1. INTRODUCTION

The origin of fermented milks in the diets of humans date back many thousands of years and predates the existence of written records of their production and consumption (Campbell-Platt, 1987). According to Pederson (1971), fermented milks were produced some 10,000-15,000 years ago as man's way of life changed from being food gatherer and hunter to food producer. It is likely that this transition may have occurred at different times in different parts of the world. However, archaeological evidence shows some civilizations e.g. the Sumarians and Babylonians in Mesopotamia, the Pharoes of ancient Egypt and the Indians in Asia were well advanced in agricultural and animal husbandry methods and kept cows and buffalos for milk production, which was either consumed as such or processed into other products (Abou-Donia, 1984). There are many sketches that illustrate the milking and milk processing in these areas (Abou-Donia, 1984). Sour milk has been known, from time immemorial, to be more stable and advantageous than fresh milk in the diet (Vedamuthu, 1979; Robinson and Tamime, 1981). Fermentation preserves the high quality nutrients present in a form that has a longer shelf-life (Oberman and Libudzisz, 1998).

Production of traditional fermented milk is widespread throughout Africa (O'Mahony and Peters, 1987; Mutukumira *et al*., 1995). Traditional fermented milk products constitute an important part of people's diet (O'Mahony and Peters, 1987; Chamberlain, 1990). Apart from providing nutrients to foods, traditional fermented milks have the advantage of long shelf life due to low pH (Feresu and Nyati, 1990; Kimonye and Robinson, 1991), they also provide other benefits which may include the presence of lactase enzyme (β-galactosidase) for lactose intolerant consumers, including other inhibitory compounds effective against several pathogenic and spoilage bacteria (Speck, 1977; Deeth and Tamime, 1981; Nout, 1994). The fermented milk provides income to the rural poor, especially to women and children for household food security and is a source of employment in rural areas (Bachmann, 1985; Joubert and De Lange, 1992).

The quality problems of traditional fermented milks have already been identified and are associated with poor technology, hygiene, sanitation, sensory aspects, shelf-life, syneresis, viscosity and unattractive presentation to consumers and have been documented by Nout, (1985); Kimonye and Robinson, (1991). Sources of contamination of traditional fermented milks have also been traced to the cow, the handlers, the utensils, water, air and environment (Marshall, 1992; Olasupo and Azeez, 1992).

However, the public health problems associated with the consumption of traditional fermented milks with a low pH ($\leq$ 4.5) have not been a major issue due to inhibition of pathogens by organic acids (Nout *et al.,* 1987; Aryanta *et al.,* 1991), antibacterial substances such as bacteriocins (Olsen *et al.,* 1995) and lowering of redox potential (E*h*) as documented by (Leistner, 2000; Kim, *et al.,* 2000). These compounds have been reported to render the products safe for human consumption.

Omashikwa is the Owambo tribe's name for traditional fermented buttermilk produced in Namibia. *Omashikwa* is processed with the root of the *Omunkunzi (Boscia albitrunca)* tree and is consumed as a refreshing drink, thirst-quencher and as a condiment for other foods like gruel and stiff porridge (*Oshithima or Oshifima)* made from maize, pearl millet or sorghum flours. Pieces of the root (12-15) of approximately 2 cm^3 (Fig. 1.1) are added into about 20 liters of cow's milk and allowed to ferment naturally in a gourd at ambient temperatures ranging from 27° to 36°C for 2-3 days. After fermentation, the pieces of root are removed and the coagulum is agitated for 2-3 hours to churn into butter. The butter is scooped off, washed to remove buttermilk, packed in recycled containers and sold separately or processed into *ghee* (cooking oil) by the Herero community. The resulting sour buttermilk, the *Omashikwa* is consumed while still wholesome. The product is characterized by a slimy texture, bitter and rancid taste with high acid flavour and peculiar *B. albitrunca* root taste and smell (Chapter 4.1).

Fig.1.1: Pieces of root of *Boscia albitrunca* tree used for processing *Omashikwa*

In many rural areas of northern Namibia, *Omashikwa* is sold by small-scale milk producers and vendors to consumers at the open markets or to workers on road and building sites. *Omashikwa* is brought to market in 20-40 L plastic containers and retailed in ½ L plastic mugs for direct consumption or in 2-5 L recycled plastic bottles for wholesale distribution (Figs. 1.2 & 1.3).

Fig. 1.2: Fermented buttermilk (*Omashikwa*) displayed for sale in the open market in northern Namibia with lumps of butter. Note the sliminess.

Owing to uncertainty in consistency of flavour, viscosity, syneresis, acidity, bitterness, rancidity and dirt or filth, consumers tend to be selective when purchasing *Omashikwa*. They go from one seller to the next, sampling and tasting, before making decision as to which one to buy. Observations by the author revealed that *Omashikwa* can contain high numbers filth-like splinters, small flying insects and dirt (Chapter 4.1). No information is available on the bacteriology and compounds contributing positively to the characteristics of *Omashikwa*.

Fig. 1.3: *Omashikwa* **display in recycled bottles for sale at Omuthiya northern Namibia. Note that there is no whey separation**

However, the growing demand for *Omashikwa* in the northern regions of Namibia, where the majority of the Namibian population lives, gives an incentive to expand quality production through technology, unit operations and good manufacturing practices. This would create a market for rural community small-scale *Omashikwa* production and micro enterprises for income generation, job creation, nutrition and household food security.

Industrial buttermilk processed from pasteurized cream by the Namibian Dairies Ltd, a byproduct of butter, is fermented with mesophilic commercial lactic acid cultures and branded as *Omashikwa* for commercial purpose. *Omashikwa* is a popular traditional fermented buttermilk product of the largest communities in Namibia, the Owambo and Herero tribes. *Omashikwa* simply means shaking or agitating the milk to churn. Industrial

buttermilk has nothing to do with traditional *Omashikwa* as such. *B. albitrunca* root is not added and it is processed by modern industrial method of milk separation, cream pasteurization, butter churning and buttermilk fermentation, a process similar to that of commercial sour cultured milk. In addition, preservatives such as potassium sorbate and sugar, including stabilizer (pectin) to preserve and improve consistency are added and packed for distribution.

This study therefore investigated the processing technology, physico-chemical properties, microbiological, viscosity and sensory quality of traditional *Omashikwa* and were compared to those of laboratory made *Omashikwa*. The remedial measures to curb inconsistency and poor quality of traditionl *Omashikwa* processed in Namibia were also investigated and scientific measures were proposed for production of quality *Omashikwa* for marketing to a wider community.

2. LITERATURE REVIEW

Detailed reviews of traditional fermented milks and the processing technologies, preservation, microbiology, sensory aspects and nutritional values have been published (Keller and Jordan, 1990; Walshe *et al.,* 1991; Olasupo and Azeez, 1992; Mutukumira, 1995a). These reviews provide important references for detailed information on the quality of traditional fermented milks in general. Traditional herbs and wood from some tree species used for smoking of milk and fermenting vessels that are used in processing, preservation and improving sensory quality of traditional fermented milks in Africa will be reviewed. Lactic acid bacteria used in fermentations will also be looked at. Examples of some popular traditional African fermented milks, their physico-chemical, microflora and utilization will also be reviewed. Work that has been done elsewhere to improve the production of some traditional fermented milks such as *Maziwa lala* or *Maziwa mgando* in Kenya and Tanzania, into commercial fermented milk such as *Mala* will be reviewed. *Mala* is processed with mesophilic lactic acid bacteria, it is heat treated under good hygienic and sanitary conditions and good manufacturing practices on unit operations and packaged for distribution.
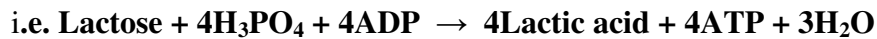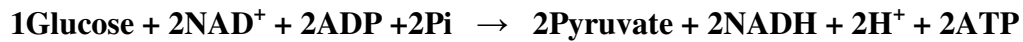
2.1 FERMENTATION

Strictly speaking, lactic acid fermentation is a metabolic process in which milk sugars orlactose and other related compounds are oxidized by lactic acid bacteria with the release ofenergy in the absence of any external electron acceptors (Jay, 1978; Cogan and Jordan1994; Sanders, 1995). In the process, lactic acid bacteria produce considerable amounts oflactic acid (De Vos and Hugenholtz, 2004). Lactic acid bacteria are the most wide spread of desirable micro-organisms in food fermentations. They are found in fermented cereal products, milks, cheeses and fermented meats and vegetables (Campbell-Platt, 1987). Lactic acid bacteria convert the available carbohydrate to organic acids and lower the pH of the food. These acids as well as other flavour compounds which include diacetyl, acetaldehyde and acetoin contribute to the desired taste and flavour of food (Pederson, 1971). The low pH that is created also makes the food unfavourable for the proliferation of spoilage and pathogenic bacteria (Djien, 1982).

