Below this range it is a misuse of resources because of LPS inactivation by pasteurisation *i.e.* there is no need for LPS treatment, as the act of immediate pasteurisation will take care of the resident microbes and will at an instant stop the activity of the introduced LPS. The method should not be practised by individual farmers but at suitable collection centres. The centres must be equipped with proper facilities for cleaning and sanitising the vessels used to hold and transport milk.

The personnel responsible for milk collection should be made in charge of milk treatment and should receive appropriate training in general milk hygiene to increase perfection.

A need do exist of an appropriate control mechanism on the application of LPS technology. This can be monitored by the dairy plant processing the treated milk to avoid the misuse of the method through testing the quality of the milk prior to processing. The method should only be used to prevent undue bacteria multiplication in raw milk during collection and transportation to the dairy processing plant.

The use of LPS should not exclude the necessity of pasteurisation of the milk before human consumption. Neither should it exclude the normal precautions and handling routines applied to ensure high hygienic quality standard of the raw milk (CAC/GL-13 FAO, 1991).

2.2.2.6 The primary advantages of LPS

The primary advantages of the LPS use are:

* Simplicity of the use or practical application of the LPS preservative method.
* Low cost in the region of 1-3% of the farm gate price. (0.007US$), *i.e.* more economic milk collection system.
* Substantial reduction of the loss in quality.
* Suitable for inaccessible zones where preservation using refrigeration is not possible due to the lack of sufficient energy and communication infrastructures.

* Allow substantial expansion of the milk collection areas followed by subsequent setting up of new collection centres. The potential collection radius can be estimated as 20-150 km depending on the mode of transport.
* Milk, which would otherwise be lost, can be transported to distant processing and milk consuming centres without having recourse to refrigeration, where other measures for transporting milk in transits cannot be adopted.
* The use of LPS can help to stimulate milk production in remote areas by providing better marketing facilities and high price to the producer and thereby help to improve their standard of living.
* The use of this method enables the dairy industry and the government concerned to offer the population the additional quantities of milk produced in the same country and thus provide a source of valuable animal protein, mineral. Protein are particularly important in nutrition, especially in technically less developed countries.
* The LPS has a much more specific antibacterial effect than general oxidative effect of hydrogen peroxide. This minimises the risk of any negative effects on the processing or nutritive value of the milk.
* The effect of LPS on the bacterial flora in raw milk is largely of bacteriostatic nature. Therefore it is not possible to improve the basic hygienic quality of initially low quality milk by use of the LPS. This is a most important aspect as good basic hygiene is thus a prerequisite for successful use of the LPS.
* The residual thiocyanate makes it possible to monitor the usage of the method; over dosage can easily be revealed by excessively high thiocyanate levels in the milk.
* Activated LPS in milk can reduce the content of aflatoxin M1 which is carcinogenic

this is especially important for children.

* The method offers a means for meeting emergencies caused by power cut or break down of refrigerated machinery in dairy plants and factories.
* Milk production, collection and storage is made possible in areas with high ambient temperatures and poor milk handling facilities.
* The use of LPS improves the bacteriological and chemical quality of the milk plus the subsequent products (Korhonen, 1980; Vasavada and Cousin, 1993; Claesson, 1999a, Claesson, 1999b(Claesson, 1999a, Claesson, 1999b); FAO 1999).

2.2.2.7 The effect of LPS on the milk constituents

LPS application in milk appears to have no measurable effect on the milk constituents when added in small amount. The vitamin content is slightly reduced; this is especially for the vitamin C. The other milk constituents are not affected *e.g.* protein, fats, lactose or mineral content. It is obvious that milk destined for liquid milk market is not adversely affected when small amount of LPS is added (Vasavada and Cousin, 1993).

2.2.2.8 Control of the LPS usage

The dairy processing plants that receive the milk must control the use of LPS for preserving raw milk. A combination of standard acceptable test ought to be performed to ascertain the qualities, these include; Titratable acidity, Methylene blue, Resazurin, Total viable count and analysis of Thiocyanate concentration in milk (IDF, 1988). Since SCN^- is not consumed in the reaction, treated milk arriving at the plant would contain

approximately 10 mg above the natural amount of SCN^- per litre of milk, i.e. analysing untreated milk from the same area (IDF, 1988). Testing ought to be done at random whenever SCN^- is too low/high, (outside specification) an investigation must be made to establish the reason. The plant should also control the use and supply of chemicals used for the activation of LPS in milk. Analysis of bacteriological quality ought to be carried out to ensure adherence to basic milk hygienic practices (TZS120: 1981). Such tests reveal initial bacterial count since LPS is predominantly bacteriostatic.

Other chemical milk preservatives include; the use of potassium sorbate, carbon dioxide and bacteriosins, this is just to mention a few (Vasavada and Cousin, 1993).

2.2.2.9 Other usage of LPS

Some dental products in the markets have been made utilising activated LPS biotechnology *e.g.* Zendium developed by Akzo dental research and Biotine from Laclade research laboratory. Other plausible usage includes LPS in milk replacers for calves, this improves calves growth rate and result in reduced frequency of diarrhoea, inhibition of *Listeria monocytogenes* in cheese, prevention of outgrowth of E. coli during manufacture and ripening of Camembert cheese, killing of *Campylobacter jejun* and the inhibition of *Salmonella typhimurium* plus *Escherichia coli* in infant milk formula (Björck, 1990). Activation of LPS appears to have great potential in Africa and Asia situation where many remote rural milk producers could be linked to the urban consumers by the extension of raw milk shelf life.

2.2.3. The effect of temperature on activated Lactoperoxidase system in milk

Since activated Lactoperoxidase system is an enzyme catalysed reaction it must be sensitive to temperatures changes. Increase in temperature will result in increase in the rate of reaction up to a certain limit above which the enzyme is denatured, because enzymes are protein in nature. The predominant effect from the temperature rise is an increase in the rate of reaction as predicted by chemical kinetic theory. Always enzymes operate efficiently in certain temperature range above which the opposing factor namely thermal denaturation becomes increasingly important. This denaturation will decrease the effective concentration of the enzyme and consequently decrease the reaction rate up to the point where a catalytic function will be destroyed (Kimambo, 1994).

Lactoperoxidase is an iron containing protein accounting for about 1% of total whey protein content, the enzyme is heat-stable and can be retained in normal pasteurisation of milk at 63^0C/30 minute or 72^0C/15 seconds but it is destroyed at 80^0C in 2-5 seconds (Korhonen, 1980). The production of hypocynide ($OSCN^-$) antibacterial compound under activated LPS reactions is temperature dependent with rapid velocity at higher temperatures.

2.2.4 Mechanical preservation

2.2.4.1 Pasteurisation

Pasteurisation means heating below the boiling point (Rosenthal, 1991). It may be defined as the heat-treating of every particle of the milk or milk product to a specific temperature for a specific period of time without allowing recontamination of the milk or

milk product during the heating process. It is the process of heat-treating a liquid particularly milk to a temperature between 65^0C and 70^0C to destroy harmful bacteria without materially changing the composition, flavour, or nutritive value of the liquid. The extent of microorganisms inactivation depends on the combination of temperature and holding time. The minimum temperature and time requirements for milk pasteurisation are based on the thermal death time studies of the most heat resistant pathogen found in milk, which is *Coxiella burnettii*. Milk is made safe for human consumption by pasteurisation. Pasteurisation must have minimum impairment to the chemical and physical properties of the milk. Usually pasteurisation should be followed with both rapid cooling and subsequent storage at temperatures below 10^0 or 5^0C (Foster, 1958, Hall and Trout, 1968, Henderson, 1971, FAO 1973 and Lewis, 1986). There are two types of pasteurisation; Low temperature long time (LTLT)/batch pasteurisation *e.g.* 63^0C/30 min. and high temperature short time (HTST)/continuous method *e.g.* 72^0C/15sec. The chief advantage of (HTST) is its capacity to heat treat milk quickly and adequately while maintaining rigid bacteriological and quality control. Milk pasteurisation kills all Yeasts and Moulds plus most vegetative cells of bacteria in milk. The surviving bacteria are termed thermoduric. The most important ones are, the non-spore formers high temperature *lactis e.g. Enteroccoci, Streptococcus thermophilus*, high temperature *Lactobacillus bulgaricus, L. lactis* and species of *Mycobacterium*. Certain species of *Micrococcus*, some species of *Streptococcus* and *Lactobacillus* are thermophilic as well as thermoduric. Sporeforming thermoduric bacteria falls into two main groups; - species of *Bacillus* i.e. aerobic to facultative sporeforming *Bacilli* of which *B. cereus* usually is the most numerous but *B. licheniformis, B. coagulans, B*

polymixa (gas-forming), *B. cacidolactis* and other species sometimes are of importance. Species of *Clostridium* anaerobic sporeforming rods, some of which are saccharolytic e.g. *Butyricum* and other proteolytic and saccharolytic *e.g. C. sporogenis*. The traditional pathogens in raw milk are *Microbacterium bovis* which causes bovine tuberculosis (which also affects humans), *Brucella abortus* which transmit brucellosis (undulant fever) to man, *Salmonella* which are responsible for gastrointestinal diseases and typhoid fever and *Campyrobacter jejun* a common factor in enteritis (Rosenthal, 1991). Most of micro-organisms that grow in milk also form gas. Miscellaneous other bacteria may survive pasteurisation but do not grow well in milk (Frazier, 1967).

Milk is an excellent culture medium for many kind of micro-organisms for its high in moisture, nearly neutral in pH and rich in microbial food. The pasteurisation of milk kills the more active acid forming bacteria but may permit the survival of heat resistant Lactics *e.g. Enterococci, Streptococcus thermophilus* and *Lactobacilli* that will cause lactic acid fermentation if the holding temperature is high enough (Bannert, 1989).

The main purpose of pasteurisation is under health aspect, *i.e.* the thermal killing of pathogens, which are micro-organisms, causing diseases in humans. This makes milk and milk products fit for human consumption. Keeping quality aspect is the additional side benefits from pasteurisation which is achieved through destruction of the large part of milk spoilage micro-organisms and deactivation of some natural milk enzymes like lipase, which can adversely affect the quality of the manufactured products (Rosenthal, 1991).

Pasteurised milk is not sterile but the pasteurisation treatment upset the balance of flora in milk by killing all sensitive organisms approx. 90-99% of the bacteria including the common Lactic acid bacteria and leaving the heat resistant types thermodurics and spores. Therefore in the heat-treated milk the surviving flora might deteriorate the proteins and produce unpleasant taints, or in certain cases, cause "sweet curdling" without souring. Nevertheless thermophiles and thermodurics, which may withstand pasteurisation, do not grow rapidly enough to normally cause spoilage in refrigerated milk. Therefore when pasteurised milk sours rapidly it is almost invariably due to post pasteurisation contamination. The presence of Coliform in pasteurised milk, which is otherwise sensitive to heat treatment, saves as dependable indicator of such contamination. Other mechanical preservations include refrigeration, radiation, thermisation, ionisation, microwave heating, sterilization, evaporative cooling, bactofugation *etc.* The purpose of the pasteurisation of milk is:

⇒ To kill all pathogenic organisms including viruses and bacteriophage.

⇒ To kill organisms that are harmful to the quality of the final product.

⇒ To inactivate enzymes that are harmful to the quality of the final product.

⇒ To produce dairy products with a constant quality.

⇒ To increase the keeping quality of the milk (IPC, 1999).

2.2.5 Fermented milk

Fermentation is another way of milk preservation that exploits the advantage of increased acidity on microbes. Advantages of fermented milk include;

▪Simplicity of manufacture, the mesophilic culture applied have the ambient

temperature ranging between 15 -30^{0}C hence can be kept under room temperature,

- Ease of preservation as fermented milk does not produce more than 1.1% Lactic acid,
- Has a minimum keeping quality of 2-4 days under room temperature and 2-3 weeks at temperatures lower than 10^{0}C.

Fermented milk performs excellently in warm (poor) developing countries with shortcomings like poor infrastructures *e.g.* cold chains or absence of power supply to operate such systems. In fermented milk, Lactic acid bacteria overgrows the others, while the acidity produced help to preserve the milk by suppressing (microbes) pathogens- proteolytic and lypolitic bacteria. Acid causes the protein to flocculate and formation of gel like consistency. Fermented milk classification depends on the type of milk used, flora and the processing done before and after fermentation (Lampert, 1988). Natural fermentation exploits lactic acid producing Streptococci and Lactobacilli bacteria present in any milk.

2.3 Milk storage

Milk should be stored at temperatures below 10^{0}C immediately after milking in order to avoid further bacterial development and therefore result in increased shelf life. Refrigeration of milk is rarely done in the remote rural areas because of absence of source of energy to operate such systems, supportive infrastructures, economic, social and organizational problems. The use of evaporative cooling is strongly recommended

for such circumstances in the tropics (Robinson, 1981; Harding, 1995).

2.3.1 Evaporative cooling

Evaporative cooling occurs when heat needed to evaporate water is taken from the air. The effectiveness of evaporative cooling is determined by the area of the wetted surface and by the closeness of its contact with the dry air (Dempsey, 1998).

2.3.1.1 Advantage of evaporative cooling

- The cost of acquisition and operation is a fraction of conventional air conditioning or mechanical refrigeration system.
- Maintenance costs are minimal requiring simple procedures and lower skilled maintenance people.
- Cost pay back due to its low energy use and extended shelf life of the food is encouraging.
- It is effective due to its simplicity, low cost and effectiveness. (Use simple mechanisms).
- Evaporative cooling systems are easy to operate and maintain hence low cost of production. (Lower running cost by up to 80%)
- Evaporative cooling helps to curb excessive use of energy (Lower energy use).
- It helps clean up the atmosphere since it has no CFC's or HCF's. Evaporative cooling helps to avoid air pollution by avoiding the over use of chlorofluorocarbons, which may burn a hole in the ozone layer resulting in global

warming.

- Evaporative cooling is a 100% fresh air-cooling, which also helps clean the air it cools. It allows thorough ventilation with a plenty of fresh air.
- It is economical, effective and has health benefit as it improves workers and equipment efficiency and provides a much-needed alternative to conventional mechanical refrigeration.
- Lower installation cost than refrigeration system by up to 50%.
- Does not require complex electrochemical equipments.
- Requires no input of electric energy or other conventional fuels.

(Dempsey, 1998; Rusten, 1985)

2.3.1.2 Performance of evaporative cooling

Evaporative cooling cools best on the hot dry days *e.g.* at 40^0C, expect a 20% drop resulting in about 15^0 C. When water evaporates sensible heat in the water is converted to latent heat in water vapour, thereby resulting in the loss of sensible heat and hence cooling (Dempsey, 1998).

Evaporative cooling can be used where: -

- Temperatures are high
- Humidity is low
- Water can be spared for this use
- Air movement is available

Air velocity plays an important role in the evaporative cooling process. Along with temperature and humidity air velocity reduces the effective temperature. The air change rate is also important. Evaporative cooling use nature's most efficient means of converting sensible heat to latent heat through the evaporation of water to remove heat from the air. When relative humidity is very high (60%) the air content absorbs much moisture and hence no cooling will takes place (Rusten, 1985).

2.3.1.3 Basic principles of evaporation and evaporative cooling

Evaporative cooler operates in the principle of a fan drawing hot air from outside through a wet pad into the building. The hot air is cooled by evaporating water, which changes sensible heat in the water to latent heat in the vaporised moisture resulting in temperature drop. Air temperature reduction in buildings such as 11^0C can be achieved through evaporative coolers during hot season (Bengston and Whitaker, 1986). Evaporative cooler is recommended for small scale dairy application due to the fact that it requires no source of power of which usually is literally available in rural areas *e.g.*, electricity (Bengston *and* Whitaker, 1986).

Evaporation is the process of changing liquids into gas. Water is unique in that it requires a relatively large quantity of heat energy to change from liquid to gas. It is this characteristic that enable evaporating water to lower substantially the temperature of its environment (Rusten, 1985).

The principle is relatively simple; air moving past water will cause water to evaporate

the heat necessary to cause evaporation is drawn out of the passing air stream and hence the air is cooled. The human body uses this principle to control the body temperature by varying the amount of water on the skin surface (persipiration/evapotransipiration in trees). The evaporation of this moisture cools the skin and helps to lower the body temperature (Rusten, 1985).

Since evaporative cooler is continuously evaporating water it therefore, naturally requires constant supply of water. But in order to avoid salt concentration formations in water that could be caused by increased salt concentration in the tank or salt precipitates on the pads due to evaporation, one has to think of a continuous bleeding system. Increased salt concentration leads to corrosion of metal component and reduction in cooling performance. With time there is a build up of pollen and dust that would also need frequent removal. When not in use the tank should be drain out completely to avoid algae and the likes proliferation (Rusten, 1985).