2.1.1 Lactic acid fermentation

Lactic acid fermentations can be divided into two broad categories distinguishable by the end products of glucose hydrolysis, namely; homofermentation and heterofermentation (Stainer *et al.* 1980). Homofermenters such as *Lactococcus* spp., *Lactobacillus delbrueckii* subsp. *bulgaricus, Lactobacillus acidophilus* and *Streptococcus thermophilus,* convert glucose to glucose 1,6- diphosphate using the Embden-Meyerhof (EM) pathway (Dirar and Collins, 1972). The enzyme aldolase cleaves fructose-1, 6-diphosphate between C_3 and C_4 to give the phosphate esters dihydroxyacetane phosphate and D-glyceraldehyde-3-phosphate. The reaction favours the production of the glyceraldehydes isomer at equilibrium. The end product in this fermentation pathway is the production of more than 90% of lactic acid, which is responsible for the sharp refreshing taste, preservation of fermented milk products and gel formation (de Vries and Stouthamer, 1968; Doelle, 1975; Mayra-Makinen and Bigret, 1993; Cogan, 1995; Sanders, 1995) (Fig.2.1). Although lactic acid is the predominant metabolite of glycolysis, other metabolites which may be present in small concentrations are also important for product's flavour (Imhof *et al,* 1994).

The overall lactic acid fermentation pathway of the above can be expressed as follows:

$$\text{1Glucose} + \text{2NAD}^+ + \text{2ADP} + \text{2Pi} \rightarrow \text{2Pyruvate} + \text{2NADH} + \text{2H}^+ + \text{2ATP}$$

$$\text{i.e. Lactose} + \text{4H}_3\text{PO}_4 + \text{4ADP} \rightarrow \text{4Lactic acid} + \text{4ATP} + \text{3H}_2\text{O}$$

The homolactic acid fermentation pathway is important in the dairy products. It is the pathway responsible for souring milk and is used in the production of yoghurt, cottage cheese, cheddar cheese, cultured buttermilk and cream cheeses (Atlas, 1995) (Figure 2.1a).

Heterofermentative lactic acid bacteria, such as *Leuconostocs lactis* and *Leu. mesenteroides* subsp. *cremoris* and *Lactobacillus fermentum,* lack aldolases and therefore cannot ferment sugar via the glycolytic pathway (Cogan and Jordan, 1994; Vedamuthu, 1994). The pentose phosphate pathway (Fig. 2.1b), is used instead of the EM pathway of glycolysis. This type of fermentation produces ethanol and carbon dioxide in addition to lactic acid in the molar ratio 1:1:1 (Cogan and Jordan 1994; Prescott *et al*. 1993).

The ethanol and the CO_2 come from the glycolytic portion of the pathway (Fig. 2.1b). There are two possible ways by which ethanol is formed (Caldwell, 1995). Acetaldehyde formed by cleavage of pyruvate decarboxylase is reduced in the presence of alcohol dehydrogenase to form ethanol. Ethanol can also be formed by a combination of acetyl CoA reduction to acetaldehyde followed by reduction of acetaldehyde by ethanol dehydrogenase Lactate is formed by direct pyruvate reduction with lactase dehydrogenase. Formic acid and acetyl CoA are produced by the action of pyruvate-ferredoxin on pyruvate. Acetyl CoA is converted to free acetic acid. Formate is converted to CO_2 and H_2 by the joint operation of formic dehydrogenase and hydrogenase (Volk and Wheeler, 1984).

The overall reaction for the heterofermentation can be summarized and expressed as follows:

$$\text{Glucose} + \text{ADP} + \text{Pi} \rightarrow \text{Lactic acid} + \text{Ethanol} + \text{CO}_2 + \text{ATP}$$

$$\text{i.e. Lactose} + \text{2H}_3\text{PO}_4 + \text{2ADP} \rightarrow \text{2 Lactic acid} + \text{2 Ethanol} + \text{2CO}_2 + \text{2ATP} + \text{H}_2\text{O}$$
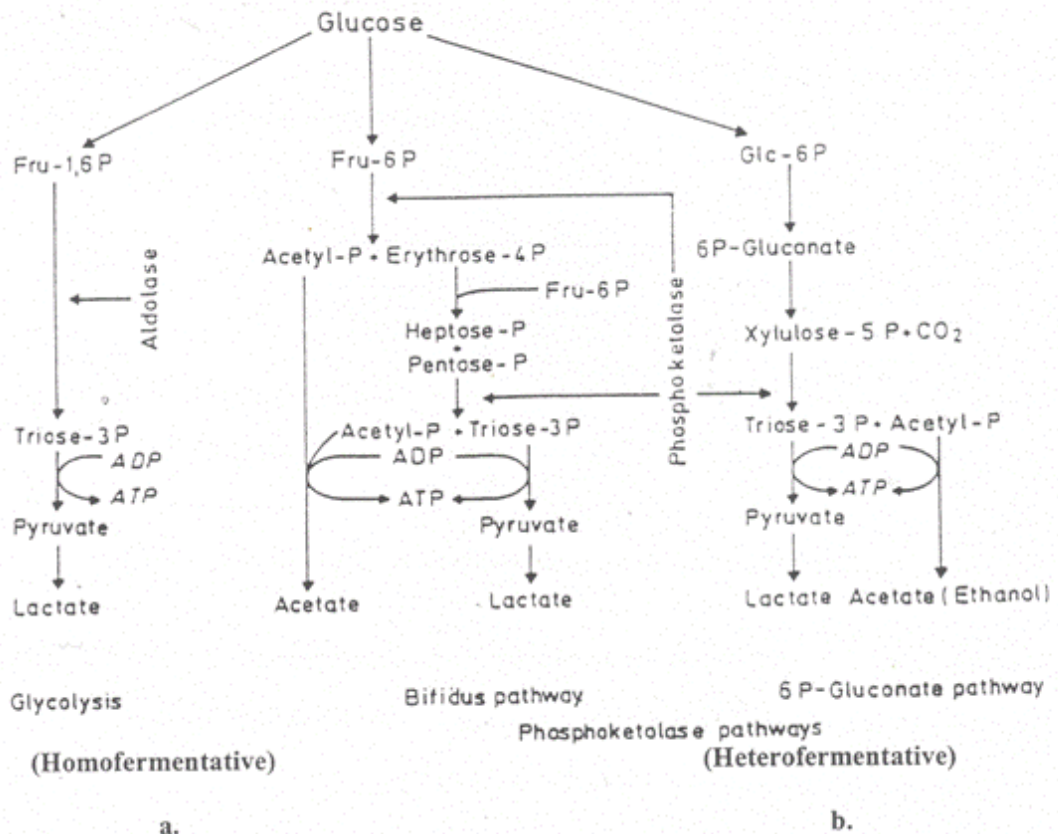
Fig. 2.1: The pathway for glucose dissimilation by homofermenters and heterofermenters (Kandler, 1983)

Mixed acid fermentation is a third type of fermentation that is carried out by the members of the family *Enterobacteriaceae* that includes *Escherichia coli* and members of the genera *Salmonella* and *Shigella* (Prescott *et al.,* 1993). Glucose is fermented by the EM pathway instead of pentose phosphate pathway of lactic acid bacteria, to form pyruvate which is converted to succinate, ethanol, lactate, CO_2 and H_2 (Fig. 2.2). According to Atlas (1995), succinate is formed by the carboxylation of phosphoenol pyruvate by phosphoenol pyruvate carboxylase to produce oxaloacetate. The oxaloacetate is converted to succinate by the combined actions of malate dehydrogenase, fumarase and fumarase reductase. The remaining products of mixed acid fermentation arise from pyruvate metabolism. Lactate is formed by direct reduction of pyruvate with lactate dehydrogenase. Formate and acetyl CoA are converted to free acetic acid with the intermediate formation of acetyl phosphate by the actions of phosphotransacetylase and acetyl kinase. This allows the CoA bond energy of

10

acetyl CoA to generate ATP. A portion of acetyl CoA is converted to ethanol by the actions of aldehyde and alcohol dehydrogenases. Formate is converted to CO_2 and H_2 by the joint operation of formic dehydrogenase and hydrogenase, as shown below:
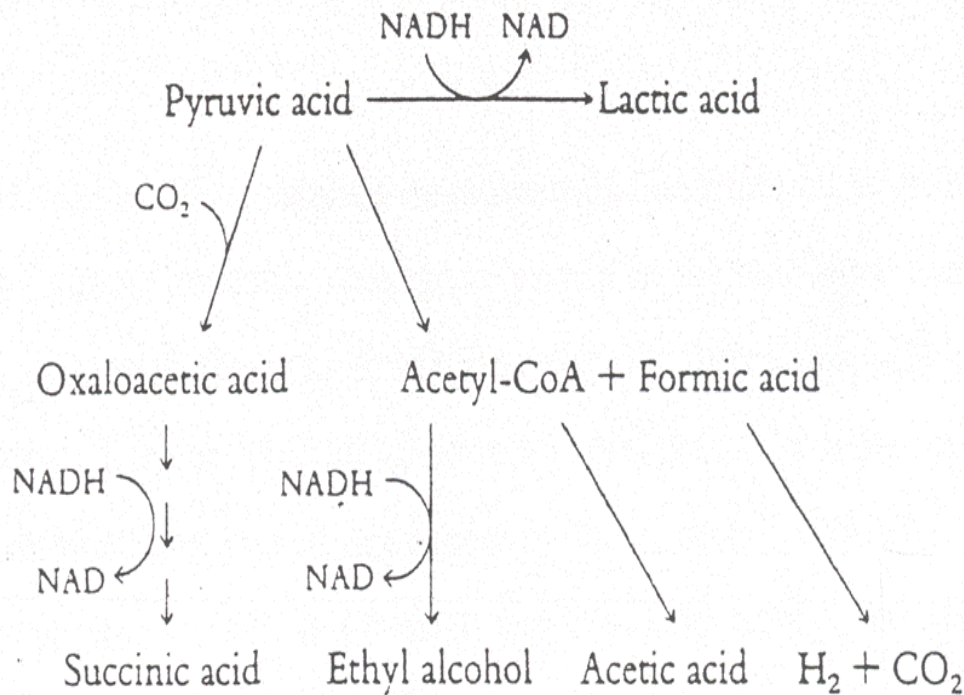