Evaporative cooling works best in dry climates though; it could still provide reasonable comfort in higher humidity areas, by simply increasing the rate of airflow provided that the air temperature is not too high. Evaporative cooling works everywhere except in areas with both high temperature and high moisture content (during wet season). The amount of water vapour that can be held by the air depends on temperature and water availability. The amount of water vapour in the air is termed as humidity. There are two ways of measuring the humidity of the air;

- Absolute humidity- measures the actual quantity of water (measured in grams) in

a given volume of air (measured in cubic meters or litters).

- Relative humidity- is the measurement of water vapour in the air as the percentage of the maximum quantity of water vapour that the air would be capable of holding at specific temperature.

Air that is fully saturated has a relative humidity of 100% while air that has a half as much water vapour as it possibly could hold at a specific temperature has a relative humidity of 50%. The relative humidity varies with temperature. As air cools (*i.e.* looses energy), its ability to hold water vapour decreases which results in an increase in the relative humidity. This is because the ability of the air to hold water vapour is reduced by the drop in temperature but the absolute humidity (the actual amount of water vapour in the air) remains unchanged. If the temperature continues to fall the relative humidity will approach 100% or complete saturation. The point at which the air is fully saturated is referred to as the dew point. At temperature lower than the dew point, water vapour condenses out of the air onto cooler surfaces (Rusten, 1985).

2.3.1.4 Factors affecting evaporation

As the rate of evaporation increases so does the rate of cooling. To make the most effective use of this technology it is important to understand the factors that influence the rate of evaporation and the relationship that exists between these factors. There are four major factors that influence the overall rate of evaporation and extent of cooling (Rusten, 1985).

a) Relative humidity

Evaporation occurs when water absorbs sufficient energy to change from liquid to gas (vapour). A relatively high air temperature will be able to stimulate the evaporative process and also capable of holding relatively great quantities of water vapour. Therefore in areas with high rates of evaporation more cooling will take place. The opposite is always true with lower air temperatures *i.e.* less water vapour can be held hence less evaporation and cooling will take place. Under such conditions of high relative humidity evaporative cooling may not be effective. However, in many areas with high relative humidity *e.g.* the humid tropics, evaporative cooling may be effective if a desiccant (*e.g.* silica gel) is used to remove moisture from the air before it is cooled (Rusten, 1985).

b) Air temperatures

A relatively high air temperature will be able to stimulate the evaporative process and also be capable of holding great quantities of water vapour. Therefore areas with high temperature will have higher rates of evaporation and more cooling effect. With lower air temperature less water vapour can be held and less evaporation and cooling will take place (Rusten, 1985).

c) Air movements

Air movement either natural (wind) or man-made (with fan) is an important factor that influences the rate of evaporation. As water evaporates from the surface, it tends to raise the humidity of the air that is closest to the water's surface. If this humid air remains in

place, the rate of evaporation will start to slow down as humidity rises. On other hand, if the humid air near the water's surface is constantly being moved away and replaced with dry air the rate of evaporation will either remain constant or increase (Rusten, 1985).

d) Surface areas

The area of the evaporating surface is equally important in controlling the rate of cooling. The greater the surface area from which water evaporates, the greater the rate of evaporation the higher the rate of cooling. For instance consider the following two situations

i) If water poured in narrow glass container with only about 16 cm^2 the surface exposed to the air.

ii) If 1L of water poured into a large shallow pan with about 180 cm^2 of surface exposed to the air.

Because of the large surface area the large pan of water would dry up much sooner than the jar if both were exposed under the same environmental condition (Rusten, 1985).

2.3.1.5 Maximum cooling potential

The extent to which evaporation can lower the temperature of the container or the air depends upon the difference between the wet and dry bulb temperatures. Theoretically it is possible to bring about a change in temperature equal to the difference in these two temperatures. But depending on the environmental conditions and the method of evaporative cooling used, it should be possible to achieve between 50 and 80% of the

theoretical maximum drop in temperature (Rusten, 1985).

2.3.1.6 Design variation

There are two general methods of evaporative cooling, direct and indirect. Direct evaporative cooling involves the movement of air past or through a moist material where evaporation and therefore cooling occurs. In contrast to this, indirect evaporative cooling uses some form of heat exchange that uses the cool moist air, produced through evaporative cooling to lower the temperature of the drier air. This cool dry air is then used to cool the environment, and the cool moist air is expelled. In our case it is better to use the less complex and less costly direct evaporative cooling process. The method of evaporative cooling used, (direct or indirect) depends on;

- The specific need of the environment that will be cooled.
- The availability and cost of commercial energy.
- The amount of money and skill available.

Before attempting to implement any of the evaporative cooling systems, it is necessary to determine whether the environmental conditions particularly the relative humidity, is suitable for the evaporative cooling process (Rusten, 1985)

Principally natural heat from the milk is transferred to a cooling agent through a separation wall. The refrigerant or cooling agent absorbs the heat of the milk. The final temperature depends on the design of the equipment. Some systems cools milk to a temperature only a few degrees higher than that of a cooling agent. The rate of cooling among other factors depends on: - the temperature difference between the milk and the

cooling agent, the surface area, the thickness, the material of the separation wall and the movement of the liquids along the wall. Large difference in temperature promotes the rate of cooling, as does the smoothness of the surface of the walls. Any cooling is better than no cooling at all (Van den Berg, 1988).

2.4 Fresh milk handling in the tropics

In the tropics and sub tropics under conditions of low ambient temperature in the high altitude, milk of good hygienic quality may be stored without refrigeration for more than 12 hours without any noticeable changes (Van den Berg, 1988). The higher the ambient temperature the quicker the milk spoils. Microorganisms develop faster in severely contaminated milk where hygiene is poor. Refrigeration is essential to achieve results, which might be partially comparable with good hygiene and little cooling. The value of cooling depends on the hygiene of production. Refrigeration of milk of good hygienic quality results in longer shelf life as compared to milk of poor hygienic quality.

Fresh milk storage includes, ice cones, water tankers, cooling rings, immersion coolers, farm tanks coolers, charcoal cooler, tubular and plate heat exchanger. Depending on conditions sometimes it is impractical or too expensive to cool or refrigerate milk against spoilage. This is true when the milk quantities are too small to warrant application of expensive cooling systems. The other stumbling block could be the energy availability/accessibility to operate such systems (Van den Berg, 1988).

2.5 Milk packaging

One of the main problems facing small-scale dairy processing in developing countries is the high capital cost of the conventional stainless steel equipments for pasteurisation and packaging of fresh milk. Many resort to heating milk in cans or boilers to make the milk safe and extend the shelf life. The higher temperature used do affect the milk natural quality and the risk of post pasteurisation contamination is very high during handling (FAO, 1999). One can easily recommend opaque plastic sachet packaging as a break through to the milk post contamination challenge (Brian, 1999).

2.6 Dairy cooperatives and milk collection

Some parts of the country are potentially high milk producers due to environmental condition, number and types of cattle breeds raised. In these areas milk production is in excess of the family subsistence level hence a need for producer organisation is important in order to dispose the constant daily milk surplus. These associations would organise collection, processing, and transportation of the milk in order to link the dairy farmers to the markets. The marketing of the milk surplus would have provided impetus for increased milk production, which would, then guarantee employment for the rural society. The failures of milk marketing do frustrate increased milk production. Again producers organisations are essential for the dairy development in the country as they bridge the gap left behind by the collapsed parastatals and other government institutions which used to provide the necessary support to the farmers throughout the country. A producers association provides common approach towards production problems and gives farmers produce marketing and bargaining power besides provision of credit facilities.

Usually the roles of dairy producer associations are Milk handling, collection, processing transportation, distribution and marketing. Others are milk quality control, advertisement, disease prevention and control, extension services, feed supply, veterinary services, breeding, artificial insemination and training. Therefore a dairy producer association is an indispensable rural community organisation without which dairy commercial production from the remote rural areas in the humid tropics would be questionable, as dairy products are easily perishable.

2.6.1 Milk handling problems experienced by cooperatives

Management problems observed include; lack of milk preservation facilities *e.g.* Cooling, processing and storage facilities. Others were; lack of transport, the milk load being not economically large enough to warrant daily milk transportation over long distances and lack of appropriate milk storage and transportation tanks. Transport has always been potentially important in the marketing costs involved as it commands a big share in the programmes, therefore it is important that an economical load size is reached before transportation or otherwise negative returns might be realised. Alternatives like widespread adoption of second hand cooling facilities imported from Europe came with disappointing poor performance as this machines were not designed for tropical environment and needed constant improvement and maintenance. The other problem has always been types, availability and erratic supply of energy to run these systems. Technologies adoption like water bath coolers- packed with ice cubes can with stand prolonged power cut only if sufficient ice bank is maintained. One can easily adopt this cheap alternative technology and shy away from expensive standby generator. The

guiding principle has always been local solution for the local problem. (Kurwijila R.L., 1990)

2.7 Conclusion from literature reviewed

Simple and cheap milk preservation techniques shall always be recommended for the remote rural small-scale dairy producers because of the general nature of milk production (dairy industry) in this hot humid environment. The situation is worsened by remoteness of the milk sources from the consumers, scattered small-scale dairy farmers, inaccessibility, absence of supportive infrastructures like roads and electricity (energy), level of economy or ability of the farmers to afford cooling systems and technical know how.

The workable solution to such serious problems involves the use of chemical preservatives and through the use of physical means like pasteurisation and cooling. The guiding principle behind all these efforts is the control and suppression of the microbial growth or their subsequent elimination from milk in order to increase milk shelf life and product values, which would have been otherwise difficult to archive.

The challenge ahead shall always be the search for cheap, simple, appropriate and acceptable milk preservation technology for the huge, remote rural surplus milk. The technology should be able to increase milk shelf life tremendously to allow efficient and profitable processing and the marketing. In the absence of mechanical refrigeration the use of activated LPS and evaporative cooling may be more appropriate options to consider and apply. Their successful application would

stimulate local milk production and the dairy industry expansion through increased farmer income resulting from the sales of milk and milk product.

CHAPTER III

3.0 MATERIALS AND METHODS

3.1 Study areas

The study was carried out at both Sokoine University of Agriculture (SUA) and Mwakaleli ward, Rungwe District. Laboratory work was done at SUA and Uyole Agricultural Centre, while fieldwork was executed at Mwakaleli.

3.2 Sample collection

Milk samples were collected early in the morning from Magadu dairy farm at SUA, Morogoro. Sub-samples were drawn from the bulk milk stored in the collection cans to make composite sample. Before collection, the milk was stirred to ensure homogenous mixing. The samples were distributed into two batches one with and the other without activated LPS treatment as a control. The milk was then sealed in 0.5 L. plastic sachets ready for pasteurisation at 80^0C for 1 minute, this was done for both LPS treated and untreated milk. A total of 20 L. of milk sealed in plastic sachets were prepared for each situation *i.e.* treated and untreated milk. The whole activity was repeated three times.

3.3 Determination of naturally occurring thiocyanate in milk

Thiocyanate was determined in milk after deproteinization with trichloroacetic acid (TCA) as the ferric complex by measuring the absorbance at 460 nm. The minimum level of detection by this method is 1-2 ppm of SCN^-. This was done in the laboratory at

SUA. The determination involved 4.0 ml of milk being mixed with 2.0ml of 20% TCA solution. The mixture was blended well then allowed to stand for at least 30 minutes. This was then filtered through Watman no. 40 filter paper. The clear filtrate was mixed with 1.5 ml of the ferric nitrate reagent followed by absorbance measurement at 460 nm using a Philips PU8620 UV/VS/NIR spectrophotometer (Pyne Unicam ltd., UK). A mixture of 1.5ml ferric nitrate and 1.5ml water was used as a blank. The measurement was carried out within 10 minutes from the addition of the ferric nitrate solution, as always the coloured complex is not stable for a longer time. The concentration of Thiocyanate was determined through comparison with standard solution of known Thiocyanate concentration e.g. 0, 10, 15 and 30 mg of Thiocyanate.

3.4 Activation of Lactoperoxidase system on pasteurised milk shelf life

The activation of the LPS in the milk was achieved through addition of sufficient amount of thiocyanate (SCN^-) and H_2O_2 to give an initial concentration of about 0.0015% and 0.00085% respectively. This antibacterial effect was attained by addition of thiocyanate as sodium thiocyanate (NaSCN) and hydrogen peroxide as sodium per carbonate (Na_2CO_3 x3/2 H_2O_2). In order to achieve the above concentration 14 mg of NaSCN was added per litre of milk. The milk was then thoroughly mixed to ensure even distribution of SCN^-. This was followed by addition of 30 mg of Sodium percarbonate per litre of milk. The milk was then stirred for about 2-3 minutes to ensure complete dissolving of the Sodium percarbonate and even distribution of Hydrogen peroxide. Alternatively special LPS activator in pre packed sachets for 50 litres was used (FAO 1999; Claesson 1995; Bachman, 1980). These are provided as activator 1 and activator 2 under trade

name MIPS1 and MIPS2 respectively. The two pre-packed bags contain 4 ml NaSCN (32%) for MIPS1 and 1.5g Na_2CO_3 x3/2 H_2O_2 for MIPS2; these were enough for 50 litres of milk. They were added into 50 litres of milk strictly one after the other i.e. MIPS1 followed by MIPS2 respectively. Milk was stirred for 30 seconds after addition of the MIPS1 this was followed by the MIPS2, which was also stirred for another two minutes. Usually the enzymic reaction begins immediately whenever Hydrogen peroxides i.e. sodium per-carbonate (MIPS2) is added. The reaction is completed within five minutes after which no H_2O_2 can be detected in the milk. The milk was then stored in a dark well-ventilated place at room temperature ready for processing. Under field condition milk collection had to be done once per day. Since it was not possible to have pre packed activator for varying small amounts of milk, common collection centres had to be established in the remote rural areas were-by farmers could pool their milk together for LPS treatment not more than two hours after milking.

3.5 Pasteurisation

In the laboratory milk samples sealed in sterile opaque half-litre plastic sachets were pasteurised in the water bath at 80^0 C for 1 minute. This was achieved by filling milk into half litre opaque plastic (utilising plastic electric sealer) sachets before pasteurisation. This was for both LPS treated and untreated milk. Continuous milk shaking facilitated the even distribution of heat; this was necessary for an achievement of an appropriate heating effect. Pasteurisation was done at 0, 3, 6, 9 and 12 hours after activated LPS treatment and then stored at room temperature (26-27^{0}C), charcoal cooler (18-20^0 C), air-conditioned room (14-15^0 C) and under refrigeration condition (5-8^{0}C). The shelf life

testing was done at 0, 6, 12 hours and daily thereafter.

3.6 Control treatment

Milk sealed in half litre opaque plastic sachet without LPS treatment acted as a control and was subjected into different corresponding treatments and storage conditions; room temperature, cold room, charcoal cooler and under refrigeration.

3.7 Evaluation of shelf life

3.7.1 Clot on boiling (COB)

This was done using small amount of milk aseptically drawn from the sample. The milk drawn was boiled under flame in a test-tube to see whether or not milk forms clot. The milk that clots was taken to have failed the test, hence rejected. (If milk develops about 0.1% lactic acid it always clots on boiling and therefore rejected). Though the test is simple, quick, cheap and easy to operate, but it is insensitive to the low acidity level hence the need for further tests. Milk failing the test was taken to have achieved an advanced stage of souring. Whenever milk contains much acid or rennet-producing microorganisms or when the milk has abnormal high percentage of proteins *e.g.* Colostrum, such milk cannot stand high heat treatment hence should be rejected. The fact that milk at high altitude boils at lower temperature makes the test even more lenient was taken into consideration (TZS120: 1991, DTI, 1997).

3.7.2 Titrable acidity

Exactly 9mls of milk drawn aseptically from the samples was titrated against 0.1N NaOH solution, using 3-4 drops of 1-% alcohol phenolphthalein indicator to monitor lactic acid development. The milk sample together with the indicator in the conical flask was titrated against NaOH under continuous mixing until a faint pink colour appeared (APHA-13, 1985, Richardson, 1985, IDF, 1990).

3.7.3 Milk pH

The shelf life of the test milk from different storage conditions was monitored by observing the change in pH by use of a pH meter (HANNA INSTRUMENTS HI 8519). This was done in the laboratory daily (after 24 hrs) till spoilage, confirmed by COB test

3.7.4 Alcohol test

Two ml of milk was mixed with equal volume of 68% alcohol in a graduated test tube. The 68% alcohol solution was prepared from 68 ml 96% (absolute) alcohol and 28 ml distilled water. If there was no flocculation further 2 ml of alcohol used was to be added. The absence of flocculation after addition of 4 ml of alcohol was taken to be as enough indicator that milk had acceptable low developed acidity (TZS120: 1981). Good quality milk formed no coagulations, clotting or precipitations. The first clotting due to acid development was expected to be seen at between 0.21%-0. 22% lactic acid.