Fig. 2.2: Mixed acid fermentation by some enteric bacteria

2.1.2 Taxonomy of lactic acid bacteria involved in fermentation

The lactic acid bacteria are spherical or rod-shaped microorganisms (Cogan and Accolas, 1996; Axelsson, 1993). Their name derives from the fact that ATP is synthesized through fermentation of carbohydrates, which yields lactic acid as a major and sometimes the sole end-product (Stanier *et al.,* 1980). Lactic acid bacteria are all facultative anaerobes which grow readily on the surface of solid media exposed to air. However, they are unable to synthesize ATP by respiration, a reflection of their inability to produce cytochromes and other haem-containing enzymes (Prescott *et al.,* 1993). Lactic acid bacteria are also unable to mediate the decomposition of hydrogen peroxide according to the following reaction:

$$2H_2O_2 \quad \rightarrow \quad 2H_2O \quad + \quad O_2$$
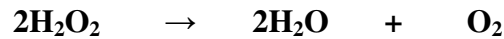
Another distinctive physiological feature of lactic acid bacteria is their tolerance of acid. Although the spherical lactic acid bacteria can initiate growth in neutral or alkaline pH, most of the rod-shaped forms cannot grow in media with an initial pH greater than 6. Growth of all lactic acid bacteria continues until the pH has fallen to a value of 5 or less (Jay, 1992).

Lactic acid bacteria differ with respect to the isomers of lactic acid that they produce (Schleifer *et al.,* 1992b). This is determined by the specificity of the lactic dehydrogenases which mediate pyruvate reduction. Some species contain only D-lactic dehydrogenases of differing stereospecificity and form racemic lactic acid (Stanier *et al.,* 1980). The ability to convert carbohydrates to lactic acid, acetic acid, ethanol and carbon dioxide with only minor changes in the other food components has made this group of micro-organisms extremely important in the preservation of food (Cogan and Accolas, 1996). The lactic acid produced during fermentation is effective in inhibiting growth of other bacteria that may decompose the food or make it toxic (Jeppessen and Huss, 1993; Leisner *et al.,* 1995). The most important genera of LAB are *Lactobacillus, Lactococcus, Enterococcus, Streptococcus, Pediococcus, Leuconostoc, Weissella, Vagococcus, Carnobacterium, Lactosphaera, Oenococcus, Tetragenococcus* and *Bifidobacterium* (Adams and Nicolaides, 1997; Jay, 1998; Klein *et al.,* 1998). However, the common lactic acid bacteria in starter cultures are classified into five genera namely, *Lactobacillus, Weissella, Streptococcus, Pediococcus, Leuconostoc, Lactococcus.*

2.1.2.1 Lactobacilli/Weissella

Lactobacilli are Gram-positive, catalase-negative rods that often occur in long chains. They can also be almost coccoid. They grow poorly in air but better under reduced oxygen tension. Although those in foods are typically microaerophilic, many true anaerobes also exist. They are chemoorganotrophic and require complex media. Their metabolism is fermentative and at least one of the carbon end-products is lactic acid. *Lactobacilli* are commonly found in dairy

products and they are rarely pathogenic (Jay, 1998). Their optimum growth temperature is 30 to 40°C (Adams and Nicolaides, 1997; Jay, 1998; Klein, *et al.,* 1998).

2.1.2.2 Pediococci and Streptococci

All members of the genera *Pediococcu*s and *Streptococcus* are homofermentative, Gram-positive, catalase-negative cocci. *Pediococci* divide in two planes at right angles to produce tetrads of cells but sometimes only pairs of cells are seen. Single cells are rare and chains are not formed. They are facultative anaerobes although some strains are inhibited on incubation in air. They are also chemoorganotrophic and require nutritionally rich media and a fermentable carbohydrate (mainly mono- and disaccharides). Glucose is fermented with the production of acid but no gas. The major product is DL or L (+) lactate. The optimum growth temperature is 25 to 40°C. *Streptococci* divide in one plane to produce chains (Stanier *et al.* 1980). The cells are spherical or ovoid, occurring in chains or pairs. Some species are encapsulated. They are facultative anaerobic chemoorganotrophs which require nutritionally rich media for growth and sometimes 5% carbon dioxide. Growth is generally restricted to a temperature of 25 to 45°C (optimum 37°C) (Adams & Nicolaides, 1997; Jay, 1998).

2.1.2.3 Leuconostocs

Leuconostocs are Gram-positive, catalase-negative cocci that are heterofermentative. The cells are spherical or sometimes longer than broad when in chains or pair. Sometimes short rods with rounded ends occur in long chains. They grow rather slowly, producing small colonies that may be slimy on media containing glucose. They are facultative anaerobes and chemoorganotrophic and have obligate requirements for a fermentable carbohydrate as well as a nutritionally rich medium. Glucose is fermented with the production of D (-)-lactate, ethanol and carbon dioxide (CO_2). The optimum growth temperature is 20 to 30°C (Adams & Nicolaides, 1997; Jay, 1998).

2.1.2.4 Lactococci

Lactococci are Gram-positive, catalase-negative, non-motile spherical or ovoid cells that occur singly, in pairs or in chains. Endospores are not formed. They are non-motile and

without capsules. They are facultative anaerobes which can grow at 10°C but not at 45°C (optimum of 30°C). They are chemoorganotrophs with fermentative metabolism. A number of carbohydrates are fermented with the production of L (+)-lactic acid but no gas. Their nutritional requirements are complex (Adams & Nicolaides, 1997; Jay, 1998).

2.1.3 Factors controlling fermentation

The factors discussed in this section constitute an inclusive, rather that exclusive, list of intrinsic and extrinsic factors that may be considered when determining whether a food or category of foods requires time/temperature control during storage, distribution and handling to ensure consumer protection.

A number of intrinsic and extrinsic factors influence the intensity and particular type of fermentation (Tomkins *et al.,* 1988). Intrinsic factors are those that are characteristic of the food itself or are parameters that are an inherent part of the food product and include pH, moisture or water activity, redox potential or oxidation-reduction potential (E_h) and nutrient content (Jay, 1978; Jay, 2000,).

2.1.3.1 Intrinsic factors

The pH of a solution describes the hydrogen ion concentration in food: [pH= -$\log_{10}$ (H+)]. (Conn *et al*.,1987). Increasing the acidity of foods, either through fermentation or addition of weak organic acids, has been used as a preservation method since ancient times. Bacterial growth rates are greatly influenced by pH values and the effects are mainly based on the nature of proteins (Kimonye and Robinson, 1991). Charge interactions within the amino acids of a polypeptide chain strongly influence the secondary and tertiary structure and folding of a protein (Ludescher, 1996). This change in shape of the active site of enzymes affects their function. Enzymes are normally inactive at very high and very low pH values (Atlas, 1995).

Most bacteria grow best at pH values around 7 (6.6 to 7.5). Lactic acid bacteria will grow at lower pH (<pH 4) and through the production of lactic acid, the pH is lowered further (Tomkins *et al.,* 1988). Many bacteria, particularly the spoilage and pathogenic bacteria, do not grow at such a low pH. This has important consequences with regard to the shelf-life and safety of fermented milk products (Feresu and Nyati, 1990).

All bacteria require water in an available form for growth and reproduction. Water is an essential solvent and is needed for all biological reactions in living systems (Jay, 2000). The availability of water has a marked influence on bacterial growth rates (Jay, 1992). Most spoilage bacteria do not grow below a_w of 0.91. With respect to food poisoning bacteria, *Staphylococcus aureus* has been found to grow at water activity as low as 0.8 (Farber *et al.,* 1992; Christian, 1980) while *Clostridium botulinum* does not grow at water activity below 0.95 (Jay, 1992). Generally, water requirement of microorganisms is described in terms of water activity (a_w) of the food or environment. Water activity is defined as the ratio of water vapour pressure of the food substrate to the vapour pressure of pure water at the same temperature (Jay, 2000): $a_w = p/p_o$, where p = the vapour pressure of the solution and p_o = the vapour pressure of the solvent, usually water (Atlas, 1995). The concept is related to relative humidity, (R.H) in the following way: Relative humidity = 100 x a_w. The a_w of a food describes the degree to which water is "bound" in the food, its availability to participate in the chemical/biochemical reactions, and its availability to facilitate growth of microorganisms.

Micro-organisms display varying degrees of sensitivity to the oxidation-reduction potential (O/R, E_h) of their growth media. Redox potential of a substance is defined in terms of ratio of the total oxidizing (electron accepting) power to the total reducing (electron donating) power of the substance and it is measured in millivolts (Jay, 1992; Morris, 2002). The E_h of a substrate is referred to as the ease with which the substrate loses or gains electrons. A substance that readily takes up electrons is a good oxidizing agent, while one that readily gives up electrons is a good reducing agent (Jay, 1992; Morris, 2002). The more highly oxidized a substance is, the more positive will be its E_h and the more highly reduced a substance is the more negative will its electrical potential be. Aerobic micro-organisms such as those belonging to the genus *Bacillus* require positive E_h values (oxidized) for growth, while anaerobic bacteria such as those belonging to the genus *Clostridium* require negative E_h values (reduced). Some aerobic micro-organisms grow better under slightly reduced conditions and are often referred to as microaerophilic (Jay, 1992; Morris, 2002). Examples of microaerophilic bacteria are *Lactobacilli* and *Streptococci.*

Microorganisms require certain basic nutrients for growth and maintenance of metabolic functions. The amount and type of nutrients required range widely depending on the microorganism. These nutrients include water, a source of energy, nitrogen, vitamins and minerals (Mossel *et al.,* 1995; Ray, 1996; Jay, 2000). In order to grow, micro-organisms must draw from the environment all the nutrients that they need for the synthesis of their cell materials and for the generation of energy (Stanier *et al.,* 1980). Water accounts for 80-90% of the total weight of cells and is therefore always the major essential nutrient in quantitative terms (Stanier *et al.,* 1980). Microorganisms also require carbon as a sourse of energy and fermentative ones get it from organic nutrients. The nitrogen and sulphur requirements are often met by organic nutrients containing amino acids, proteins, or products of complex protein degradation such as peptones. Growth factors are a group of nutrients that the micro-organisms cannot synthesize. They include amino acids, constituents of protein, purines and pyrimidines required as constituents of nucleic acids and vitamins (Frazier, 1967). Vitamins are a diverse collection of organic compounds which form parts of the prosthetic groups or active centres of certain enzymes. Microorganisms may require B vitamins in low quantities, and almost all natural foods tend to have an abundant quantity for those organic compounds that microorganisms are unable to synthesize for their essential requirements.

2.1.3.1 Extrinsic factors

Extrinsic factors are those that refer to the storage environment surrounding the food such as types of packaging/atmospheres, effect of time/temperature conditions on microbial growth, storage/holding conditions and processing steps (Jay, 1992; ICMSF 1996; Loss and Hotchkiss, 2002).

Temperature is an important factor. All microorganisms have a defined temperature range in which they grow, with a minimum, maximum, and optimum. The relationship between temperature and growth rate constant varies significantly across groups of microorganisms. Four major groups of microorganisms have been described based on their temperature range as for growth; thermophiles, mesophiles, psychrophiles and psychrotrophs (ICMSF, 1980). Low temperatures reduce membrane fluidity and hence restrict transport of essential

nutrients. Such temperatures also slow down enzyme reaction rates in microorganism (Mossel *et al.,* 1995). Below the minimum growth temperature, metabolic processes are too low to meet the requirements of the cell. Within the growth range for a particular micro-organism, there is an optimal growth temperature at which enzyme reaction rates are at their peak, the highest rate of reproduction occurs and growth rate is minimal (Atlas, 1995). At high temperatures, structural cell components become denatured and inactivation of heat-sensitive enzymes occurs. The microorganisms reproduce the shortest doubling time at their optimum temperature (Lund *et al.,* 2000; ICMSF, 1996 and Doyle *et al.,* 2001). Mesophilic lactic acid bacteria tend to grow best between 10 and 40°C with an optimum around 30°C and they include *Lactococcus lactis* subsp. *lactis, Lactococcus lactis* subsp. *cremoris* as well as *Leuconostoc mesenteroides* subsp. *cremoris* (Oberman and Libudzisz, 1998). Thermophilic lactic acid bacteria such as those that are used to produce yoghurt are represented by two species; *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus salivarius* subsp. *thermophilus.* Some thermophilic species such as *Lactobacillus delbrueckii* subsp. *leichmannii,* which has now changed to subsp. *lactis*, is homofermentative, will grow at temperatures of between 48 and 50°C (Lund *et al.,* 2000; Doyle *et al.,* 2001). Raising the temperature above the maximum usually kills the microbial cell by denaturizing protein and irreversibly damaging molecules essential to the cell's survival (Mossel *et al.,* 1995).

These various factors, particularly in traditional fermented milk products where a spontaneous fermentation is often relied upon, result in a sequence of different micro-organisms responsible for the fermentation. Lactic fermentation is initiated by spherical bacteria such as *Leuconostoc* and *Lactococcus* species. The rod-shaped bacteria take over and lower the pH to around pH 3.6 (Tomkins *et al.,* 1988). Then yeasts may grow in the final product and spoil it. Alternatively, in alcoholic beverages, the initial fermentation would be alcoholic due to yeasts, followed by a bacterial lactic acid fermentation, which would ultimately spoil the product. The complexity of such systems poses great challenges when attempting to elucidate the micro-organisms of importance in a particular product.

The relative humidity of the storage environment is important both from standpoint of water activity (a_w) within foods and the growth of microorganisms at the surfaces (Jay, 1996).

When the a_w of a food is set at 0.60, it is important that this food be stored under conditions of R.H. that do not allow the food to pick up moisture from the air and thereby increase its own surface and subsurface a_w to a point where microbial growth can occur. When foods low in a_w values are placed in environment of high R.H., the foods pick up moisture until equilibrium has been established. Likewise, foods with a high a_w lose moisture when placed in an environment of low R.H. Foods such as cheeses and fermented milks that undergo surface spoilage from molds, yeasts and certain bacteria, should be stored under conditions of low R.H.

In case *Omashikwa* quality will develop into packaging state in the near future, the information given below will be important and should be known in advance in order to control the activities of microorganisms and the quality of *Omashikwa*. Studies have demonstrated the antimicrobial activity of gases at ambient and sub-ambient pressures on microorganisms important in foods (Loss and Hotchkiss, 2002). Gases inhibit microorganisms by two mechanisms. First, they can have a direct toxic effect that can inhibit growth and proliferation. Carbon dioxide (CO_2), ozone (O_3) and oxygen (O_2) are good examples of such gasses. A second inhibitory mechanism is achieved by modifying the gas composition, which has indirect inhibitory effects by altering the ecology of the microbial environment. Nitrogen (N_2) replacement of oxygen is an example of this indirect antimicrobial activity (Loss and Hotchkiss, 2002).

The storage of food in atmospheres containing increased amounts of CO_2 up to about 10% is referred to as controlled atmosphere packing (CAP) or modified atmosphere packing (MAP) (Jay, 1996). Other methods include controlled atmosphere storage (CAS), direct addition of carbon dioxide (DAC) and hypobaric storage or cold storage under partial vacuum (Loss and Hotchkiss, 2002). The MAP methods with addition of inert gas such as nitrogen (N_2) are mostly used to control food spoilage especially in meat, fish, ghee or butter oil and milk powder. Unfortunately, microorganisms are very versatile, when one restricts CO_2, aerobic microorganisms grow. Likewise, when one restricts O_2, anaerobic and facultative anaerobic microorganisms grow. Vacuum packaging is an alternative method which tends to substantially increase the shelf-life of food products. These various factors, particularly in traditional fermented milk products where a spontaneous fermentation is relied upon, results

is a sequence of different microorganisims responsible for fermentation. These factors are important to know for product development and for future packaging and storage of traditional fermented food products such as *Omashikwa*.

2.2. FLAVOUR COMPOUNDS IN FERMENTED MILK

2.2.1 Diacetyl

Diacetyl (butanedione) is a compound that gives buttermilk, cultured sour milk and some yoghurts a sweet, buttery aroma (Longo and Sanroman, 2006; Hugenholtz, 1993). Homofermentative *Lactococcus lactis* subsp. *lactis* biovar. *diacetylactis* and heterofermentative *Leuconostocs* including the strains of *Leu. mesenteroides* subsp. *cremoris*, metabolize citrate. The co-metabolism of lactose and citrate has been studied by Cogan (1987); Verhue and Tjan (1991); Hugenholtz (1993) and Ramos *et al.,* (1994). Citrate is not metabolized as an energy source, but is readily utilized in the presence of another fermentable carbohydrate. In both the *Lactococci* and the *Leuconostocs*, citrate uptake is plasmid-encoded and is coupled to translocation of protons in response to the proton-motive force generated by adenosine triphosphate (ATP) hydrolysis (Bellingier *at al.,* 1994). Carbon dioxide is released by decarboxylation to the intermediate.