3.7.5 Coliform count

Milk serial dilutions (10^{-1}, 10^{-2}, and 10^{-3}) were transferred into sterile plates. 10 to 15 ml of Violet Red Bile agar were added followed by mixing thoroughly by tilting and rotating each dish. This was then allowed to solidify on a level surface for 5-10 minutes. An overlay of about 3-4 ml. of VRB was made to cover the surface of the already solidified medium, this was important to inhibit the surface bacterial colony formation. The sample were inverted and incubated at 32^0C for 48 hours. Isolation of the coli was made from the positive plates with distinct dark red colonies. The total number of coliform was determined by multiplying the average number of colonies by a dilution factor (TZS119: 1981; APHA-13, 1985, Richardson 1985.).

3.7.6 Total plate counts (TPC)

The whole process was done under highly aseptic environment. Standard plate agar was melted in a boiling water bath and then cooled to 45^0C ready for plating. Milk samples were stirred/shaken to ensure even distribution of bacteria, then drawn aseptically from sachets into a sterilised test tube from which a sterile pipette was used to transfer milk for serial dilutions (10^{-2}, 10^{-3} 10^{-4} for pasteurised milk and 10^{-3}, 10^{-4},10^{-5}, 10^{-6},10^{-7} for raw milk). All the glassware used was sterilised in the autoclave. The plates were incubated at 32^0C for 48 hours. Plates with colonies between 25 and 250 were counted and the total number of microorganisms determined by multiplying the average number of the colonies by the dilution factor (TZS120: 1981; APHA-13, 1985, Richardson 1985.).

3.7.7 Field work

Mwakaleli Dairy Farmers Co-operative Society is situated along the Mwakaleli rift valley at Rungwe district 30 km from the district headquarters, Tukuyu and about 300km from Mbeya town. It lies at altitude of 1400 to 1700 m.a.s.l. with an average rainfall of about 8000 mm. Diurnal temperature ranges from 11.6 to 24.6^{0}C (Maganga and Matumla, 1992).

A total of 60 litres of milk was collected per day for ten sessions from Mwakaleli. The source of milk sample was from smallholder farmers at Mwakaleli ward Kandete division Rungwe district; these farmers formed Mwakaleli dairy farmers co-operative society. The milk was gathered through three collection points each with capacity to handle 20 litres of milk. Milk from farmers gathered in these centres was then transported to a common central collection centre for activation of LPS but transportation to the market, which was Mbeya town, was done in the morning once a day. Of these 60 litres of milk collected per day, 30 litres (which is a half of the milk) stood as a control *i.e.* without any treatment. Out of the total milk collected, 20 litres was from the evening milk and while 40 litres was from the morning milk but the two were kept separately. Before transporting the milk to Mbeya town the evening milk had to undergo testing to ascertain its quality. Parameters measured included clot on boiling, alcohol, organoleptic and pH tests. These tests were done at all collection centres and receiving centre i.e. Mwakaleli and Uyole agricultural research institute. The milk collected in the evening was stored under shade in the dark at well-ventilated place

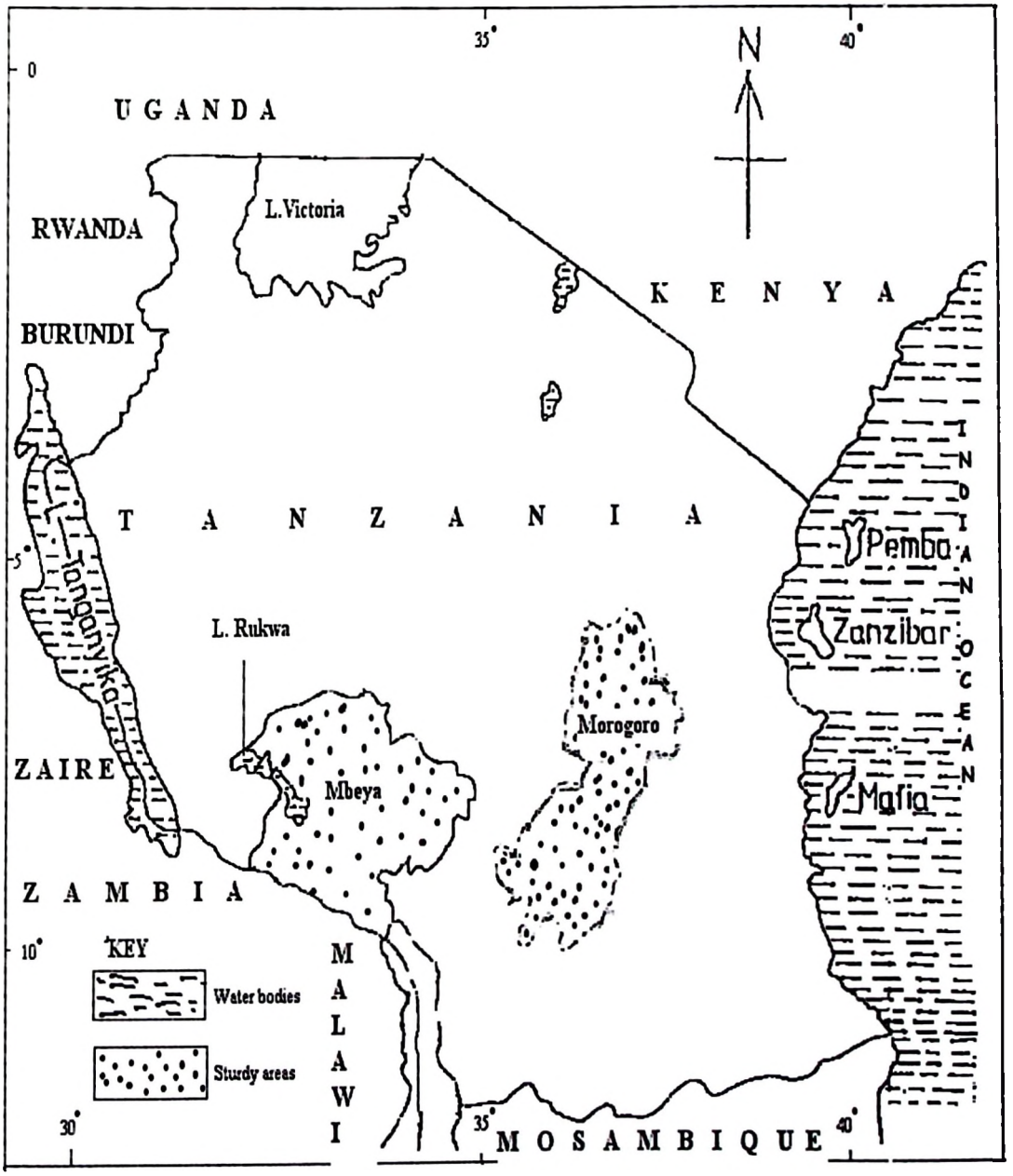

Figure. 1: A map of Tanzania showing LPS study areas

3.8 Effect of activated LPS on fermented milk quality

After pasteurisation, both the LPS treated and untreated milk was allowed to ferment naturally and as yoghurt by the use of yoghurt culture. Parameters measured were, time taken for fermentation process to complete, flavour, smell, appearance, gas production acidity and coliform counts. The untreated milk acted as a control. The milk with three replications was kept into two litres containers each for both conditions. Comparison on the two milk treatments, treated and untreated was done to ascertain the difference.

3.9 Effect of LPS activation on pasteurised milk shelf life

The milk was pasteurised at 80^0C for 1 minute. Parameters measured were COB, alcohol test, TPC, Coliform count and pH. The storage conditions were the room temperature, cold room, evaporated charcoal cooler box and under refrigerator. The milk quality test was done after every 24 hours. This process was done on the milk pasteurised after 0, 3, 6, 9, and 12 hours after LPS treatment.

3.10 Evaporative charcoal cooler

Two charcoal cooler Aluminium boxes with a lid were constructed supported on wooden frames. The cooler was surrounded throughout the body by charcoal held in position by chicken wire-mesh. The two shelves wooden frames supported the charcoal cooler storage box on the lower shelf whereas the 40L plastic container was fixed on top. The plastic container with several perforations underneath allowed even and constant water splashes distributed on the box to facilitate evaporative cooling.

A simple Charcoal cooler box was designed using principles of evaporative cooling. This cooler was evaluated on its capacity and effectiveness in preserving milk. This was as an alternative to refrigeration, which is hard to be acquired by remote rural community due to both organisation and economic reason. The effectiveness of evaporative cooling was influenced by the area of the wetted surface and by its closeness\contact to the dry air.

I). The charcoal cooler operation

The cooler was located in an open space, which was a well-ventilated area with free natural air movements. A constant water splashes over charcoal cooler was maintained by a water source on top of the box. The 40L source used to be refilled three times a day. Thermohydrography machine was used to record temperature fluctuations in the cooler.

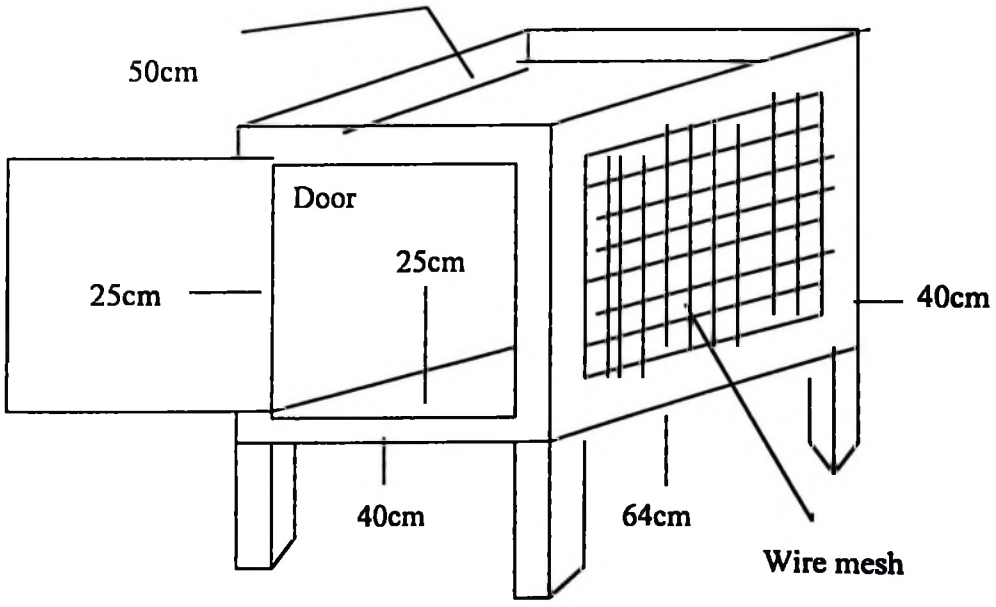

Figure 2: Charcoal cooler box

II). The dimensions of the charcoal cooler box

Dimensions	**Inside (cm.)**	**Outside (cm.)**
Length	50	64
Width	25	40
Height	25	40
Charcoal layer width	7 cm (around the box)	
Box capacity (volume)	31,250cm^3=31.25L	
(Ref. to Fig. 2)		

3.11 Experimental design

Data collected was analysed using SAS Computer software 1992. Both descriptive statistics and general linear model was utilised to process the data from both laboratory and field condition. The statistical model is:-

$$Yijkl = \mu + Ti + Hj + Sk + eijkl$$

Where as: Yijkl = Observation from lth sample of milk under ith LPS treatment at kth storage temperature pasteurised after jth time

μ = General mean.

Ti = the effect of ith LPS treatment.

Hj = the effect of jth time taken to pasteurisation

Sk = the effect of kth storage temperature.

eijkl = Random error/residual effect specific to each observation

CHAPTER IV

4.0 RESULTS

4.1 Milk Thiocyanate content.

The experimental milk was obtained from Magadu dairy farm (SUA). Thiocyanate content of this milk as quantified spectrophotometrically ranged from 2.29 ppm to 8.18 ppm with a mean of 4.09±1.1 ppm. The bacteriological and chemical characteristic of the milk used appeared to be very good as the registered TPC value of 800,000 (cfu/ml) and a Coliform count of 1900 (cfu/ml) was rated to be good (TBS, 1996). The pH of the milk used was normal (6.7)

4.2 Milk shelf life

Table 2 summarizes the maximum shelf life attained by the experimental milk under each storage condition. For instance, refrigerated LPS activated milk pasteurised after 0, 3, 6, 9 and 12 hours tagged as P0, P3, P6, P9 and P12 respectively had registered shelf life gain/loss of about 24, 144, 72, -48 and -72 hours respectively, this is when they were compared to the shelf life of the raw milk without LPS activation pasteurised immediately (R0) which remained fresh for more than 336 hours.

Milk under the charcoal cooler box remained fresh for more than 48 hours for treatment P0, P3, P6 and R0 however the remaining treatments *i.e.* P9 and P12 could not reach 48 hours. At the same time milk under cold room storage condition went above 48 hours but

could not reach 72 hours for treatment P3, P6, P0 and R0 whereas treatment P9 and P12 were registered spoiled at 48 hours. Room temperature storage condition gave a shelf life of greater than 24 hours but less than 48 hours, for all treatments. The physical characteristic of the LPS treated fresh milk appeared to be normal with no noticeable change in colour, smell or gas production.

Table 2: The effect of activated Lactoperoxidase system and the holding time to pasteurisation on pasteurised milk shelf life

[1]Treatment	Refrigerator (5 - 8°C)		[2]AC room (14 -15°C)		Charcoal cooler box (18-20°C)		Room temp. (26 -27°C)	
	shelf life (Hrs.)	Hrs. Gained	shelf life (Hrs.)	Hrs. Gained	shelf life (Hrs.)	Hrs. Gained	shelf life (Hrs.)	Hrs. Gained
P0	>360	48	>48	0	>48	0	<48	0
P3	>480	168	<72	27	>48	3	<48	3
P6	>408	96	<72	3	>48	0	<48	0
P9	>288	-24	<48	-6	<48	-6	<48	-3
P12	>264	-48	<48	-9	<48	-9	<24	-3
R0	>336	0	>48	0	<48	0	<48	0

[1]P3: LPS activated milk pasteurised after 3 hours
P6: LPS activated milk pasteurised after 6 hours
P9: LPS activated milk pasteurised after 9 hours
P12: LPS activated milk pasteurised after 12 hours
R0: Raw milk pasteurised immediately without LPS activation (CONTROL)
[2]AC: Air-conditioned

4.3 The effect of activated Lactoperoxidase system (LPS) and various holding time to pasteurisation on the initial total bacterial count of milk stored under different storage conditions

The influence of activated Lactoperoxidase system (LPS) and the holding time to pasteurisation on the initial TPC of the milk kept under various storage conditions is presented in Table 3. According to the results, no significant (P>0.05) difference in TPC

was observed among the milk treatments under test except treatment P12 which was significantly ($P<0.001$) different from the rest. Treatment P3 (LPS activated milk pasteurised after 3 hours) registered the lowest TPC, while the highest was recorded under treatment P12 (LPS activated milk pasteurised after 12 hours).

Table 3: Initial (0 hours) total plate count (cfu/ml x 10^3) of Lactoperoxidase system (LPS) activated pasteurised milk across and within storage conditions

[1]Treatment	Storage conditions			
	Charcoal cooler box ($18\text{-}20^0$C)	[2]AC room ($14\text{-}15^0$C)	Refrigerator ($5\text{-}8^0$C)	Room temp ($26\text{-}27^0$C)
P0	6.3 ± 0.12^b	6.3 ± 0.12^b	6.3 ± 0.12^b	6.3 ± 0.12^b
P3	6.8 ± 0.12^b	6.8 ± 0.12^b	6.8 ± 0.12^b	6.8 ± 0.12^b
P6	6.8 ± 0.12^b	6.8 ± 0.12^b	6.8 ± 0.12^b	6.8 ± 0.12^b
P9	9.1 ± 0.12^b	9.1 ± 0.12^b	9.1 ± 0.12^b	9.1 ± 0.12^b
P12	23.0 ± 0.12^a	23.0 ± 0.12^a	23.0 ± 0.12^a	23.0 ± 0.12^a
R0	6.8 ± 0.12^b	6.8 ± 0.12^b	6.8 ± 0.12^b	6.8 ± 0.12^b

[1]P0: LPS activated milk pasteurised immediately
P3: LPS activated milk pasteurised after 3 hours
P6: LPS activated milk pasteurised after 6 hours
P9: LPS activated milk pasteurised after 9 hours
P12:LPS activated milk pasteurised after 12 hours
R0: Raw milk pasteurised immediately without LPS activation
[2]AC:Air-conditioned
Means within the same column (for the same parameter) and raw with the same superscript are not significantly different ($P>0.05$).

According to Table 3, all storage conditions provided milk with similar initial chemical and bacteriological status across storage conditions. TPC values showed no significant ($P>0.05$) difference among them.

4.4 The effect of activated Lactoperoxidase system and various holding time to pasteurisation on both initial total plate count (TPC) and pH of milk before and after pasteurisation

As depicted in Figure 3 below, before pasteurisation there was no significant difference ($P>0.05$) in the initial TPC between R0 and either P0 or P3 but there was a significant difference ($P<0.001$) in the initial TPC between R0 and P6, P9 and P12.

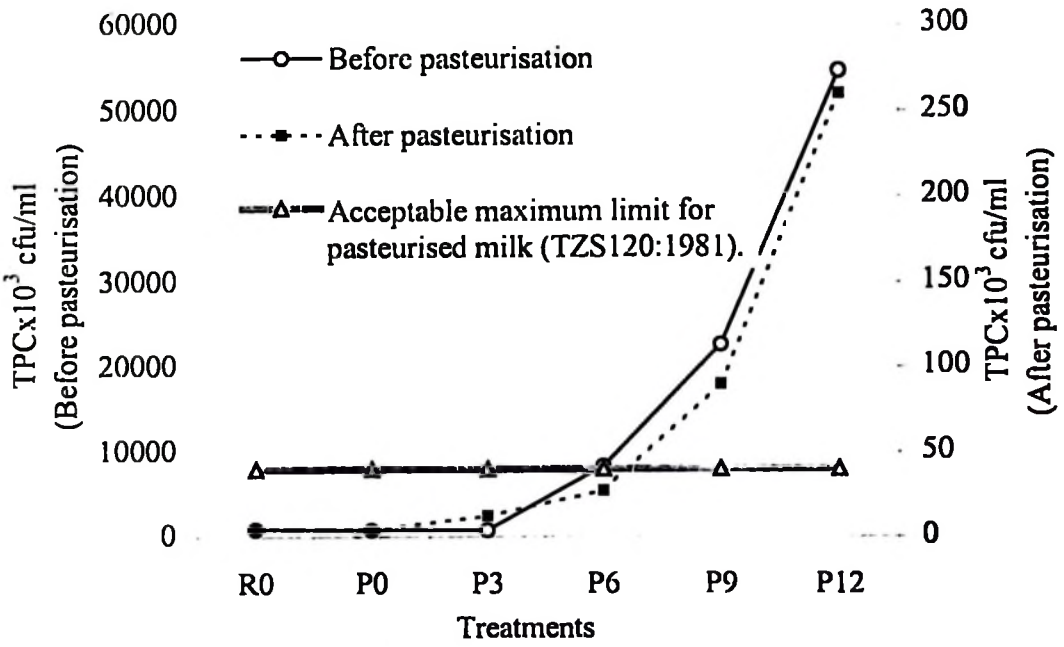

Figure 3.The effect of LPS activation and various holding time before and after pasterisation on the test milk total plate count

In case of pasteurised milk there was no significant difference ($P>0.05$) in the initial TPC among all treatments (LPS treated and untreated milk), this fact was also true for the within treatment results except for the holding time above 6 hours *i.e.* P6, P9 and P12 were significant differently ($P<0.001$) from the rest.

The pH results obtained before pasteurisation showed there was no significant (P>0.05) difference in the initial pH between untreated milk R0 and P0, P3 and P6 but, there was significant difference (P<0.001) in the initial pH between R0 and either P9 or P12 (*i.e.* significant pH difference started to be observed after 6 hours)

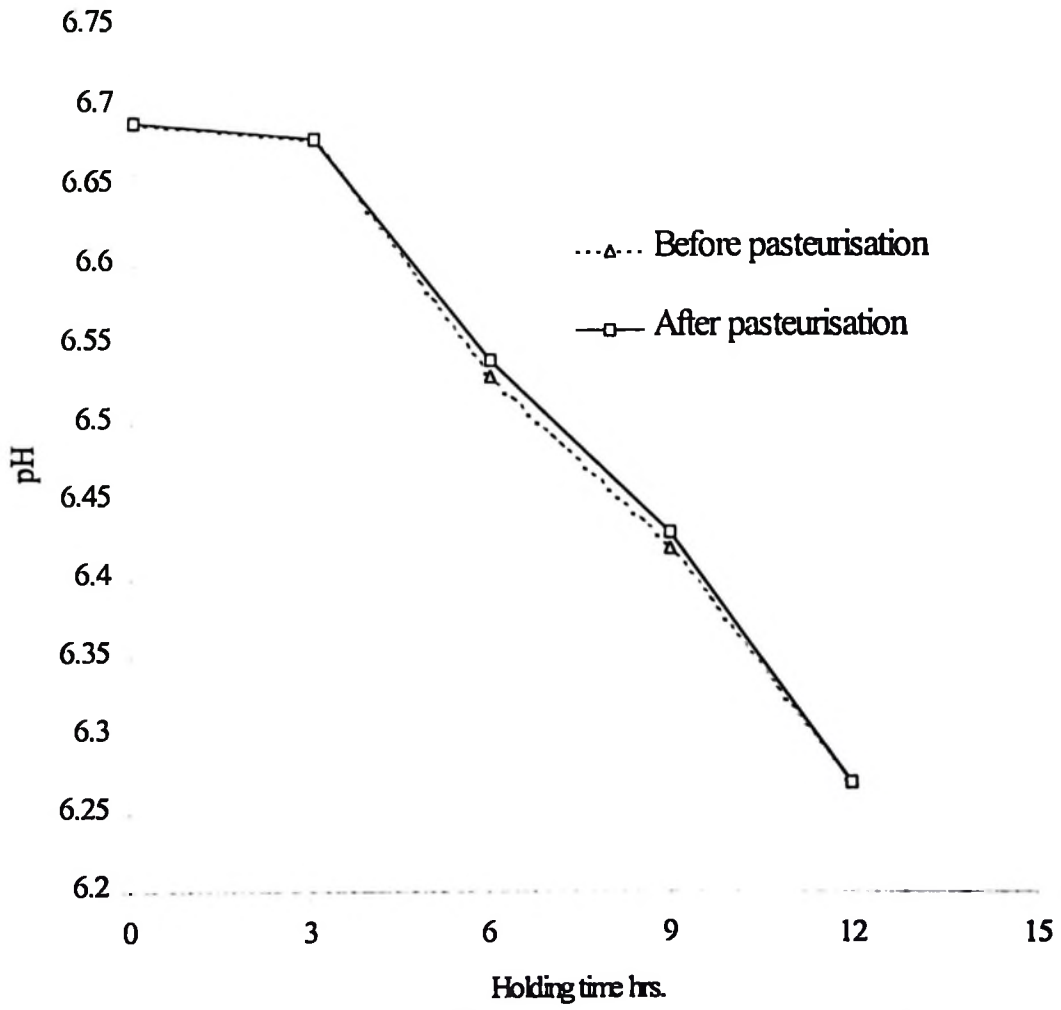

Figure 4. The effect of LPS activation and various holding time to pasteurisation on the test milk initial pH

When comparisons were made within treatment before and after pasteurisation no significant different (P>0.05) in the initial pH was observed. This observation was true

for all treatments.

The Initial pH difference among pasteurised milk treatments started being evident at the holding time of 6 hours and thereafter (*i.e.* P6, p9, and p12). No significant difference ($P>0.05$) in the initial pH was realised when R0 was compared to P0 and P3. Further clarity can be visualised under Fig. 4 above.

4.5 The effect of activated Lactoperoxidase system and various holding time to pasteurisation on the milk total plate count (TPC) after 24 hours exposure under various storage conditions

As is shown under Table 4(a) and 4(b), the total plate count values under charcoal cooler box ranged from $8.6x10^6$ to $18.2x10^6$ cfu/ml under treatment P3 and P12 respectively. Significantly ($P<0.001$) the highest TPC was obtained under LPS activated milk pasteurised after 12 hours (P12). Next to treatment P12 was P9 with a TPC value that was significantly ($P<0.001$) higher than that registered by treatment R0. Treatment P0 had significantly ($P<0.001$) lower TPC than that of R0. Significantly ($P<0.001$) the lowest TPC was realised by treatment P3. Next to P3 was P6 with significantly ($P<0.001$) lower TPC than that recorded by P0.

The total plate count (TPC) observed for the milk stored under air-conditioned room ranged from $4.1x10^6$ to $14.8.0x10^6$ (cfu/ml) under treatments P3 and P12 respectively.

Table 4(a) The effect of specific LPS activation periods on Total plate count (TPC) (x 10^6 cfu/ml) of milk after 24 hours storage under various storage conditions.

[1]Treatment	Storage conditions			
	Charcoal cooler box (18-20°C)	Air conditioned room (14-15°C)	Refrigerator (5-8°C)	Room temp (26-27°C)
P0	11.0±90^{d}	6.5±90^{c}	0.078±90^{a}	16.4±90^{d}
P3	8.6±90^{f}	4.1±90^{e}	0.046±90^{a}	16.0±90^{e}
P6	9.8±90^{e}	5.2±90^{d}	0.062±90^{a}	17.0±90^{d}
P9	15.0±90^{b}	12.9±90^{b}	0.210±90^{a}	20.0±90^{b}
P12	18.2±90^{a}	14.8±90^{a}	0.350±90^{a}	25.0±90^{a}
R0	13.8±90^{c}	6.3±90^{c}	0.08±90^{a}	18.2±90^{c}

Within a column means with different superscripts are significantly different (P<0.05)

Table 4(b) The effect of storage conditions on the total plate count (TPC x 10^6 cfu/ml) of milk pasteurised after specific LPS activation periods and 24hrs. storage.

[1]Treatment	Storage conditions			
	Charcoal cooler box (18-20°C)	Air conditioned room (14-15°C)	Refrigerator (5-8°C)	Room temp (26-27°C)
P0	11.0±90^{b}	6.5±90^{c}	0.078±90^{d}	16.4±90^{a}
P3	8.6±90^{b}	4.1±90^{c}	0.046±90^{d}	16.0±90^{a}
P6	9.8±90^{b}	5.2±90^{c}	0.062±90^{d}	17.0±90^{a}
P9	15.0±90^{b}	12.9±90^{c}	0.210±90^{d}	20.0±90^{a}
P12	18.2±90^{b}	14.8±90^{c}	0.350±90^{d}	25.0±90^{a}
R0	13.8±90^{b}	6.3±90^{c}	0.08±90^{d}	18.2±90^{a}

[1]P0: LPS activated milk pasteurised immediately
P3: LPS activated milk pasteurised after 3 hours
P6: LPS activated milk pasteurised after 6 hours
P9: LPS activated milk pasteurised after 9 hours
P12:LPS activated milk pasteurised after 12 hours
R0: Raw milk pasteurised immediately without LPS activation
Within a row means with different superscripts are significantly different (P<0.05)

Significantly (P<0.001) the highest TPC was observed under LPS activated milk pasteurised after 12 hours. Next to P12 was P9 with a TPC value, which was significantly (P<0.001) higher than that recorded by both treatments R0 and P0 whose TPC values were significantly (P<0.001) higher than that of treatment P6. Significantly

(P<0.001) the lowest TPC was recorded under treatment P3. (See Table 4(a) and 4(b)).

Refrigerated milk TPC values ranged from $0.046x10^6$ to $0.35x10^6$ (cfu/ml) under treatment P3 and P12 respectively. All treatments registered no significant (P>0.05) TPC value difference among them except P12 and P9 whose TPC values were significantly (P<0.001) the highest with no significant (P>0.05) TPC difference between them.

The TPC values for milk stored under room temperature ranged from $16.0x10^6$ cfu/ml to $25.0x10^6$ (cfu/ml) under treatment P3 and P12 respectively. Treatment P12 registered significantly (P<0.001) the highest TPC value followed by P9 whose TPC value was significantly (P<0.001) higher than that recorded under R0. The TPC values recorded under treatment P3. was significantly (P<0.001) the lowest though was not significant (P>0.05) different from both P0 and P6 whose TPC value was not significant (P>0.05) different but significantly (P<0.001) lower than that registered under treatment R0.

TPC values across storage conditions indicated under Table 4(b) shows. refrigeration produced milk with the lowest total plate count under each treatment while the highest TPC was always recorded under the room temperature storage environment. The total plate count ranged from $0.046.0x10^6$ which was the lowest. registered by treatment P3 under refrigeration to $25.0x10^6$ (cfu/ml). which was the highest TPC. obtained from P12 under the room temperature storage environment. Refrigeration provided the best storage environment by recording significantly (P<0.001) the minimum TPC values under each treatment. Next to refrigeration was the air-conditioned room. which was

significantly ($P<0.001$) better in TPC than the charcoal cooler box. Room temperature under this observation proved to be the poorest storage environment as all treatments registered under it recorded significantly ($P<0.001$) the highest TPC.

4.6 The effect of activated Lactoperoxidase system and various holding time to pasteurisation on the milk total plate count (TPC) after 48 hours exposure under various storage environment

Table 5, shows that the total plate count of milk stored under charcoal cooler box ranged between $17.0x10^6$ to $32.0x10^6$ (cfu/ml) under treatments P3 and P12 respectively. Significantly ($P<0.001$) the highest TPC was registered under treatment P12 followed by treatment P9. Next were both treatments R0 and P0 with no significant ($P>0.05$) difference between them but with significantly ($P<0.001$) higher TPC than treatment P6. Treatment P3 registered significantly ($P<0.001$) the lowest TPC followed by treatment P6 whose TPC value was significantly ($P<0.001$) lower than that registered by both treatments R0 and P0.

The highest total plate count recorded under air-conditioned room was 26.0×10^6 while the lowest was 13.0×10^6 (cfu/ml) under treatment P12 and P3 respectively. Treatment P3 registered significantly ($P<0.01$) the lowest total plate count though was not significantly ($P>0.05$) different from P6. Next was treatment P0 which had significantly ($P<0.001$) lower total plate count values than treatment R0. Significantly ($P<0.001$) the highest TPC was recorded under treatment P12 followed by P9 whose TPC value was significantly higher ($P<0.001$) than that under treatment R0.

The total plate count for refrigerated milk with various holding time to pasteurisation but receiving the same level of LPS activation is presented in Table 5. There was no significant (P>0.05) difference in total plate count between treatments P12 and P9. Treatment P0, P6 and R0 TPC values were not significantly (P>0.05) different from each other but had significantly lower (P<0.001) TPC value than that recorded under treatment P12 and P9. Treatment P3 registered significantly (P<0.001) the lowest TPC.

Table 5: Total plate count (TCP 10^6 cfu/ml) of LPS activated pasteurised milk after 48 hours storage under different conditions.

[1]Treatments	Storage conditions			
	Charcoal cooler (18-20°C)	Air conditioned (14-15°C)	Refrigerator (5-8°C)	Room temp. (26 –27°C)
P0	22.0 ± 210^{c}	13.8 ± 210^{d}	0.12 ± 210^{a}	38.0 ± 210^{c}
P3	17.0 ± 210^{a}	13.0 ± 210^{a}	0.084 ± 210^{a}	28.0 ± 210^{a}
P6	20.0 ± 210^{c}	13.2 ± 210^{f}	0.16 ± 210^{b}	32.0 ± 210^{f}
P9	25.0 ± 210^{d}	18.6 ± 210^{e}	0.36 ± 210^{b}	41.0 ± 210^{e}
P12	32.0 ± 210^{e}	26.0 ± 210^{b}	0.62 ± 210^{a}	56.0 ± 210^{d}
R0	23.0 ± 210^{b}	15.0 ± 210^{c}	0.14 ± 210^{a}	39.0 ± 210^{b}

[1]P0: LPS activated milk pasteurised immediately
P3: LPS activated milk pasteurised after 3 hours
P6: LPS activated milk pasteurised after 6 hours
P9: LPS activated milk pasteurised after 9 hours
P12:LPS activated milk pasteurised after 12 hours
R0: Raw milk pasteurised immediately without LPS activation
Within a column means with different superscripts are significantly different (P<0.05)

The total plate count recorded under room temperature storage condition ranged between 28.0 x 10^6 and 56.0 x 10^6 (cfu/ml) under treatment P3 and P12 respectively. Treatment P12 was significantly the highest (P<0.001) in total plate count followed by treatment P9 whose total plate count value was significantly higher (P<0.001) than

that recorded by the treatment P0. Treatment P3 registered significantly the lowest ($P<0.001$) total plate count. Next to it was P6 whose total plate count value was significantly ($P<0.001$) lower than that recorded under treatment R0.