Acetaldehyde (ethanal) (Walstra *et al.,* 1999). This active aldehyde is likely to remain associated with the enzyme pyruvate decarboxylase, which requires thiamine pyrophosphate as a cofactor (Hugenholtz, 1993). Decarboxylation may be followed by a number of different transformations resulting in diacetyl, acetoin and or 2, 3-butanediol. Citrate lyase, the first enzyme of the pathway, and acetolactate synthase which gives rise to acetolactate from which acetoin is formed, have been purified from *Lactococcus lactis* subsp. *lactis* variants which carry the *cit* plasmid (Bowien and Gottschalk, 1977). The citrate lyase enzyme is inducible in *Leuconostocs*, but is constitutive in the biovar. *diacetylactis* of *Lactococcus lactis* subsp. *lactis*. The organisms involved are called aroma-forming bacteria.

There are two metabolic routes which yield diacetyl. Oxidative decarboxylation of acetolactate and condensation of acetaldehyde-TPP with acetyl CoA (Verhu**s** and Tjan, 1991). The former may not be the major route according to Cogan and Jordan, (1994) as the strains do not generally produce large amounts of acetolactate. Degradation is dependent on

pH and redox potential (Eh) (Cogan et $al.$ 1981; Bassit et $al.$ 1993). Citrate is transported into the cell by citrate permease as shown below. At first, citrate is hydrolyzed into acetate, CO_2 and pyruvate by citrate lyase according to:

$$COOH.CH_2.C(OH)COOH.CH_2.COOH \rightarrow CH_3.COOH + CO_2 + CH_3.CO.CO$$

2.2.2 Acetaldehyde

Acetaldehyde (ethanal) is essential for the characteristic yoghurt flavour and aroma. It is predominantly accumulated by rod-shaped lactic acid bacteria that have no alcohol dehydrogenase enzyme (Narvhus et $al.,$1998). The yoghurt bacteria, $Lactobacillus$ $delbrueckii$ subsp. $bulgaricus$ and $Streptococcus$ $thermophilus$, including $Lactococcus$ $lactis$ subsp. $lactis$ biovar. $diacetylactis,$ however, do not have this enzyme so that any intracellular acetaldehyde will be excreted as an end-metabolite (Marshall and Cole, 1983). Examples of acetaldehyde-accummulating bacteria are found among strains of $Lactococcus$ $lactis$ subsp. $lactis$ biovar. $diacetylactis$ and $Lactobacillus.$ $delbrueckii$ subsp. $bulgaricus.$ The latter bacterium and $Streptococcus$ $thermophilus$ also produce acetaldehyde from the free amino acid threonine according to:
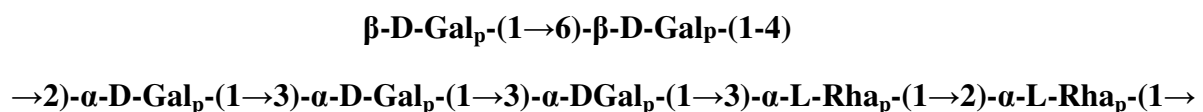
$threonine$ $aldolase$

threonine $\rightarrow$ acetaldehyde + glycine

Far more acetaldehyde is accummulating via this pathway than via carbohydrate metabolism pathway (Cogan and Accolas, 1996; Walstra et $al.,$ 1999).

2.2.3 Production of polysaccharides (slime)

Most strains of lactic acid bacteria, namely *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus*, including some strains of *Lactococcus lactis* subsp. *cremoris* and subsp. *lactis*, produce polysaccharides (Nakajima *et al.,* 1990). A number of authors have investigated the viscous (ropy) nature of milk after fermentation with mesophilic and thermophilic lactic acid bacteria. It is generally accepted that ropiness is related to the synersis and excretion of exopolymers (Nakajima *et al.,* 1990; Gruter *et al.,* 1992 and 1993; Bubb *et al.,* 1997). A layer of these polysaccharides built of galactose and other sugar residues can envelop the bacteria cells (capsule). This is called a glycocalix. The polysaccharides can also be excreted into the medium in the form of slime, and then are called exopolysaccharides. The substances either homopolysaccharides (dextrans) from mesophilic *Leuconosctocs* or heteropolysaccharides from *Lactococci* and the *Lactobacilli,* are important for the properties of stirred yoghurt and other fermented milks. These latter polymers are branched and will differ in composition depending on the carbohydrate source on which they are grown (Cerning *et al.,* 1994; Bubb *et al.,* 1997). The exocellular polysaccharide of *Streptococcus thermophilus* is a heteropolymer of D-galactopyranose and L-rhamnopyranose residues in the molar ratio 5:2. (Bubb *et al.,* 1997). The polysaccharide has a branched heptasaccharide repeating unit with the following structure:

β-D-Gal$_p$-(1→6)-β-D-Gal$_p$-(1-4)

→2)-α-D-Gal$_p$-(1→3)-α-D-Gal$_p$-(1→3)-α-DGal$_p$-(1→3)-α-L-Rha$_p$-(1→2)-α-L-Rha$_p$-(1→

2.3   THE EFFECT OF FERMENTATION ON FOOD SAFETY

Diarrhoeal diseases are among the major causes of death in most developing countries and also a major factor in malnutrition in young children (United Nations Children's Funds - UNICEF, 1988). In Africa, milk mixed with gruels or porridges from cereals are generally used as weaning food for infants. It can therefore be assumed that a large part of the diarrheal diseases are food-borne and it has been confirmed that these weaning foods and rural water are important sources of pathogens (Mathur and Reddy, 1983).

The bacteria that are most common agents of diarrhoea include enterotoxinogenic *Escherichia coli, Campylobacter, Salmonella , Shigella,* and *Vibrio cholera* (Fernandes *et al.,* 1987). Lactic acid bacteria in fermented milk products significantly suppress the growth of food-borne pathogens (Adams and Hall, 1998; Soomro *et al.,* 2002). The lactic and acetic acids produced during fermentation lower the pH to less than pH 4.5. This strongly inhibits the pathogenic bacteria which do not grow at such low pH and also slows down the rate of bacterial spoilage of food (Jay, 1992). For maximum benefits, fermented milk and milk products must not be heat-treated because this will eliminate viable bacteria in the product that may be beneficial to health (Hargrove and Alford, 1980). The importance of hygiene and sanitation before and during fermentation cannot, therefore, be underestimated.

2.3.1 Organic acids

Lactic acid fermentation in food products like *Omashikwa* is characterized by the accumulation of organic acids, primarily lactic and acetic acids, and the accompanying reduction in pH (Cogan, 1983; Adams and Hall, 1988; Kandler, 1983; Cogan and Accolas, 1996). Acid production is an efficient agent for inhibiting pathogenic and spoilage bacteria since they have broad antibacterial activities (Kociubinski *et al.,* 1996). Milk fermentation is traditionally used for improving food safety and shelf-life (Adams and Hall, 1988). Levels and proportions of organic acids produced during fermentation depend on the types of micro-organisms involved, chemical composition of the culture and the physical conditions encountered during fermentations (Sanni, 1993).

The preservation action of acids may be partly due to the depression of internal (cytoplasmic) pH (Russell, 1992; Daly and Davis, 1998; Soomro *et al.*, 2002). Undissociated acid molecules are lipophilic and pass readily through the plasma membrane by diffusion. In the cytoplasm (pH 7) acid molecules dissociate into charged anions and protons (Eklund, 1985). These cannot pass across the lipid layer and accumulate in the cytoplasm thus reducing the pH. The acidified cytoplasm in turn inhibits metabolism, in particular the enzymes of metabolism (Krebs *et al.,* 1983; Stratford and Anslow, 1998).

In principle, a target for many antibacterial compounds is the plasma membrane and an alternative mechanism which may explain how acids act as preservatives, is that they eliminate the proton motive gradient (Eklund, 1985). The selective permeability of the plasma membrane to protons allows cells to create a pH gradient and an electrical potential of which together form the proton motive force (Eklund, 1985). In the chemiosmotic theory, the energy contained in the proton motive force is then used to drive the uptake of essential nutrients such as amino acids (Bracey *et al.,* 1998). The accumulation of charged acid particles in the cytoplasm disrupts the proton motive force and prevents uptake of amino acids (Prescott *et al.,* 2005).

2.3.2 Bacteriocins

Gram-positive bacteria including all dairy and food fermenting genera of lactic acid bacteria are well-known for their production of antimicrobial proteins or peptides collectively known as bacteriocins (Gross and Morell, 1971; Kociubinski *et al.,* 1996). These protein complexes are active against Gram-positive bacteria and normally known to display a narrow range of inhibitory activity that affects closely related species within *Lactobacilaceae* (Klaenhammer, 1988; Klaenhammer, 1993). Bacteriocins have been isolated from fermented milks and dairy products (Litopoulou-Tzanetaki, 1987) and may also be present in traditional fermented milks like *Omashikwa* as preservatives and also for safety of consumers in the rural enviroment.

The ability of many bacteriocins to inhibit some food-borne pathogens makes them attractive as potential food preservation agents. The best characterized bacteriocin produced by lactic acid bacteria is nicin. It is produced by *Lactococcus lactis* subsp. *lactis* and has been available commercially in concentrated form since 1959 (Holzalpfel *et al.,* 1995; Coventry *et*

al., 1997). Most micro-organisms require an intact plasma membrane (Bracey *et al.,* 1998). Nicin is strongly attracted to phospholipids in bacterial and liposomal membranes. The cationic nicin molecule initially interacts by electrostatic attraction within anionic membrane phospholipids. The molecules reorient themselves in the membrane in such a way that they form non-selective pores (Von Mollendorff *et al.,* 2006; Sezer and Guven, 2009). The net result is that nicin makes cytoplasmic membrane permeable, which causes the release of accumulated amino acids from the cells as well as membrane vesicles of sensitive bacteria by leakage (Klaenhammer, 1993; Holzalpfel *et al.,* 1995).