The total plate count within the same treatment under different storage condition are also presented in Table (5). The TPC values ranged from $0.084.0x10^6$ to 56.0×10^6 (cfu/ml) under treatment P3 and P12 stored under refrigeration and room temperature storage conditions respectively. Each treatment registered the highest total plate count under room temperature while the lowest was obtained under refrigeration condition.

In all cases of LPS treatment storage temperature had significant ($P<0.05$) influence on the pasteurise milk shelf life, with refrigeration giving the longest shelf life followed by air conditioned room, charcoal cooler and the room temperature.

4.7 The effect of activated LPS and the holding time to pasteurisation on the milk total plate count after 72 hours exposure under various storage conditions.

Treatment P3 and P6 under air-conditioned storage were able to reach a shelf life of 72 hours with total plate count values expressed in Table 6. The total plate count of P3 was significantly ($P<0.001$) lower than that recorded under treatment P6. This shows P3 performed better than P6 in TPC values.

Reference is made on Table 6 in which the total plate count values under refrigeration are presented. The total plates count results ranged between $1.8x10^6$ to $0.12x10^6$ (cfu/ml) under treatments P12 and P3 respectively. Treatment P12 recorded

significantly (P<0.001) the highest TPC value of all other treatments. Next was treatment P9 with a total plate count value which was significantly (P<0.001) greater than that recorded by treatment P6, P0 and R0 but with no significant (P>0.05) TPC difference among them. Significantly (P<0.001) the lowest TPC was recorded by treatments P3. The test milk from other storage conditions could not reach a shelf life of 72 hours.

Table 6: TPC ($x10^6$ cfu/ml) of LPS activated pasteurised milk after 72 hours under air-conditioned room and refrigeration

[1]Treatment	Storage conditions		Remarks
	Air conditioned room (14-15°C)	Refrigeration (5-8°C)	
P0	-s	0.18 ± 0.02^{b}	
P3	20.0 ± 0.02^{b}	0.12 ± 0.02^{a}	***
P6	23.0 ± 0.02^{a}	0.16 ± 0.02^{b}	***
P9	-s	0.66 ± 0.02^{c}	
P12	-s	1.8 ± 0.02^{d}	
R0	-s	0.19 ± 0.02^{b}	

[1]P0: LPS activated milk pasteurised immediately
P3: LPS activated milk pasteurised after 3 hours
P6: LPS activated milk pasteurised after 6 hours
P9: LPS activated milk pasteurised after 9 hours
P12:LPS activated milk pasteurised after 12 hours
P0: Raw milk pasteurised immediately without LPS activation
-s: Spoiled
***- Significant at P<0.001
Means within the same column and the same raw with the same superscript are not significantly different (P>0.05).

4.8 The effect of LPS activation and variation of holding time to pasteurisation on the refrigerated milk shelf life (96 to 480 hours.)

As represented in table 2 above, treatment P12 under refrigeration storage condition remained fresh for about 240 hours (*i.e.*10days). The TPC at 240 hours ranged from $0.85x10^6$ to $21.9x10^6$ (cfu/ml) under treatment P3 and P12 respectively. Treatment P12 TPC value was significantly the highest followed by P9, R0, P0 and then P6. Treatment P3 registered significantly ($P<0.001$) the lowest TPC value. For all treatments the pH values followed the same trend by being significantly ($P<0.001$) different from one another. Treatment P9 got spoiled after 264 hours followed by R0 at 288 hours. Treatment P0 was next to R0 followed by P6 with a shelf life of 336 hours and 408 hours respectively. The highest shelf life was recorded under treatment P3 which remained fresh up to 456 hours. (19days).

Treatment P3 excelled in both TPC and pH values up to the 20^{th} day when it got spoiled. In fact the treatment remained significantly ($P<0.001$) different from the rest in both pH and TPC up to the end. Next to it was treatment P6 then P0.

4.9 The effect of activated LPS and the holding time to pasteurisation on the milk pH under various storage conditions.

4.9.1.Charcoal cooler box (18-20^{0}C)

The initial pH of the test milk ranged between 6.7 for treatments P0, R0 to 6.55 under treatments P12. This initial pH used to be decreasing with the increase of the holding

time to pasteurisation as shown in Fig. 5.

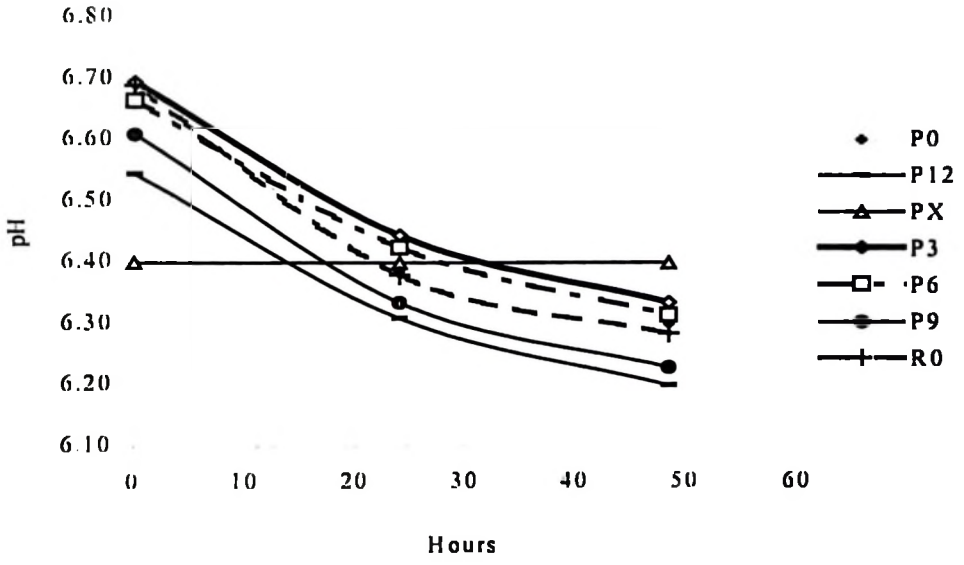

Figure 5. The effect of activated LPS on the pH of pasteurised milk stored under the charcoal cooler box

Key

P0: LPS activated milk pasteurised immediately
P3: LPS activated milk pasteurised after 3 hours
P6: LPS activated milk pasteurised after 6 hours
P9: LPS activated milk pasteurised after hours
P12: LPS activated milk pasteurised after 12 hours
R0: Raw milk without LPS activation pasteurised immediately
PX: pH acceptable limit (O'Connor, 1995)

The pH activity in milk remained static for about 2 hours from the time LPS activation was made, after this period pH development started being noticeable. Milk under all treatments at 0 hours remained acceptably fresh except treatment P12 which was below the acceptable limit.

Most treatment managed to remain fresh for a period of between 7 hours and 12 hours with the exception of P9 and P12 whose pH value had already been registered as unacceptable. Throughout the experimental period the pH recorded by treatment P3 was always above others, this was followed by that of treatments P6, P0, R0 and lastly P12 whose pH value was always the lowest followed by P9. Most of the treatments were recorded spoiled after 48 hours except P12, which was recorded spoiled after 24 hours.

4.9.2. Air-conditioned room (13-14^0 C)

The maximum shelf life for the milk stored under this storage condition was registered by both treatment P3 and P6 whose shelf life could not go beyond 72 hours. All other treatments shelf life performed below 72 hours but were above 48 hours except for treatment P12 which was reported spoiled at 48 hours. All the milk samples started with an acceptable initial chemical property by recording pH values above acceptable limit (pH 6.64) as displayed by Fig. 6. Treatment P9 and P12 behaved differently as their pH values were below acceptable limit and they were reported spoiled after 48 hours.

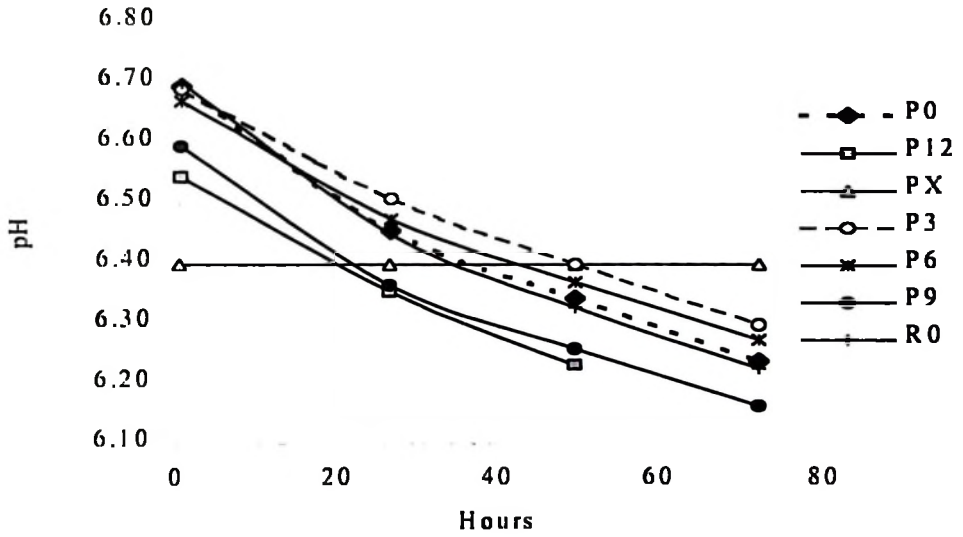

Figure 6. The effect of activated Lactoperoxidase system on the pH of pasteurised milk stored under air conditioned room (14-15°C)

Key

P0: LPS activated milk pasteurised immediately
P3: LPS activated milk pasteurised after 3 hours
P6: LPS activated milk pasteurised after 6 hours
P9: LPS activated milk pasteurised after 9 hours
P12: LPS activated milk pasteurised after 12 hours
R0: Raw milk without LPS activation pasteurised immediately
PX: pH acceptable limit (O'Connor, 1995)

4.9.3. Refrigeration (5-8^{0}C)

The milk chemical characteristics under refrigeration storage can be visualised from Fig 7. Treatment P12 milk quality was below standard/acceptable limit just from the beginning. As depicted in other storage environments the initial milk chemical characteristic was the same for each treatment across storage conditions as it was derived from the same source and hence continued to record the minimum pH throughout the

observation period. Treatment P12, pH values went outside acceptable limit after 54

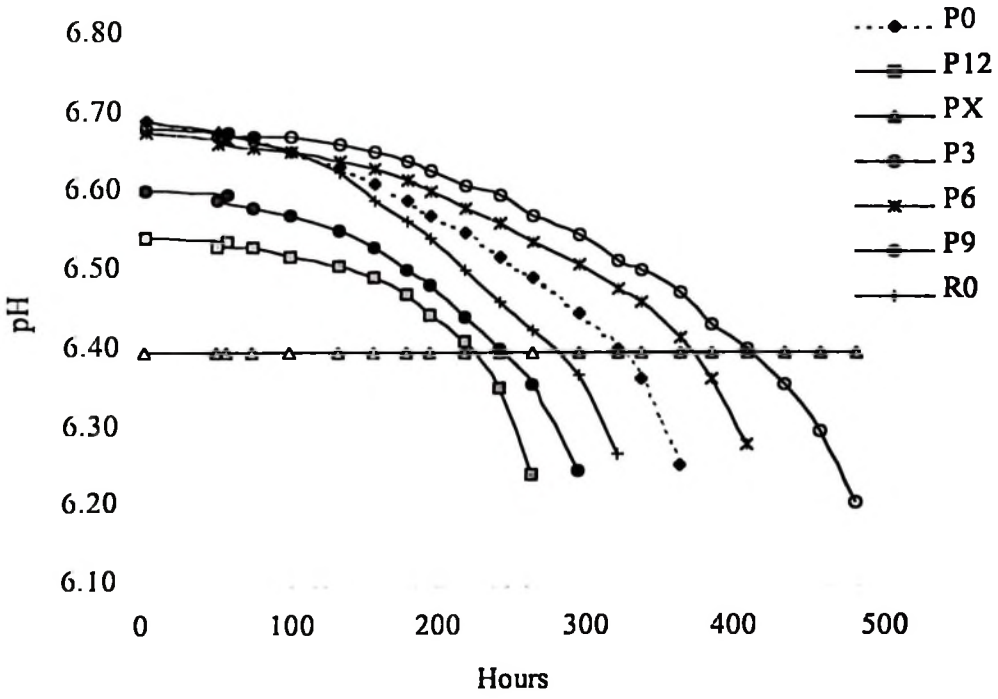

Figure 7. The effect of activated lactoperoxidase system on the pH of pasteurised milk stored under refrigeration (5-8°C)

Key

P0: LPS activated milk pasteurised immediately
P3: LPS activated milk pasteurised after 3 hours
P6: LPS activated milk pasteurised after 6 hours
P9: LPS activated milk pasteurised after 9 hours
P12: LPS activated milk pasteurised after 12 hours
R0: Raw milk without LPS activation pasteurised immediately
PX: pH acceptable limit (O'Connor, 1995)

4.9.4. Room temperature (26-27^{0}C)

The pH behaviour of the milk under this storage condition is displayed in the graphs below (Fig. 8). All the milk samples were registered spoiled after 48 hours except P12 and P9, which were spoiled after 24 hours. The graph under P3 was always above all other

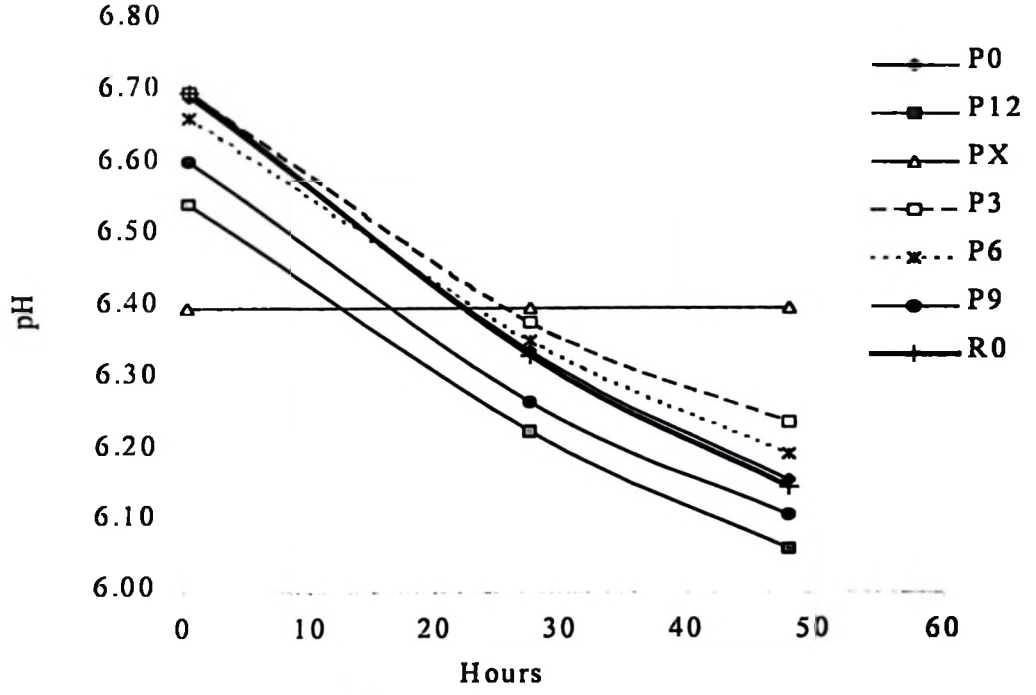

Figure 8. The effect of activated lactoperoxidase system on the pH of pasteurised milk stored under the room temperature (26-27°C)

Key
P0: LPS activated milk pasteurised immediately
P3: LPS activated milk pasteurised after 3 hours
P6: LPS activated milk pasteurised after 6 hours
P9: LPS activated milk pasteurised after 9 hours
P12: LPS activated milk pasteurised after 12 hours
R0: Row milk without LPS activation pasteurised immediately
PX: pH acceptable limit (O'Connor, 1995)

treatments while the lowest pH graph was possessed by P12. Compared to other storage

environment milk under room temperature storage proved to have the shortest shelf life followed by the charcoal cooler box then the air-conditioned room. Refrigeration storage produced milk of the longest shelf life as compared to other given storage conditions.

4.10 Coliform test.

All the treatments did not favour the growth of coliform bacteria. This test was done to the milk immediately after having been pasteurised and daily thereafter. No coliform growth was observed in all the treatments. Hence forth, there was no difference ($P>0.05$) between the two treatments (*i.e.* LPS activated and untreated milk).

4.11 The effect of activated LPS and holding time to pasteurisation on the pH and TTA of natural fermented milk and yoghurt.

Table 7 shows that, after 24 hours incubation period under room temperature the mean pH of the fermented milk ranged from 3.73 in P12 to 4.06 in P3. Treatment P3 registered the highest pH though there were no significant ($P>0.05$) difference observed between treatment P3 and treatment P0, R0 and P6. Treatment P9 was significantly ($P<0.001$) different from the rest except P6 and R0. Next to treatment P9 was P12, which had significantly ($P<0.001$) the lowest pH from the rest.

According to Table 7 the TTA of the natural fermented milk ranged from 0.96±0.02 to 1.15±0.02 under treatment P3 and P12 respectively. Treatment P12 registered Significantly ($P<0.001$) the highest TTA of all other treatments. This was followed by

both P9 and P6 with no significant (P>0.05) difference between them. No significant (P>0.05) difference was observed on treatments R0, P0, P3 and P6 and between P6 and P9.

After 3.30 hours incubation period the pH of yoghurt ranged from 4.35 to 4.09 under P3 and P12 respectively. Treatment P12 had significantly P<0.05) the lowest pH from the rest. Next was treatment P9 with no significant (P>0.05) difference from P6 but significantly (P<0.05) difference from the remaining treatments. Treatment R0, P0, P3 and P6 registered no significant (P>0.05) difference among themselves.

Table 7: pH and TTA of natural fermented milk and yoghurt made from LPS activated fresh milk exposed under different holding time to pasteurisation

[1]Treatment	[3]pH		[2]TTA	
	Yoghurt	Naturally fermented milk	Yoghurt	Naturally fermented milk
P0	$4.33 \pm .03^{c}$	4.03 ± 0.03^{c}	0.88 ± 0.01^{c}	0.98 ± 0.022^{c}
P3	$4.35 \pm .03^{c}$	4.06 ± 0.03^{c}	0.89 ± 0.01^{c}	0.96 ± 0.022^{c}
P6	$4.28 \pm .03^{bc}$	3.97 ± 0.03^{bc}	0.91 ± 0.01^{c}	1.03 ± 0.022^{bc}
P9	$4.23 \pm .03^{b}$	3.87 ± 0.03^{b}	0.94 ± 0.01^{b}	1.08 ± 0.022^{b}
P12	$4.09 \pm .03^{a}$	3.73 ± 0.03^{a}	0.98 ± 0.01^{a}	1.15 ± 0.022^{a}
R0	$4.31 \pm .03^{c}$	3.98 ± 0.03^{bc}	0.90 ± 0.01^{c}	0.98 ± 0.022^{c}

[1]P0: LPS activated milk pasteurised immediately
P3: LPS activated milk pasteurised after 3 hours
P6: LPS activated milk pasteurised after 6 hours
P9: LPS activated milk pasteurised after 9 hours
P12: activated milk pasteurised after 12 hours without LPS activation.
R0: Raw milk pasteurised immediately without LPS activation
[2]TTA: Titrable acidity
[3]pH: Positive (+ve) hydrogen ion concentration in milk
Means within the same column with the same superscript are not significantly different (P>0.05).

The titrable acidity values for yoghurt ranged from 0.88±0.01 to 0.98±0.01 under

treatment P0 and P12 respectively. Treatment P12 had significantly ($P<0.001$) the highest TTA from the rest. Next to P12 was treatment P9 who's TTA was significantly ($P< 0.001$) different from the remaining treatments i.e. P0, R0, P3 and P6. The highest TTA value was observed under treatment P3 but with no significant ($P>0.05$) difference from that of P0, R0, and P6.

4.12 Temperature comparisons between the environment and the charcoal cooler

During the experimental period the minimum environmental temperature recorded was 18^0C; where as the charcoal cooler box recorded 13.2^0C as its minimum. During this experimental period 36.5^0C was registered as the maximum environmental temperature, while the charcoal cooler box managed to achieve 26.2^0C as its maximum temperature.

Table 8: Temperature and relative humidity variations under charcoal cooler box and room temperature storage conditions

	Parameters		
Storage	Maximum	Minimum	Mean(±se)
Charcoal cooler box °C	22.86±1.66	15.67± 1.39	19.27 ± 0.13[a]
Room temperature °C	31.81±2.48	21.12±1.04	26.41 ± 0.14[b]
Relative humidity	48.0	100.0	93.72± 7.66

Source: SUA Farm Meteorological station

Table 8 shows that the average maximum environmental temperature obtained was 31.81^0C where as 21.12^0C was its average minimum realised. Meanwhile 22.87^0C and 16.67^0C were the average maximum and minimum temperatures attained by the cooler. The mean temperature difference between the environment and that recorded in the charcoal cooler for the maximum and minimum temperatures were 8.95^0C and 4.45^0C

respectively. The environmental humidity during experimental period ranged from 48% RH to 100% RH with the mean of 93.72± 7.66% RH. When the environmental temperature was compared against that of charcoal cooler, the cooler registered significantly ($P<0.001$) lower temperature than the surroundings (Ref. to Table 8).

4.13 Field data results

The chemical and bacteriological quality of the milk used was within acceptable range, this is in accordance to Tanzania bureau of standards (TBS 1996) Ref. to Tab.9. The resident Thiocyanate (SCN^-) content of Mwakaleli milk was not determined.

Table 9: Characteristic of the milk used.

Source	[2]TPC ($x10^6$ cfu/ml)	[1]TTA
Mwakaleli (Morning)	1.5	0.18
Mwakaleli (Evening)	1.7	0.18

[1]TTA: Titrable acidity
[2]TPC: Total plate count (cfu/ml)

4.13.1 Shelf life of LPS treated evening milk

As displayed in Fig. 9, all activated LPS treated milk samples that were collected the previous evening remained fresh up to the next morning where as for the untreated milk, 30% were spoiled, this is after a holding period of about 14 hours. Milk quality tests showed, there was no significant ($P>0.05$) difference between the two treatments after being kept overnight.

The distance between the sources of this milk (Mwakaleli) to Mbeya receiving centre is

about four hours travel, therefore plus the previous 14 hours it makes a total of about 19 hours ultimately when the previous evening milk reached Mbeya receiving centre no significant (P>0.05) difference was observed between the two treatments as all untreated milk and most of the LPS treated milk samples had already been spoiled.

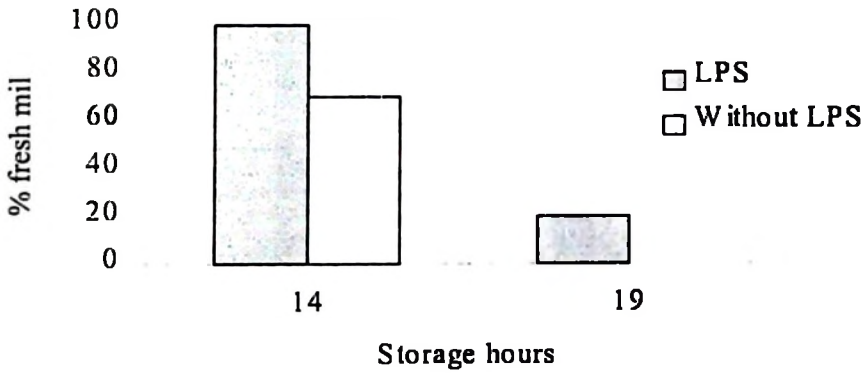

Figure 9: The effect of activated LPS on the evening milk shelf life (16-20.2^{0}C)

4.13.2: Shelf life of LPS treated morning milk

There was no significant (P>0.05) difference between the LPS treated and untreated fresh milk below 6 hours where as at between 6 and 12 hours there was significant (P<0.01) difference between the two. No significant (P>0.05) difference was observed after 12 hours as most of the samples from both treatments were spoiled. (Ref. to Fig.10).

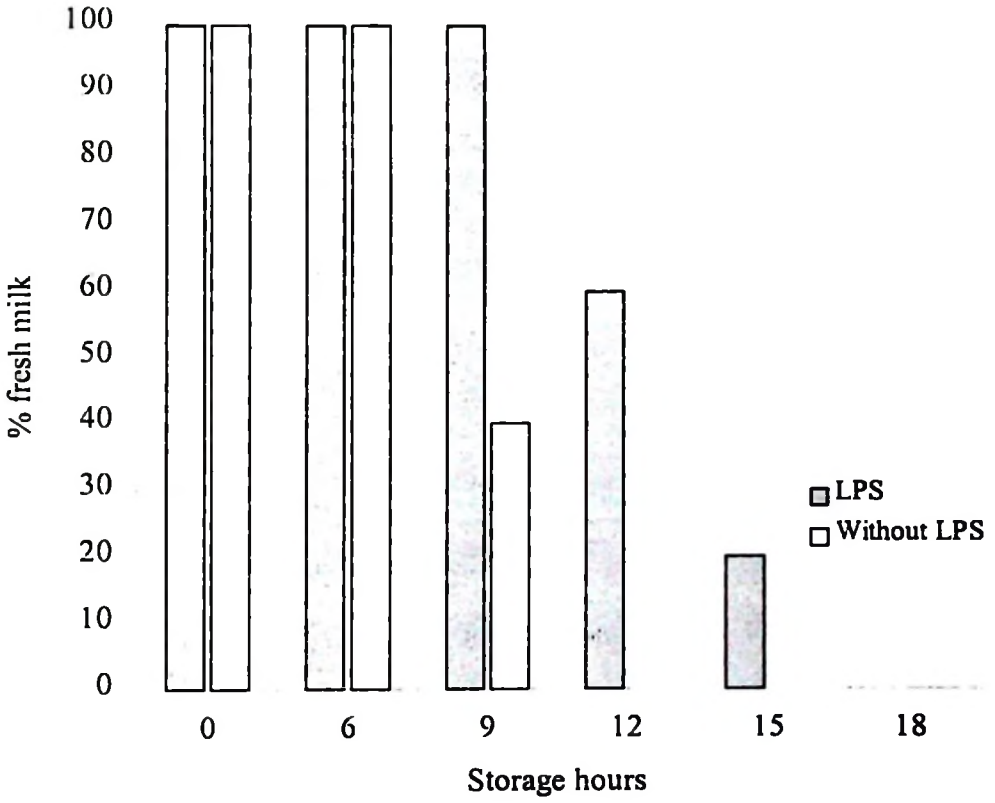

Figure 10: The effect of activated LPS on morning fresh milk shelf life (17-22.2^{0}C)

4.13.3 Milk physical characteristics

There was no immediate or subsequent change in colour or texture of the milk after LPS activation. The taste and smell of the milk seemed to be normal. No coagulation and gas production was observed in the treated milk.

CHAPTER V

5.0 DISCUSSION

5.1 Thiocyanate content of experimental milk

Thiocyanate (SCN^-) content of milk (4.09±1.1 ppm) observed from Magadu dairy farm (SUA) is within normal range normally found in milk, which is between 2-7 ppm (IDF, 1988). However, the level of thiocyanate found in milk are fairy variable as levels of between 10-15 ppm have also been reported by Boulogne (1959) and Lawrence (1970). The important source of thiocyanate content in milk is the feeds an animal is exposed to, for instance vegetation like cauliflower and white Cabbage contains 88 ppm and 31 ppm respectively (Korhonen, 1980). Thiocyanate level in milk is more variable and is mainly usually derived from glucosides present in Brassica feed, rapeseed and *Rahanus spp.* in the feed. Different vegetation has different levels of thiocyanate content depending on the soil and the type of species (Lawrence, 1970, Reiter, 1978, IDF, 1988)

The recommended external addition of thiocyanate for the activation of LPS from the special ready-made sachets bases on the inherent normal SCN^- concentration of between 2-7 ppm SCN-. It is recommended that after external addition the total SCN^- concentration should not exceed 15 ppm. It is obvious therefore that the experimental milk obtained from Magadu dairy farm (SUA) was within normal range in SCN^- concentration and the activated LPS biotechnology can definitely be applied without any problem.

Although SCN^- is a normal electrolyte in many secretions, it is well established that high serum level of SCN^- result in impairment of thyroid gland functioning *i.e.* Hypothyroidism (IDF, 1988). But according to Finnish investigation doses of between 200-400mg are necessary to induce thyrostatic effect (Dahl *et. al.*, 1985, IDF, 1994). Equally well pharmaco-kinetics studies of Nitro prucide, which is used in hypertension treatment, have clearly demonstrated that serum levels above 18-20 ppm of SCN^- are necessary to cause impaired thyroid functioning (Dahl *et. al.*, (1985)). This investigation indicates that the level of SCN^- (4.09ppm) obtained from the experimental milk warrant recommended external addition of the SCN^- (10ppm) for proper LPS activation of which the total (14.09ppm) could not exceed the limit (15ppm) (FAO, 1999). The results obtained are in agreement with other investigations and are within the normal range expected. The milk in question can withstand recommended SCN^- external addition for activated LPS without a negative effect to human health and especially not causing any disturbance on the Thyroid gland functioning. (Dahl Van den Berg *et al*, 1985)

5.2 Milk shelf life

Throughout the experiment under all storage conditions LPS treated pasteurised milk performed significantly better than the untreated immediately pasteurised milk (R0). This was true for all LPS treatments except P9 and P12 whose performance was generally below the control R0. This shows that the time limit of LPS holding time before pasteurisation is between 6 hours and 9 hours as its activity diminishes tremendously after 6 hours. This was proved by P9 and P12 performance being below R0. It is obvious therefore that in the tropics the proper LPS activation resident time before processing

should be close to 6 hours but should not reach 9 hours at room temperature as the bacteriostatic/bactericidal effect of activated LPS declines after 6 hours.

Treatment performance under charcoal cooler storage recorded relatively longer shelf life as compared to room temperature storage, this was true for within treatment across storage. The result obtained under Charcoal cooler storage encourages the applicability of this simple and cheap technology to increase fresh milk shelf life in the remote rural areas where there is no electricity, and there is absence/poor communication infrastructures. It is obvious therefore that the realised shelf life increase would give the milk enough time to reach the lucrative markets in the urban centres.

Charcoal cooler storage gives an additional increase in milk shelf life to the LPS treated milk this is true when one compares to the under room temperature storage. Therefore the application of both LPS treatment alone and or in combination with charcoal cooler storage technologies can therefore reduce rural milk spoilage, increase product value, facilitate marketing and in the final analysis encourage increased production since the technology is simple, cheap, easy to apply and it is user friendly.

As expected refrigeration storage condition produced milk with the longest shelf life above all other storage conditions (i.e. Air conditioned room, charcoal cooler and under the room temperature), this was true for all treatments. The source of variation between treatments within storage has always been the holding time to pasteurisation after LPS activation while the observed shelf life difference (pH and TPC development) within

treatment across storages is brought about by the difference in environmental temperature under each storage condition. The temperature under refrigeration as compared to other storages was recorded to be the minimum. After refrigeration (5-8^{0}C) were the air-conditioned room (14-15^{0}C), the charcoal cooler (18-20^{0}C) and then the room temperature (26-27^{0}C). The difference in fresh milk keeping quality within treatment across storages is the manifestation of unequal microbial development in milk, which has been brought about by the difference in storage temperatures. The changes realised (chemical, physical and biological characteristics) express microbial metabolism in milk whose multiplication and development is activated by the rise in temperature (within acceptable limit). Hence the higher the temperature (within limit) the faster the multiplication rate culminating in shorter milk shelf life. The above fact explains the difference in milk shelf life within treatment across storages was realised. Among other things increased fresh milk shelf life is a function of the surrounding environment storage temperature. It is obvious therefore that across storages the causative agent of the observed milk shelf life variations is non other than the corresponding different environmental temperatures registered under each storage condition. This is why milk under refrigeration storage had relatively longer shelf life followed by the air-conditioned room, charcoal cooler box and then the room temperature. The refrigeration relatively records extremely low temperature, which allowed minimal microbial proliferation hence longer fresh milk keeping quality. (Henderson 1971, Van den Berg 1988).

Though refrigeration storage condition provided the best storage environment followed by the air-conditioned room but they have the limitation of not being generally available

in the rural areas due to the absence of the supportive infrastructures like a reliable energy source to operate such systems, cost of operation and maintenance, initial capital investment, availability plus a constant flow of spare parts and technicians, technical know how and poverty. Therefore according to this study the best alternative storage technology for the tropical remote rural areas is non other than the charcoal cooler and activation of the LPS in milk.

5.3 Milk quality

The initial chemical and biological quality of the milk used from both SUA and Mwakaleli village was within acceptable limit (TZS, 1981) The milk physical and organoleptic quality was normal without any abnormality likewise was the milk quality after the LPS activation. The absence of colour, smell, gas production, thickness plus any visible abnormality in LPS treated fresh milk supported its suitability as a preservative.