Nicin exhibits broad spectrum inhibitory activity against Gram-positive bacteria, including spore-forming bacteria (Klaenhammer, 1988). It inactivates thermophilic spoilage microorganisms in canned foods (Stevens *et al.,* 1991). Nicin and pediocin, a bacteriocin produced by *Pediococcus* species, have been shown to be effective in controlling *Listeria monocytogenes* in white pickled cheese, skim milk, yoghurt and other foods (Schaack and Marth, 1988; Ming *et al.,* 1997).

2.3.3 Anti-cancer effects

Intestinal microflora may be involved in colon carcinogenesis (Gustafsson *et al.,* 2005; Fonden *et al.,* 2000). Anaerobes such as *Peptostreptococcu*s and *Clostridium* as well as *Escherichia coli* produce high amounts of β-glucuronidase and nitroreductase enzymes that increase the rate of conversion of indirectly acting carcinogens into proximal carcinogens (Cole *et al.,* 1985). *Beta*-glucuronidase influences the enterohepatic circulation of carcinogenic conjugates and nitroreductase enhances the formation of reactive N-nitroso and N-hydroxy intermediates thereby converting aromatic nitro-compounds into potentially harmful amines (Ling *et al.,* 1994). There is interest in understanding how the colonic flora can be modified through diet. Diet influences the activities of these enzymes in the intestinal tract (Goldin and Gorbach, 1984). Among the food products studied as potential modifiers of the colonic microflora, dairy products containing viable organisms have received the most attention (Marteau *et al*., 1990; Jay, 1992; Sreekumar and Hosono, 2000). Ling *et al.,* (1994) confirmed a reduction in faecal β-glucuronidase and nitroreductase activities after ingestion of *Lactobacillus.* They suggested that the partial replacement of the flora in the gastrointestinal tract by *Lactobacilli* could reduce the levels of these enzymes.

2.4 PHYSIOLOGICAL BENEFITS OF FERMENTED MILKS

2.4.1 Lactose utilization

Lactose intolerance describes a situation in which an individual lacks adequate ability to digest lactose. This inability is for the most part due to insufficient amounts of the enzymes β-galactosidase and β-phosphogalactosidase in the small intestine (Swagerty *et al.,* 2002; Beyer, 1989). The former acts on lactose, while the latter acts on lactose phosphate to split it into monosaccharide components. The products of the reaction are glucose and galactose 6-phosphate. The galactose is then catabolized via the Tagatose pathway at the same time as the glucose is catabolized via the EM pathway. The usual symptoms associated with these problems include cramps, flatulence and diarrhea following the consumption of milk products (Beyer, 1998).

Lactose-intolerant individuals can consume certain fermented dairy products such as *Omashikwa* without harmful effects. Where beneficial effects have been found, they have been attributed to the reduced level of lactose in the fermented product and to the production of β-galactosidase and β-phosphogalactosidase by fermenting micro-organisms following ingestion of the products (Blanc, 1984; Vesa *et al.,* 1996). The bacteria used to make yoghurt and other fermented milks such as *Omashikwa,* contain the enzyme β-galactosidase which can improve lactose utilization by lactose-intolerant individuals. Being intracellular, β-galactosidase of yoghurt starter culture bacteria seems to be able to survive passage through the stomach to reach the small intestine (McFeeters, 1988; Fernandes and Shahani, 1989).

2.4.2 Hypocholesterolemia activity

Risks of heart attacks in hypocholesterolemic individuals can be significantly reduced by lowering their plasma cholesterol (Fuller, 1989). It has been claimed that cholesterol is lowered due to a factor produced or enhanced by the action of the starter culture bacteria during fermentation (Jay, 1992) such as in *Omashikwa*. The factor may lead to decreased synthesis of cholesterol and removal of cholesterol or its precursors from the gastrointestinal tract. The factor may also inhibit cholesterol synthesis in the body (Danielson *et al.,* 1989; Akalin *et al.,* 1997). Hypocholesterolemic effects of *Lactobacillus gasseri* were found and were attributed to the ability of the culture to suppress the reabsorption of the bile acids into

the enterohepatic circulation and to enhance the excretion of acidic steroids in faeces of hypocholesterolemia rats (Usman, and Hosono, 2000).

2.4.3 General microbial interference (antagonism)

This phenomenon refers to the general nonspecific inhibition or destruction of one microorganism by other members of the same habitat or environment. The souring of milk is due primarily to the fermentative conversion of lactose to organic acids, mainly lactic and acetic acid. The pH is lowered from 6.8 to less than 4.6. This leads to an increased shelf-life and safety of fermented milks with regards to some food pathogens e.g. *Streptococcus aureus*, *Bacillus cereus*, *Salmonella* spp. and *Clostridium* spp. (Nout *et al.,* 1987; Aryanta *et al.,* 1991). *Lactobacillus acidophilus* and *Bifidobacterium bifidum* are well known for inhibitory activity towards the commonly known food-borne pathogens (Fuller, 1989). Both micro-organisms have been shown to be both preventive and therapeutic in controlling intestinal infections through administration of milk containing one or both micro-organisms. The exact mechanism is not clear but it is likely that the organic acids produced by the micro-organisms may be involved in such antagonistic action. The inhibitory activity of the acids is governed by the dissociation constant (pK_a) and acid concentration at a given pH. Therefore, an organic acid of high pK_a value has more acid in the undissociated form and has a stronger antimicrobial activity. For example, the activity and pK_a values of some organic acids are: lactic (3.83) < benzoic (4.19) < acetic (4.73) < propionic (4.87) (Gould, 1991; De Vuyst and Vandamme, 1994), and it has been reported by Lindgred and Dobrogosz, (1990) that acetic acid has up to four times more of the acid in the undissociated form at pH 4.0-4.6 when compared with lactic acid. Furthermore, the undissociated forms of lipophilic acids can penetrate a microbial cell, dissociate to produce hydrogen ions, interfere with metabolic function and cause an inhibitory effect. The phenomenon of a lactic acid bacterium inhibiting or killing closely related and food-poisoning or food-spoilage organisms in cultures have been observed to be associated with antibiotics (bacteriocins or bacteriocin like factors such as nicin), hydrogen peroxide, depressed pH, diacetyl and nutrient depletion (Marteau and Rambaud, 1993; Abee, 1995).

2.5 EFFECT OF FERMENTATION ON NUTRITIONAL VALUE OF MILK

Milk fermentation is a very complex process since it normally involves the interaction between the product and a group of microorganisms. This means that any changes that occur during fermentation will depend on the available nutrients and nutrient precursors in the raw milk, the metabolic activities of the microorganisms responsible for the fermentation and any possible interactions of these elements (McFeeters, 1988).

2.5.1 Proteins

The total amino acid composition of yoghurt and other fermented milk products does not differ substantially from that of the milk which they originate (Fernandes *et al.,* 1992). However, during fermentation some lactic acid bacteria utilize milk proteins as a nitrogen source to ensure their growth. *Lactobacullus helveticus,* in particular, is recognized as possessing efficient protease and peptidase activities with respect to milk proteins. The Protein Efficient Ratio (PER) of milk proteins is already very high and it was found that the bioavailability of yoghurt proteins, as measured using rats, was not improved significantly above that of milk (Fernandez *et al.,* 1994). Fermentation has been found to increase the total free amino acids as well as the quantity of the essential amino acids lysine, tryptophan and methionine (Umoh and Fields, 1981; Chavan and Kadam, 1989; Steinkraus, 1994). Contrary to the above, results in fermentation studies done with foods of higher nutritional value such as milk, available lysine was reduced by 40%, which is from 56.4 g/100 g to 22.6 g/100 g, when skim milk was fermented with *Lactobacillus acidophilus* (Rao *et al.,* 1982).

2.5.2 Vitamins

Fermentations may result in changes in vitamin content by several mechanisms. These include synthesis of vitamins by fermenting microorganisms, loss of vitamins by metabolism of fermenting microorganisms and of the fermenting food, loss of vitamins by chemical reactions not directly related to fermentation, increase or decrease in stability of vitamins due to pH changes and heating losses associated with preparation of raw material prior to or after fermentation (McFeeters, 1988).

During the manufacture of yoghurt, heat treatment of the milk causes losses in the amount of vitamin B$_{12}$ (Rasic and Panic, 1961). Some lactic acid bacteria require B vitamins for growth, while several lactic acid bacteria are capable of synthesizing them. *Lactobacillus delbrueckii* subsp. *bulgaricus* require folic acid (Deeth and Tamime, 1981; Gilliland, 1990). Vitamins that increase during the manufacture of yoghurt are niacin and folic acid because they are actively synthesized by *Streptococcus salivarius* subsp. *thermophilus. Lactobacillus acidophilus* was found to increase folic acid levels in skim milk (Deeth nd Tamime, 1981; Friend, *et al*., 1983).