Both initial pH and TPC obtained under various treatments was the function of the holding time before pasteurisation. The LPS holding time of 3 hours (P3) was found to be significantly effective from all other LPS holding times by recording the longest fresh milk shelf life. The noticeable chemical (pH) change started to be conspicuous after the holding time of 6 hours (P6) from milking (*i.e.* turning point) and likewise was the bacteriological activity. The reason behind this could have been the slowly expiry of the useful active resident time of the activated LPS. It appears LPS did manage to halt microbial development in milk under room temperature beyond 6 hours since initial introduction, which is a manifestation of its bacteriostatic/bactericidal nature. These

results are in agreement with a similar work done by Claesson (1995).

5.4 Performance of the charcoal cooler box

When temperature comparisons were made between the environments against that of charcoal cooler box, temperature under charcoal cooler box was found to be significantly ($P<0.001$) lower than that of the environment. Results show 36.5^0C was the maximum environmental temperature recorded where as 26.2^0C was registered as its minimum. Meanwhile charcoal cooler box had 23^0C as its maximum temperature and 13.2^0C registered as its corresponding minimum. On the other hand the average maximum and minimum temperatures attained by the charcoal cooler box was 22.87^0C and 15.67^0C where as 31.81^0C and 21.12^0C were the environments maximum and minimum respectively. The mean temperature difference between the two conditions was 8.94^0C and 5.45^0C for the maximum and minimum temperatures respectively. All these fact testifies the ability of the charcoal cooler box to maintain 8.94^0C below the environmental maximum temperatures and 5.45^0C below that of its minimum. The charcoal cooler performance (Grand mean 19.27^0C) under Morogoro (SUA) environment *i.e.* low land (Grand mean 26.47^0C) was very close to the environmental temperature (Grand mean18.1^0C) that would generally be achieved in the highlands (Mwakaleli)

When the temperature variations within the charcoal cooler box were compared to that of the environment, the variations in the cooler was found to be moderate. This shows that the environmental temperature had little influence on that of the cooler; this was especially true during the daytime when the temperature shoots up tremendously.

Maintenance of more or less constant temperature fluctuations in the charcoal cooler box could be emanating from the excellent insulative property of the cooler surroundings. The average minimum temperature realised by the cooler *i.e.* 15.67^{0}C was found to be a bit higher than that of Bengstone and Whytaker, (1986) and Dulle (1991) who managed to obtain 11^{0}C and 11.19^{0}C as their average minimum temperatures respectively. The difference could be due to the factors affecting evaporative cooling, whose variations is a function of the difference in weather pattern that include the prevailing environmental temperature, relative humidity, and airflow. Definitely these factors must have affected the charcoal cooler performance. The other equally important reasons must have been the difference in geographical locations and seasons of the year *i.e.* hot/cold season, dry/humid season, presence or absence of the geographical physical barriers like mountains, plains, shrubs /trees, buildings, clouds, fogs, latitude, longitude and the altitude of the region. These facts explain the reasons for variations in minimum temperature records in various days with the same or different minimum environmental temperature and relative humidity.

Studies show that most benefits of milk preservations by cooling are obtained by cooling milk to temperatures between 10^{0}C to 15^{0}C for dirty and clean milk respectively (FAO, 1972). This fact supports the performance achieved by the charcoal cooler box because the environment it can provide (mean 15.7^{0}C) is not very far from the above recommended fresh milk storage temperatures limits only that the milk used should be of good initial bacteriological quality.

5.5 Room temperature storage

Within each treatment milk preserved under room temperature registered significantly ($P<0.001$) the highest TPC and the lowest pH ($P<0.01$) than any other storage conditions (Tab 4 and 5 and Fig. 8). The higher TPC and the lower pH obtained could have been due to increased bacteriological activities resulting in corresponding shorter milk shelf life. The rapid changes in physical, chemical and biological characteristic of the milk under room temperature storage could have been due to the relatively higher storage temperature recorded under room temperature storage environment. The increase in temperature has an effect on the rate of chemical reactions (cellular metabolism) through activating microbial enzymes, which result in faster cell division hence shorter milk shelf life.

The great temperature difference existing between the room temperature and charcoal cooler box without mentioning the other available storage conditions could be the source of the shorter milk shelf life under room temperature storage. Usually warmer environments favour increased microbial activity and thus relatively higher microbial multiplication rate, which cause immediate milk spoilage (Van den Berg 1988).

5.6 The effect of activated LPS and holding time to pasteurisation on milk pH, and total bacterial count (TPC)

The absence of initial pH difference in milk before pasteurisation among treatments R0, P0 and P3 and the corresponding difference witnessed when R0 was compared to P6, P9

and P12 could have been derived from the bacteriostatic bactericidal action of the LPS on milk micro-organisms. This LPS action extends milk lag phase in between 3 and 6 hours due to the continual diminishing antimicrobial potential of the LPS with time. This fact results in continued resurgence of microbial growth in milk after 3 hours. The increased growth rate is the function of the corresponding increased growth freedom, which is noticeable after 3 hours. Usually the length of the natural milk microbial lag phase depends on the initial microbial load and the prevailing storage temperature.

In case of pasteurised milk the absence of initial TPC difference among treatments could be due to the effectiveness of pasteurisation temperature/time combination used plus the LPS administered in milk i.e. very few thermodurics could survive both activated LPS and the pasteurisation temperature/time combination used (80^0C/ for one minute). While within (TPC) treatment difference observed after 6 hours before and after pasteurisation is derived from the upsurge of the microbes in milk brought about by the continuing LPS diminishing activity, which result in increased microbial growth freedom after 3 hours.

In case of pH no significant ($P>0.05$) difference was observed in the initial pH within treatment while a significant initial pH difference between treatments was realise at 6 hours and thereafter. The absence of the statistical pH difference within treatment is because the act of pasteurisation could not change the already realised chemical activity brought about by the microbial activity in the test milk. The initial pH difference observed among treatments before pasteurisation from 6 hours and thereafter could be resulting from resumption of microbial proliferation in milk, which is obtained from reduced LPS

antimicrobial action after 3 hours. (Tab.4 and Fig. 3 and 4)

The highest pH and the lowest TPC were registered under treatment P3; this is in accordance to the data provided by Table 5. This shows that treatment P3 performance was the best from the rest. It's obvious therefore that the best holding time to pasteurisation after LPS activation was 3 hours. The reason behind this achievement by treatment P3 could be that LPS reaches the climax of its bactericidal activity curve at 3hours before its effectiveness starts diminishing progressively thereafter.

Next to treatment P3 was P6 whose pH and TPC did not differ significantly ($P>0.05$) from that of P0. This indicates that P6 and P0 were more or less on the same position on the normal distribution of the LPS activity curve.

In treatment P0, LPS had not been given enough time to exhaust its potential due to the immediate pasteurisation therefore, the resultant pH, and TPC is more of the effect of pasteurisation rather than LPS activation. Next to them (P6 and P0) was R0.

In terms of pH, treatment R0 was not significantly ($P>0.05$) different from P0 but the two were significantly ($P<0.01$) different from the rest. Similarity so obtained between the two *i.e.* P0 and R0 is due to similar initial TPC. Since the act of immediate pasteurisation after LPS activation under P0 did not give LPS enough time to exercise or exploit it's potential therefore the result obtained in both treatments *i.e.* P0 and R0 is more of pasteurisation than LPS activation.

The other reason is the immediate pasteurisation after LPS activation interfered with the LPS mode of action by inactivating Lactoperoxidase enzyme through heating (temperatures above 80^{0}C), since Lactoperoxidase enzyme is protein in nature (Nicholette *et al.*, 1999). Hence the results obtained under P0 were more of the effect of pasteurisation than LPS activation. These facts explain the reason behind similarity found between R0 and P0.

Treatments P9 and P12 were significantly ($p<0.001$) different in pH, and TPC first from themselves and then from the rest, due to the diminished level of the LPS active life as it is reported that LPS potential activity is between 7-8 hours. (FAO, 1999).

5.7 The effect of storage conditions on pH and TPC of the LPS activated pasteurised milk.

Considering all the given storages facilities available, refrigeration storage produced milk with significantly longer shelf life than all other alternative storages. Next to refrigeration were the air-conditioned room, the charcoal cooler and lastly the room temperature. Throughout the experiment, treatments performance under refrigeration storage seemed to be excellent as compared to the other alternative storages. Always in each treatment the lowest TPC and the highest pH were found under refrigeration storage. The pH and TPC values registered under refrigeration storage were significantly ($P<0.001$) different from all other storage conditions. The reason behind this was refrigeration facility managed to provide relatively very cold environment at between 5 and 8^{0}C. Such low temperatures may extend the lag time by reducing tremendously or halting temporarily all

microbiological activities in milk (Harding, 1995). In fact, this might have stopped the milk biochemical changes by immobilizing bacteria and thus long fresh milk shelf life. Therefore the growth rate and proliferation of all the bacteria surviving both pasteurisation and LPS activation (*i.e.* Thermodurics, sporeformers and from post pasteurisation contamination) is minimised by this very low temperature achieved under refrigeration.

Next was the air-conditioned room, which was significantly ($P<0.01$) different from the room temperature and the charcoal cooler box ($P<0.05$) but shared the same drawbacks as those listed under refrigeration.

The milk shelf life gain for the LPS treated pasteurised milk (P3), stored under charcoal cooler was found to be about 3hrs., above that stored under the room temperature. The untreated milk (R0) came up with similar result as above, this when subjected to similar storage condition.

Under charcoal cooler storage environment, LPS treated pasteurised milk (P3) registered 3hrs. milk shelf life gain above that of untreated pasteurised milk (R0), were as there was no gain in shelf life between the two treatments when the milk was stored under room temperature (ref. to Tab.2).

The room temperature storage was significantly different from the rest in TPC, and pH. The extreme values recorded under it seem to have been brought about by the relatively

higher temperature. The differences explained above are mainly due to the capacity of each storage condition to provide and maintain low storage temperature environment, as milk spoilage is a function of microbial activity, whose growth and multiplication is a function of time and storage temperature within acceptable limits. Temperature does influence the type of bacteria that will grow plus their spoilage characteristics (Harding, 1995). The higher the temperatures the higher the microbial plus enzymic activity hence higher microbial growth rate that result in shorter milk shelf life, the opposite of the aforesaid statement is always true.

The above tested storages try to answer the most pressing rural farmer's milk storage facility problem. Excellent milk storages (cold rooms) immediately after milking do influence milk final quality by being able to extend milk shelf life through halting microbial multiplication. Such a measure gives confidence and flexibility to the would be serious milk processors for being guaranteed constant initial milk quality.

The use of cheap and simple technology like the charcoal cooler and LPS activation application in milk would guarantee remote rural fresh milk lucrative urban market penetration

5.8 Coliform test

Absence of Coliform bacterial growth in LPS activated pasteurised milk signified the effectiveness of in-pouch pasteurisation, as their presence would have implied inefficiency in pasteurisation or recontamination from poor handling. Absence of

Coliform confirms completeness and competence of pasteurisation level utilised. From the above argument we can say that the pasteurisation temperature/time combination used was perfect (80^0C/ for one minute) and the combination of the two (LPS and in-pouch pasteurisation) ensured complete elimination of Coliform from fresh milk.

5.9 Naturally fermented milk

After 24 hours incubation period there was no significant ($P>0.05$) difference in TTA and pH for all fermented milk treatments except for P12 and P9. The disappearance of the difference in milk quality among these treatments (except P12 and P9) could be resulting from the bacteriostatic/bactericidal nature of the activated LPS in milk plus the subsequent pasteurisation, which provided more or less equal environment to the introduced culture. The pasteurisation done did stop the manifestation of the LPS activity on the introduced culture by being inactivated through denaturing of the enzyme by the introduced heat (80^0C/ for one minute.)

Significantly ($P<0.001$) the relatively high acidity values realised under P12 and P9 must be resulting from the higher initial TTA and Lower pH, which had already been realised before pasteurisation. This acidity development must be resulting from the resumption of the microbial activity, which was otherwise under the control of the activated LPS whose resident active time expires after 6hrs. (FAO, 1999).

The pasteurisation (80^0C/ for one minute) done inactivated the LPS by denaturing the Lactoperoxidase enzyme hence did provide equal environment to the fermentative

bacteria after pasteurisation. Therefore milk fermentative bacteria inoculated into this milk had equal freedom of manifesting their potential without any limitation that might have been resulting from the LPS secondary activities. The other reason could be the absence of the residual effect from LPS activity that could have interfered with the inoculums proliferation in milk. The bacteriostatic/bactericidal effect from the LPS activated milk, done immediately after milking might have made the milk metabolism to remain static for a few hours resulting in milk samples with similar chemical and physical characteristics.

It is obvious therefore that LPS treated milk must be pasteurised properly before inoculation of the culture or else, the LPS system might act on the bacteria introduced from the culture resulting in poor fermentation performance. This fact of proper pasteurisation holds true also for healthy reasons.

5.10 Yoghurt

Reference is made on table 7 under which yoghurt was made after 3 hours incubation period. The yoghurt source was LPS activated milk pasteurised after different holding time. This ativity gave pH values, which was within normal range as reported by Webb (1970). Therefore there was no significant ($P>0.05$) pH difference among all treatments except treatments P9 and P12, which had significantly ($P<0.05$) lower pH. Treatment P12 registered significantly ($P<0.05$) the lowest pH value from the rest. The yoghurt treatments acidity variation is brought about by the same reasons as what has been explained under natural fermented milk above. (Refer to section 5.9)

5.11 Applicability of LPS in the field

5.11.1. Morning milk

Field data indicates that there was significant difference ($P<0.01$) between the two treatments after the initial shelf life of 6 hours. The difference so obtained signifies the effect of LPS on milk final quality. Presence of LPS seems to have contained microbial development in treated milk where as bacterial growth was unrestricted in untreated milk, these results explain the reason behind the difference in milk shelf life between the two treatments. Therefore the realised early spoilage of the untreated milk signifies the bacteriostatic/bactericidal potency of the LPS in milk. Difference between the two treatments was maintained and continued to exist up to about 12 hours when complete total spoilage of the untreated milk was realised. Thereafter, spoilage rate of the LPS treated milk increased tremendously resulting on total spoilage after 15 hours. Under this observation, significant difference between the two treatments began after 6 hours and continued to the end.

Results obtained strongly encourage the application of this biotechnology for increased raw milk shelf life, to enable surplus remote rural fresh milk to reach the best markets in the urban centres. Hence, activated LPS may act as an alternative solution for fresh milk storage in absence of refrigeration and cold room facilities in the tropical humid remote rural areas. The lack of supportive structures to operate refrigeration systems and the likes, lack of energy, poor economic base and technical know how aggravates and impedes refrigeration technology transfer to the remote rural dairy industry. The LPS technology seems to be potentially important and appropriate for the rural fresh milk

disposal in towns and cities as a result of its ability to increase milk shelf life for more than 6 hours (at room temperature) to enable the marketing functions to operate. Such an achievement depends on the initial milk microbial quality.

Ultimately the success of this effort could act as an incentive for increased milk production which could also be motivated by the better price realised in towns and in the long run may change for the better the livelihood standard of the rural dairy producers due to increased income from milk sales.

5.11.2 Evening milk

After 19 hours storage a test on milk quality was done on the evening milk following the 14 hours test showing most of the milk samples in both tests were fresh. This was done at Mbeya receiving centre a distance of about 5 hours (300km) from Mwakaleli. The 5 hours travel plus the previous 14 hours overnight storage made a total of 19 hours storage. The difference between LPS treated and untreated milk was insignificant ($P>0.05$) as all the untreated and most of the treated samples had already been spoiled after this long holding period. Despite the treatments being similar, it appears the evening milk had a longer shelf life as compared to the morning milk this is explained by the difference in temperature between the two environments. Generally nights are cooler than day times due to the absence of solar radiations. The difference in temperatures between the two environments *i.e.* nights/daytimes do significantly affect milk microbial activity and hence the rate of spoilage. Generally there is higher microbial activity at the daytime/warm environment than during nights/dark cooler hours From the above results it shows that

LPS treated evening milk must be disposed immediately next day at a distance of less than 3 hours (to make a total of 15 hours) due to the experience obtained from day time milk as daytime milk could stay fresh up to 15 hours from milking (Ref. to Fig.9).

CHAPTER VI

6.0 CONCLUSION AND RECOMMENDATION

6.1 Conclusion

Under all storage conditions LPS activated milk had longer shelf life than the untreated milk. The maximum holding time before pasteurisation after LPS activation is 6hrs. after which milk starts to deteriorate. The holding period of 3hrs. after LPS activation appears to be the best time before pasteurisation for the milk original quality preservation. Both laboratory and field results support and demonstrate the suitability of the activated LPS on the pasteurised milk keeping quality by recording substantial hygienic quality and shelf life improvement on the treated milk. No changes in physical characteristics or organoleptic quality of the pasteurised and fermented milk were observed. The level of the natural thiocyanate content of the test milk was within acceptable limits.