2.6   TRADITIONAL FERMENTED MILKS IN AFRICA

Most traditional fermented milks resulting from natural fermentation fluctuate in quality (Nout, 1985). The process tends to be difficult to control if carried out at a large scale. The presence of a significant amount of accompanying natural microflora with their different metabolic pathways can accelerate spoilage once fermentation is completed, especially with increased holding periods between production and consumption. In order to control this problem, traditional milk fermentation processes have been manipulated by the processors in order to preserve and improve the quality. One such method is the draining of some whey (40-50%) after fermentation and mixing of the curd to a smooth consistency (Olasupo and Azeez, 1992; Mutukumira, 1995a). The process reduces the volume of the original product as some whey has to be drained off to obtain the desired consistency. Some milk producers smoke the fermenting milk containers and the milk with wood of certain tree species, as a method of improving the flavour, colour, taste and palatability (Shalo and Hansen, 1973; Bekele and Kassaye, 1987; Kurwijila, 1989; Kimonye and Robinson, 1991).

Processing of various fermented milk is widespread throughout Africa and elsewhere, and has been described by many workers. For example, Kurmann *et al.,* (1992) and Ashenafi (1994) described fermented milk from Ethiopia known as E*rgo*. Milk is allowed to ferment naturally and is accumulated over a period until the desired acidity has been achieved. The product is viscous and may be churned into butter (Fekadu and Abrahamsen, 1997).

The pastoralists of Southern Ethiopia consume traditionally fermented milk called *Ititu* (Bekele and Kassaye, 1987; Kassaye *et al.,* 1991; Kurmann *et al.,* 1992). The fermenting

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vessel (a gourd) is smoked with *Acacia nilotica* wood before the milk is added. *Ititu* contains the essential amino acids. The predominant lactic acid isolated from *Ititu* was identified as *Lactobacillus plantarum* (Kassaye *et al.,* 1991).

The nomadic tribes of Kenya use smoked gourds for the fermentation of milk (Shalo and Hansen, 1973; Kimonye and Robinson, 1991). A glowing splinter from a selected species of tree or shrub such as *Cassia didymobotrya, Olea africana, O. capensis, Lantana kitu, Rhus natalensis, Prunus africana, Euclea divinorum, Croton macrostacbyus, Acacia gerardi* and *Eucalyptus* spp. are used repeatedly to singe the inside of the gourd until a fine layer of charcoal is produced (Shalo and Hansen, 1973; Miyamoto *et al.,* 1986; Kimonye and Robinson, 1991). The fermented milk is characterized by a typical smoky flavour.

According to Kimonye and Robinson (1991) the Masais, Turkanas, Kalenjins, Somalis and Merus tribes of Kenya traditionally produce fermented milk called *Iria ri Matii.* A smoked gourd is used as a fermenting vessel. *Iria ri Matii* can be produced from the fermentation of boiled or unboiled milk. An investigation of *Iria ri Matii* by these authors on isolated bacterial colonies suggested that the dominant organism was characteristic of *Streptococcus thermophilus.* This finding is in agreement with the assertion that there may be correlation between the thermophilic microflora present in *Iria ri Matii* and hot tropical climate of East Africa (Marshall, 1987; Tamime and Robinson, 1988).

The Hausa community in Nigeria produces a naturally fermented milk product known as *Nono* (Eka and Ohaba, 1977; Atanda and Ikenebomeh, 1991; Kurmann *et al.,* 1992; Olasupo and Azeez, 1992). Studies by Olasupo and Azeez (1992) indicated that *Nono* was still produced on household scale and that the production had spread to other regions of Nigeria. Unpasteurized milk is allowed to ferment naturally in a calabash over a period of two to three days. Excess whey is drained off, and the product is stirred to obtain a uniform consistency. A nutritional study of *Nono* showed that the protein content was higher (4.7%) in this product than in Nigerian commercial yoghurt (3.0%). This result may be attributed to the removal of whey during preparation of the product. However, various Nigerian workers have described the quality of *Nono* as poor (Umoh *et al.* 1990; Atanda and Ikenebomeh, 1991). This unsatisfactory quality has been attributed to the crude method of production (Eka and Ohaba, 1977). The use of proper lactic starter culture and heat treatment with good manufacturing

practices showed that a safer and better quality product can be produced (Atanda and Ikenebomeh, 1991).

In South Africa, traditional fermented milks, *Maas* and *Inkomasi* were described by Keller and Jordan (1990). These two products were traditionally produced in clay pots and gourds which were used repeatedly. Bacteria present on the inner surface of the container are presumed to be responsible for the fermentation of the milk. Mixed fermentation by hetero- and homo-fermentative *Lactobacilli, Streptococci, Leuconostocs* and yeasts have been reported to dominate the products. Some of the whey may be drained off to obtain a product with higher viscosity. The production of *Maas* and *Inkomasi* has also been commercialized. The presence of similar products in the western Zimbabwe has been mentioned by Feresu (1989). The fermented milk product in Zimbabwe, which is known as *Mukaka wakokora,* is a result of natural fermentation of untreated fresh milk. The product has a drinkable consistency after stirring and is often consumed with a traditional maize porridge called *Sadza* or mixed with other cereal-based food products (Bachmann, 1979). Milk is left to ferment in an earthenware pot or suitable container at ambient temperature (Oliver, 1971; Feresu and Muzondo, 1989).

Other fermented milk products in Africa are quite similar in taste and flavour due to their mode of fermentation including micro-organisms reponsible. Some of the products are more viscous due to removal of excess whey to maintain a thick consistency. *Rob*, Sudanese fermented milk that contains *Strepococcus bovis, Lactobacillus fermentum* and *Enterococcus faecium* and yeasts; *Kluyveromyces marxianus* and *Issatchenkia orientalis* is related to *Laban Khad* of Egypt (Abdel-Malek, 1978) that contains *Lactobacillus plantarum, Leuconostoc mesenteroides* subsp*. cremoris, Lactococcuss* spp. and *Enterococci* spp. and to Moroccan *Iben* which contains *Leuconostoc lactis, Leuconostoc mesenteroides* subsp*. cremoris* and *dextranicum, Lactococcus lactis* subsp *lactis* biovar *diacetylactis* and *Enterococci* spp (Tantaoui-Elaraki and El Marrakchi, 1987). In Sub-Sahara Africa, *Rob* that contains species of *Lactobacillus plantarum, Leuconostoc* spp. and *Enterococci* spp. is akin to *Maziwa-lala* of Kenya (Nout, 1981; Abdel Gadir *et al.,* 2001) and *Maziwa mgando* of Tanzania (Kurwijila, 1989) which forms about 53% of the milk of those countries and to *Maas* of South Africa with dominating *Lactobacilli, Streptococci* and *Leuconostoc* spp. (Golberg *et al.,* 1945). In

West Africa, *Kadam* from Mali is used as a thirst quenching drink in the hot summer season and is dominated by thermophilic bacteria (Bekele, 1989).

The main advantage of spontaneous fermentation processes is that they are appropriate to rural situations, since they were, in fact, created by it. Nout (1987) noted that the variety of micro-organisms present in the natural fermented milk products creates rich and full flavours that are hard to imitate when employing pure starter cultures under aseptic conditions.

Mala, commercial fermented milk in Kenya originated from traditional fermented milk known as *Maziwa lala* (Kiswahili language) which simply means milk which has slept overnight or as *Maziwa mgando*, sour milk curd in Tanzania. Preparation of the traditional fermented milk, *Maziwa lala* or *Maziwa mgando*, which is carried out naturally under ambient temperatures in Kenya and Tanzania has been described by Shalo and Hansen (1973); Shalo (1987) and Kurwijila (1989). The general processing method for *Maziwa lala* is to filter the raw milk into a smoked or non smoked clay pot or gourd and stored undisturbed in a warm place for 3-4 days or until fermented (Fig. 2.3). Fresh batches of milk may be added each day with or without removal of whey, until the gourd or clay pot is full. The fermented milk may be consumed as such or some whey may be removed to increase the total solids and or viscosity (Shalo, 1987; Bekele and Kassaye, 1987).

```
                    Raw Milk
                       ↓
                    Filtered
                       ↓
              Poured in smoked Gourd
                       ↓
    →  →   Inoculated (back-slopping)
  ↑                    ↓
  ↑    Incubated in bulk until coagulated
  ↑ (Room temperature-25-30°C/2-3 days)
  ↑                    ↓
  ↑      Stirred to uniform consistency
  ←  ←        ←  ↓
                    Ready for
              dispatch or consumption
```
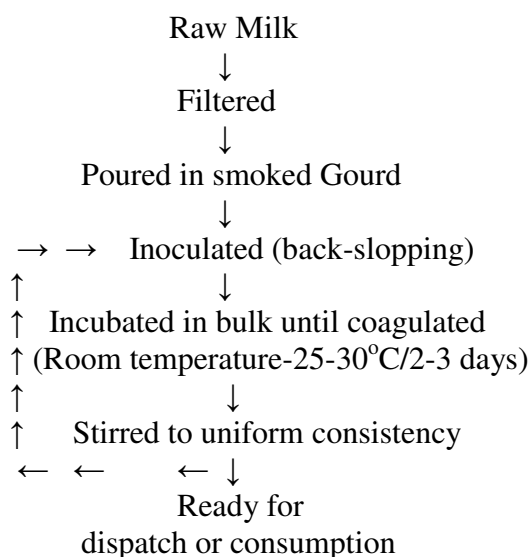
Fig. 2. 3: Traditional procedure for making *Maziwa-lala* (Adapted from Shalo, 1987)