The charcoal cooler box performed better in fresh milk storage than storage at room temperature. The charcoal cooler technology seems to be adaptable to the rural environment due to the possibility of utilising easily available resources like solar energy, air movement, charcoal and the metal box, which can easily be obtained, constructed and maintained.

6.2 Recommendations

Experience drawn from this stu dy advises us that, that under similar environmental condition (30°C) the treated milk should always be processed within 6 hours from LPS activation and should never reach 9 hours, as LPS active resident time starts to decline after 3 hours and reaches its peak at around 9hrs. (FAO, 1999, IDF, 1988). From the above reasoning, treated milk should always be pasteurised before processing to stop the LPS bactericidal/bacteriostatic activity on the introduced culture.

The storage temperature level so far achieved by the cooler is encouraging though still needs a lot of technological improvement for the perfection in operation. Therefore, for the fresh milk shelf life extension in any of the dairy production system in the tropics lacking refrigeration or cold room facilities, we strongly recommend the application of both activated LPS and charcoal cooler box for fresh raw milk storage and preservation.

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APPENDICES

Appendix 1: ANOVA Table for milk pH and Total Bacterial count (TPC)

Effect of LPS before pasteurisation

Dependent Variable: TPC

Source	DF	SS	Mean Square	F Value	Pr > F
Model	11	8760545352222223.0	796413213838384.00	207.49	0.0001
Error	24	92121494666666.1	3838395611111.08		
Corrected Total	35	8852666846888889.0			

R-Square	C.V	Root MSE	TPC Mean
0.989594	26.55	1959182.38	7380444.44

Dependent Variable: PH

Source	DF	SS	Mean Square	F Value	Pr > F
Model	11	0.915	0.083	665.6200	0.0001
Error	24	0.003	0.003	0.0001	
Corrected Total	35	0.918	0.919		

R-Square	C.V	Root MSE	TPC Mean
0.996733	0.17	0.01	6.55

Effect of LPS after pasteurisation

Dependent Variable: pH

Source	DF	SS	Mean Square	F Value	Pr > F
Treat	5	0.37842446	0.07568489	1204.78	0.0001
hours	3	2.86507622	0.95502541	5202.45	0.0001
Storage	3	2.11438755	0.70479585	11219.20	0.0001
Treat* hours	15	0.08789496	0.00585966	93.28	0.0001
Treat*storage	15	0.02956158	0.00197077	31.37	0.0001
Treat* hours*storage	36	1.18437269	0.03289924	523.70	0.0001
Corrected total	233	6.87430598			

R-Square	C.V	Root MSE	pH Mean
0.998574	0.121881	0.00792594	6.50299145

Dependent Variable: TPC

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Treat	5	686334771943075.00	137266954388615.00	8016.77	0.0001
hours	3	12017932858918550.00	4005977619639517.00	99999.99	0.0001
Storage	3	9494571512476854.00	3164857170825618.00	99999.99	0.0001
Treat* hours	15	1032595485685287.00	68839699045685.80	4020.43	0.0001
Treat* hours*storage	36	7040041762576037.00	195556715627112.00	11421.05	0.0001
Error	156	2671106267170.83	17122476071.61		
Corrected total	233	31552396660079104.00			

R-Square	C.V	Root MSE	TPC Mean
0.999915	1.470957	130852.87949299	8895762.99145299

LPS and holding timeacross storages

Dependent Variable: pH

Source	DF	SS	Mean Square	F Value	Pr > F
Model	8	0.60	0.08	3.70	0.0004
Error	291	5.91	0.02		
Corrected total	299	6.51			

R-Square	C.V	Root MSE	pH Mean
0.09	2.19	0.14	6.50

Dependent Variable: TPC

Source	DF	SS	Mean Square	F Value	Pr > F
Model		2462013047644208.00	307751630955526.00	5.18	0.0001
Error		16580331560755652.00	59427711687296.20		
Corrected total	287	19042344608399860.00			

R-Square	C.V	Root MSE	pH Mean
0.129291	109.60	7708937.13	7033499.31

Charcoal cooler box and Weather temperature

Dependent variable: Temperature

Source	DF	SS	Mean Square	F Value	Pr > F
Model	1	2128.69	2128.69	1320.55	0.0001
Error	166	267.59	1.61		
Corrected Total	167	2396.28			

R-Square	C.V	Root MSE	Temperature Mean
0.89	5.62	1.27	22.58

Fermented milk

Dependent Variable: pH

Source	DF	SS	Mean Square	F Value	Pr > F
Model	5	77054.35	15410.87	2.49	0.05
Error	30	185791.11	6193.04		
Corrected Total	35	262845.46			

R-Square	C.V	Root MSE	pH Mean
0.29	318.7	78.7	24.7

Yoghurt

Dependent Variable: pH

Source	DF	SS	Mean Square	F Value	Pr > F
Model	5	0.28	0.057	9.23	0.0001
Error	30	0.18	0.006		
Corrected Total	35	0.47			

R-Square	C.V	Root MSE	PH Mean
0.61	1.85	0.08	4.25

Appendix 2: Analysis SCN in milk

No	Mean	Std Dev	Minimum	Maximum
47	4.0697872	1.1007246	2.2940000	8.1820000

Appendix 3: Charcoal cooler box temperature and relative humidity variations

Variable	N	Mean	Std Dev	Minimum	Maximum
MAXIM	90	22.8666667	1.6603100	18.80	26.20
MINIM	90	15.6700000	1.3857582	13.20	19.20
RH	90	93.7222222	7.6555448	48.00	100.00

Appendix 4: Weather temperature and relative humidity variations

Variable	N	Mean	Std Dev	Minimum	Maximum
MAXIM	78	31.81	2.48	23.0	36.5
MINIM	90	21.12	1.04	18.0	23.8
RH	90	93.72	7.66	48.0	100.0

Appendix 5: Guidelines for the preservation of raw milk by use of the Lactoperoxidase system

(www.codexalimentarius. net/STANDARD/VOLUME12/VOL12_e.htm)

Introduction

Milk is an easily perishable material. Contaminating bacteria my multiply rapidly and render it unsuitable for processing and/or unfit for human consumption. Bacterial growth can be retarded by refrigeration, thereby slowing down the rate of deterioration. Under certain conditions refrigeration may not be feasible due to economical and/or technical reasons. Difficulties in applying refrigeration are specially a problem for certain areas in countries setting up or expanding their milk production. In these situations, it would be beneficial to have access to a method, other than refrigeration for retarding bacterial growth in raw milk during collection and transportation to the dairy processing plant.

In 1967 the FAO/WHO Expert panel on milk quality concluded that the use of hydrogen peroxide might be an acceptable alternative in the early stages of development of an organised dairy industry, provided that certain conditions were complied with. However this method has not achieved general acceptance as it has several drawbacks, most important of which is the difficulty of controlling its use: it may be disguise milk of basic hygienic quality produced under poor hygienic conditions. The toxicological aspects of the use of highly concentrations of hydrogen peroxide in the milk have also been questioned. A chemical method for preserving milk would still be of great advantage in certain situations. The search for such a method has therefore continued. Interest has recently been focused on the indigenous antibacterial systems in milk to determine if these could be applies practically to preserve raw milk. During the last decade, basic and applied research has demonstrated that one of these systems; the Lactoperoxidase/thiocyanate/hydrogen peroxide system (LP-system) can be used successfully for the purpose.

1. Scope

This Code of practice describes the use of Lactoperoxidase system for preventing bacterial spoilage of the raw milk (Bovine and Buffalos) during collection and transportation to the dairy processing plant. It describes the principles of the method, in what situations it can be used, its practical application and control of the method. It should be stressed that this method should be used when refrigeration of the raw milk is not feasible.

2. Principles of the method

The Lactoperoxidase /thiocyanate/hydrogen peroxide system is an indigenous antibacterial system in milk and human saliva. The enzyme Lactoperoxidase is present in bovine and buffalo milk in relatively high concentrations. It can oxidise thiocyanate ions in the presence of hydrogen peroxide. By this reaction, thiocyanate is converted into hypothiocynous acid (HOSCN). At the pH of the milk HOSCN is dissociated and exist mainly in the form of hypothiocyanate ions ($OSCN^-$). This agent reacts specifically with free sulphydryl groups, there by inactivating several vital metabolic bacterial enzymes, consequently blocking their metabolism and ability to multiply. As milk protein contains very few sulphydryl groups and those that are present are relatively inaccessible to $OSCN^-$ (masked), the reaction of this compound is in milk quite specific and is directed against bacteria present in milk. The effect against bacteria is both species and strain dependent. Against a mixed raw milk flora dominated by mesophilic bacteria, the effect is bacteriostatic (predominantly inhibitory). Against some gram-negative bacteria, i.e. pseudomonads, Escherichia coli, the effect is bactericidal. Due to the bacteriostatic effect of the system it is not possible to disguise poor quality milk which originally contained A high bacterial population, by applying this method.

The antibacterial oxidation products of thiocyanate are not stable at neutral pH. Any surplus of these decomposes spontaneously to thiocyanate. The velocity of this reaction is temperature dependent, i.e. more rapid at higher temperature. Pasteurisation will ensure complete removal of any residual concentration of the active oxidation products.

Oxidation of thiocyanate does not occur to any great extent in milk when it has left the udder. It can however be initiated through addition of small concentrations of hydrogen peroxide (see section 4). The concentration of hydrogen peroxide used to preserve milk (300-800). destroy the enzyme Lactoperoxidase and thereby preclude the oxidation of thiocyanate. With this method the antibacterial effect is thus an effect of hydrogen peroxide itself.

The antibacterial effect of the LP-system is, within certain limits. proportional to the thiocyanate concentration in milk (provided that an equimolar amount of hydrogen peroxide is provided). The level of thiocyanate in milk is related to the feeding of the animal and can thus vary. The practical use of the method consequently requires addition of some thiocyanate to ensure that a level necessary to achieve the desired effect is present in the milk.

The levels of thiocyanate resulting from this treatment are within the physiological levels reported to occur in milk under certain circumstances and feeding regimes They are also far below the thiocyanate level known to exist in human saliva and certain common vegetables, e.g. cabbage and cauliflower. In addition, results from clinical experiments have clearly demonstrated that milk treated according to this method will not cause any interference of the iodine uptake of the thyroid grand, neither in persons with a normal iodine status nor in case of iodine deficiency.

3. Intended utilization of the method

This method should only be used in situations when technical, economical, and/or practical reason do not allow the use of cooling facilities for maintaining the quality of raw milk. Use of the LPS in areas which currently lack an adequate infrastructure for collection of liquid milk, would ensure the production of milk as safe and wholesome food, which otherwise would be virtually impossible.

The method should not be used by individual Farmers but at suitable collection point/centre. The centre must be equipped with proper facilities for cleaning and sanitising the vessels used to hold and transport milk.

The personnel responsible for the collection of the milk should be incharge for the treatment for the milk. They should be given appropriate training including training in general milk hygiene; to enable them fulfil this in correct way.

The dairy processing the milk collected by use of Lactoperoxidase system should be made responsible for ensuring that the method is used as intended. This dairy should set up appropriate control methods (see section 5) to monitor usage of the method, raw milk quality and quality of the milk prior to processing.

The method should primarily be used to prevent undue bacterial multiplication in raw milk during collection and transportation to the dairy processing plant under condition stated in 3.1. The inhibitory effect of the treatment is dependent on the temperature of the stored milk and has been found to act for the following periods of the time in the laboratory and field experiments carried out in different countries with raw milk of an initial good hygiene standard:

Temperature (°C)	Time (hours.)
30	7-8
25	11-12
20	16-17
15	24-26

The use of the Lactoperoxidase method does not exclude the necessity of pasteurisation of the milk before human consumption. Neither does it exclude the normal precautions and handling routines applied to ensure a high hygienic standard of raw milk.

4. Practical application of the method

The Lactoperoxidase system can be activated in raw milk to give the above stated antibacterial effect by an addition of thiocyanate as Sodium thiocyanate and hydrogen peroxide in the form of sodium per carbonate by the following procedure:

14mg of NaSCN is added per litre of milk. The milk should then be mixed to ensure an even distribution of SCN^- Plunging for about 1 minute with a clean plunger is normally satisfactory. Secondly, 30 mg of sodium per carbonate is added per litre of milk. The milk is then stirred for about 2-3 minutes to ensure that the sodium per carbonate is completely dissolved and the hydrogen peroxide is evenly distributed in milk.

It is essential that sodium percarbonate and sodium thiocyanate be added in the order stated above. The enzymic reaction is started in the milk when the hydrogen peroxide (sodium percarbonate) is added. It is completed within 5 minutes from the addition of H_2O_2, thereafter no hydrogen peroxide is present in the milk. The activation of Lactoperoxidase system should be carried within 2-3 hours from the time of milking

Quantities of sodium thiocyanate and sodium percarbonate needed for the treatment of a certain volume of milk, for example 40-50 litre milk churns, should be distributed to the collection centre/point in pre packed amounts lasting for a few weeks at a time. The technical specifications for the thiocyanate and sodium percarbonate, which should be used, are stated in Appendices I and II

5. Control of the usage

The use of Lactoperoxidase system for preserving raw milk must be controlled by the dairy processing plant receiving the milk. This should be a combination of currently used acceptance test e.g. titrable acidity, Methylene blue, Resazurin, total viable count and analysis of thiocyanate concentration in milk. Since the thiocyanate is not consumed in the reaction, treated milk arriving at the dairy plant would contain approximately 10 mg above the natural amount of thiocyanate (the later can be determined by analysing untreated milk from the same area) per litre of milk. The analytical method for SCN^- is described in Appendix III. Testing should be under taken at random. If the concentration of thiocyanate is too high (0r too low), investigation must be carried out to determine why the concentration is outside specification. The dairy processing plant should also be responsible for the control of the chemicals to be used at the collection centre for the activation of Lactoperoxidase system.

Analysis of the bacteriological quality of the milk (Methylene blue, Resazurin, Total plate count) should also be carried out to ensure that good hygienic standards are not neglected. Since the effects of the system are predominantly bacteriostatic, an initial high bacterial population in the milk can still be revealed by such tests

Technical specification of sodium thiocyanate

Definition
Chemical name sodium thiocyanate
Chemical formula NaSCN
Molecular weight 81.1
Assay content 98-99%
Humidity 1-2%

Purity (according to JEFCA* specification)
Heavy metals (as Pb) < 2ppm
Sulphate (as SO_4) < 50ppm
*Joint FAO/WHO Expert Committee on Food Additives.

Technical specification of sodium percarbonate

Definition
Chemical name sodium percarbonate (*)
Chemical formula $Na_2CO_3.3H_2O_2$
Molecular weight 314.0
Assay content 85%
Commercial available sodium percarbonate recommended to be used has the following specification:
Sodium carbonate peroxihydrate > 85%
Heavy metals (as Pb) < 10ppm
Arsenic (as As) < 3ppm
(*) For information where sodium percarbonate could be obtained commercially, please apply to IDF general secretariat, 41 square Vergote, B-1040 Brucssels, Belgium.

Analysis of thiocyanate in milk

Principle
Thiocyanate can be determined in milk, after diproteinisation, with trichloroacetic acid (TCA) as the ferric complex by measuring the absorbance at 460nm. The minimum level of detection by this method is 1 to 2ppm of SCN^-

Reagent Solutions
1. 20% (w/v) trichloroacetic acid: 20g TCA is dissolved in 100ml of distilled water and filtered
2. Ferric nitrate reagent: 16.0g Fe (NO_3) 3.9H2O is dissolved in 50ml 2M HNO_3* and then diluted with distilled water to 100ml. The solution should be stirred dark and cold.

*2MHNO_3 is obtained by diluting 138.5ml 65% HNO_3 to 1000ml with distilled water

Determination
4.0ml of milk is mixed with 2.0ml of 20% TCA solution. The mixture is blended well and then allowed to stand for at least 30 minutes. It thereafter filtered through a suitable filter paper (Whatman No 40). 5ml of clear filtrate is then mixed with 1.5 ml of ferric nitrate reagent and the absorbance measured at 460nm. As a blank a mixture of 1.5ml of ferric nitrate solution and 1.5ml of water is used. The measurement must be carried out within 10 minutes from the addition of ferric nitrate solution, as the coloured complex is not stable for any length of time. The concentration of thiocyanate is then determined by comparisons with standard solutions of known thiocyanate concentration e.g. 10, 15, 20 and 30 µg/ml of thiocyanate
(CAC/GL 13-1991)