Mala has also been commercially processed by the Kenya Cooperative Creameries (1969). Milk is filtered or clarified, standardized (2.5% fat), homogenized (150-200 bar), pasteurized (65°C/30 min or 72°C/15 sec), cooled to 25-30°C and inoculated with 2% of mesophilic starter culture containing either species of *Lactococcus lactis* subsp *lactis* and or *cremoris*, and *Lactococcus lactis* subsp. *lactis* biovar. *diacetylactis* or *Leuconostoc mesenteroides* subsp. *lactis or cremoris*. Salt and pectin are added to improve its flavour and consistency. *Mala* is then packed, incubated in packets at ambient temperature of 25-30°C for two to three days and distributed as such without refrigeration (Fig. 2.4). Once the acidity reaches 0.9 to1% lactic acid (pH 4.5) from mesophilic bacteria, it stops and cannot continue to sour any further. *Mala* is generally distributed and sold with or without being refrigerated.
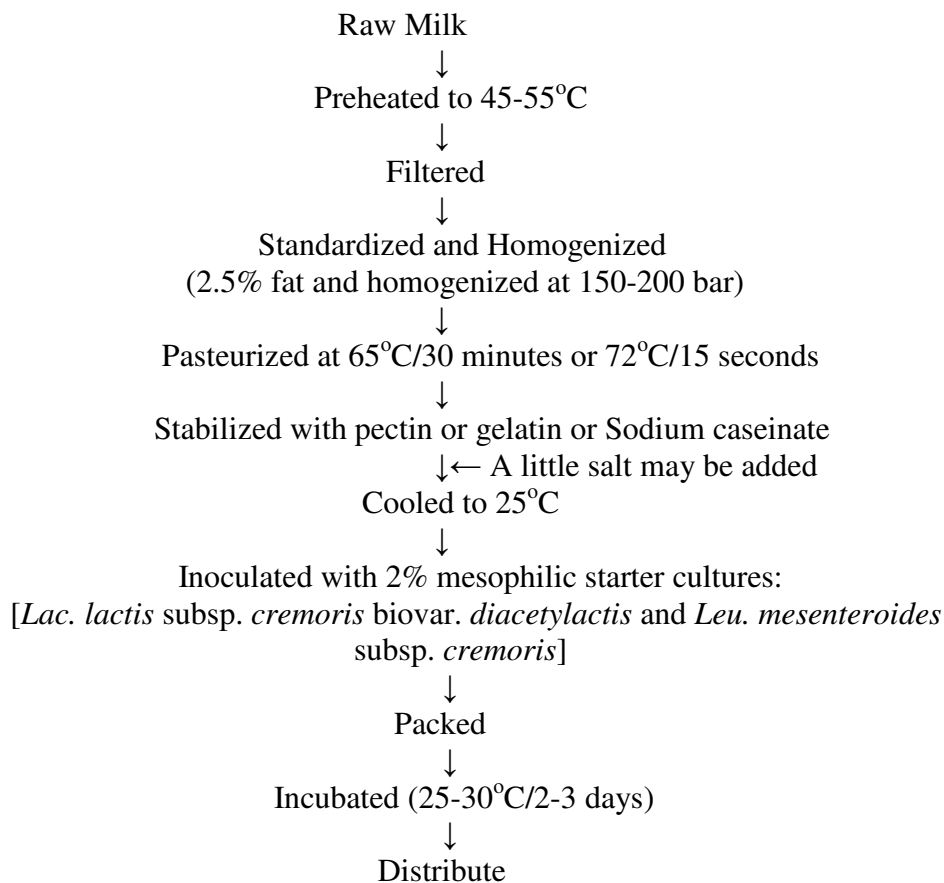
Raw Milk
↓
Preheated to 45-55°C
↓
Filtered
↓
Standardized and Homogenized
(2.5% fat and homogenized at 150-200 bar)
↓
Pasteurized at 65°C/30 minutes or 72°C/15 seconds
↓
Stabilized with pectin or gelatin or Sodium caseinate
↓← A little salt may be added
Cooled to 25°C
↓
Inoculated with 2% mesophilic starter cultures:
[*Lac. lactis* subsp. *cremoris* biovar. *diacetylactis* and *Leu. mesenteroides*
subsp. *cremoris*]
↓
Packed
↓
Incubated (25-30°C/2-3 days)
↓
Distribute

Fig. 2.4: Flow diagram for making commercial *Mala* (Kenya Co-operative Creameries, 1976).

In Namibia**,** fresh buttermilk obtained as a by product of butter processing with 0.5-0.7% fat is pasteurised, cooled to 25°C and inoculated with 2% mesophilic lactic acid bacteria, non-gas forming, mixed and packed for incubation (Fig. 2.5). Coagulation takes place in the packets to pH of 4.5-4.6. Root of *B. albitrunca* tree and natural cultures are not added but

may contain sugar, stabilizer (pectin) and preservatives (potassium sorbate). Buttermilk which is known in other parts of the world as a by-product of butter is made from sweet or cultured pasteurized cream and packed for sale instead of going to waste. The product is branded as *Omashikwa* for commercial purpose due to the popularity of *Omashikwa* among the largest ethnic groups in Namibia, the Owambo and Herero tribes. And also due to its processing method of churning or agitation, similar to the process used in churning of traditional fermented milk to make traditional *Omashikwa*.
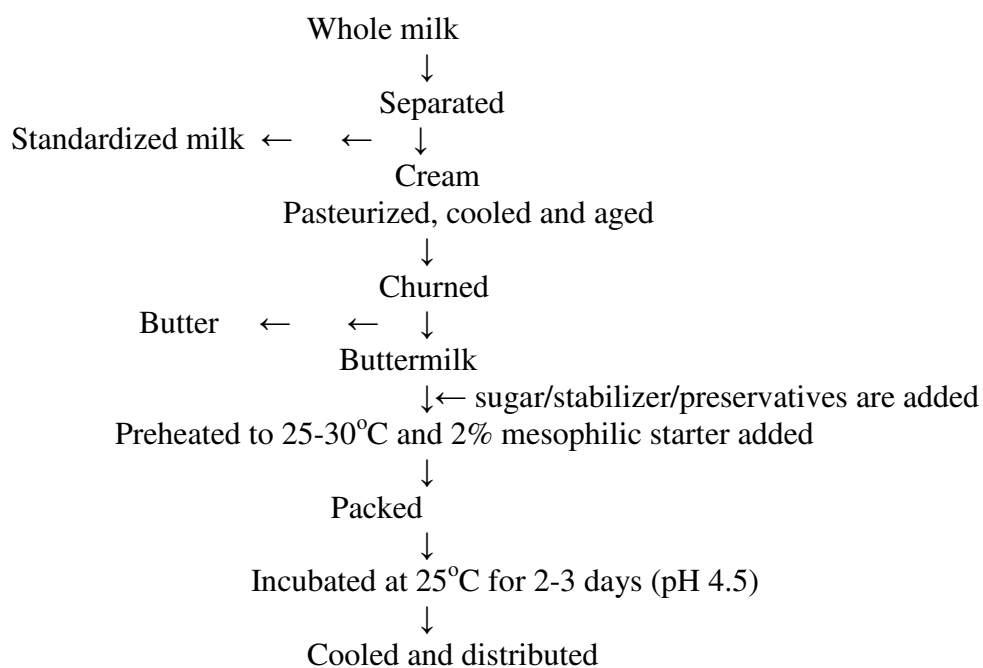
<pre>
                        Whole milk
                            ↓
                        Separated
Standardized milk ←   ← ↓
                          Cream
            Pasteurized, cooled and aged
                            ↓
                         Churned
        Butter   ←   ←   ↓
                       Buttermilk
                            ↓← sugar/stabilizer/preservatives are added
      Preheated to 25-30°C and 2% mesophilic starter added
                            ↓
                         Packed
                            ↓
          Incubated at 25°C for 2-3 days (pH 4.5)
                            ↓
               Cooled and distributed
</pre>

Fig. 2. 5: Flow diagram for commercial *Omashikwa* (Namibia Dairies Ltd, 2000).

2.7   CONCLUDING REMARKS

This study has revealed that traditional fermented milks such as *Omashikwa* have very variable quality in terms of physico-chemical and sensory characteristics. In order to improve the quality of traditional fermented milks, appropriate processing technologies need to be identified for the benefit of the local farmers. Good manufacturing practices on unit operation, especially pasteurization, hygiene, sanitation, use of proper mesophilic lactic acid starter cultures and packaging would probably improve the consistency and general quality of traditional fermented milks in terms of viscosity, syneresis and flavour for marketing to wider areas and for better prices. This in return would generate more income for food security and may create more jobs for the community.