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# Science and technological development of *Omashikwa;* Namibian traditional fermented butter milk


**By**

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**In the**


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# DECLARATION

I declare that this thesis which I hereby submit for the degree of PhD at the University of Pretoria is my own work and has not previously been submitted by me for a degree at any other University or institution of higher education.

Peter George Bille

# DEDICATION

This is dedicated to my family; Mrs. Monica Bille, daughters Marylyne and Gloria and sons Dennis and David for their support, prayers and encouragement during the five years I have been toiling, as a part time student, for the study.

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# ABSTRACT

**Science and technological development of *Omashikwa;* Namibian traditional fermented buttermilk**

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In Namibia, *Omashikwa* traditional fermented buttermilk made with the root of *Omunkunzi* (*Boscia albitrunca*) tree by local farmers is one of the most important rural food products. It provides nutrition, jobs and generates income for food security for the community. As a traditional fermented product, it is unusual as it has a viscous consistency and low syneresis. However, the quality of *Omashikwa* is inconsistent and is characterized by high acid taste, low pH, rancid flavour, root taste and smell and contains filth.

In this research, the production process of *Omashikwa*, physico-chemical properties of the traditional and laboratory prepared *Omashikwa* and the role of the root of *B. albitrunca* tree in *Omashikwa*, viscosity, syneresis, microbiology and sensory properties were studied and compared in order to improve the quality of *Omashikwa* for wider community use and for poverty alleviation. Omashikwa was found to have a protein content of about 3.3%, fat 1.6%, moisture 90%, lactose 4.6%, ash 0.7%, total solids 8.7%, lactic acid 0.9% and a pH of 3.3

The quality of traditionally processed *Omashikwa* (TO) was compared with the laboratory processed *Omashikwa* (LO), which was made without the root. Traditional fermentation was carried out with raw milk and under rural conditions. After fermentation the milk was agitated vigorously to churn into butter, whereas LO was made with pasteurized (65ºC/30 min) and filtered milk, and cream was scooped off after fermentation instead of churning. LO had a significantly ($p < 0.05$) higher pH (4.44) compared to traditional *Omashikwa* (pH 3.25), lower acidity (0.68%) compared to 0.92% of TO. Fat content was higher in LO (2.44% fat) compared to 1.56% fat in TO. LO was free from filth and had higher viscosity (2.98 Pa.s) compared to 2.54 Pa.s and lower syneresis (14.4ml/24 ml) compared to 19.6ml/24ml of TO.

It was found that extract from *B. albitrunca* root showed a low pH of 4.7 and exhibited bacterial inhibition properties on Total Plate Count Agar ring test. The root appear to specifically inhibit *Escherichi coli, Staphylococcus aureus* and Clostridium species. It also had a high content of soluble carbohydrates (hydrocolloids or gum) (19.4%).

Significant difference ($p < 0.05$) in total aerobic counts was observed in TO of 6.62 log cfu/g compared to 8.62 log cfu/g of LO and lower lactic acid bacterial counts, 6.58 log cfu/g compared to 7.87 log cfu/g of LO. Probably the most affected microorganisms in TO were the non acid formers, as lower pH of TO and inhibitory compounds in *Omunkunzi* root could have reduced them. Coliforms, yeasts and moulds counts were not significantly different ($p<0.05$). No pathogenic bacteria were found in either product. The lactic acid bacteria identified belonged to the genera *Lactobacillus (Lb.) (Weissella), Leuconostoc (Leuc.), Lactococus (Lact.) and Streptococcus (Str.)* Twenty representative strains of LAB isolates were identified to species level; three belonged to the species *Lb. delbrueckii* subsp. *lactis* and two belonged to *Lb. plantarum* and two to *Weissella confusa* (former *Lb. confuses).* Three belonged to *Str. salivarius thermophilus,* three to *Leuc. lactis,* and two to *Leuc. mesenteroides* subsp. *mesenteroides.* Three belonged to *Lact. lactis* subsp. *lactis* and two belonged to *Lact. lactis* subsp. *cremoris.*

Significant differences ($p < 0.05$) in descriptive and consumer sensory attributes scores were observed between traditional and laboratory *Omashikwa.* Sensory attributes scores of TO on

the levels of syneresis was 3.4 compared to 2.9 for LO, filth 3.0 compared to 1.8 in LO, rancidity scores were 3.4 in TO compared to 1.8 in LO, and bitterness 4.2 in TO compared to 2.5 in LO. Aroma scores were 2.6 for TO and 4.2 for LO, viscosity 2.5 (TO) and 3.8 (LO) and texture 2.7 for TO compared to 4.2 for LO. There was an 80% preference score given to the laboratory *Omashikwa* by the young consumer panelists.

The results of this study indicate justification of using *B. albitrunca* root in the processing of *Omashiwa* by the rural community to improve the quality of *Omashikwa* in terms of flavour, smell and consistency compared to other traditional fermented milk products and in the absence of modern technology. However, application of good manufacturing practices on unit operations, particularly heat treatment of milk prior to fermentation, use of lactic acid starter cultures, maintenance of good hygiene and sanitation including packaging, seem to be the effective methods to improve and sustain the quality and safety of traditional fermented buttermilk *(Omashikwa)* for a wider market and better price.

Namibia Dairies Ltd, just like any other dairies in the region and elsewhere, manufactures buttermilk, a byproduct of butter that is fermented with mesophilic lactic acid cultures and branded as *Omashikwa* for the purpose of marketing. It has nothing to do with traditional *Omashikwa* as such; *B. albitrunca* root is not added and is processed by using modern industrial method. In addition, additives such as preservatives (potassium sorbate), stabilizers (pectin) and sugar are added and packed for distribution.

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

Since laboratory processing method of *Omashikwa* gave a better quality product compared to traditional method in terms of microbiological quality, sensory attributes, viscosity, filth content, syneresis and general appearance, laboratory processing technique of processing *Omashikwa* is therefore recommended as an alternative and appropriate processing method for small scale production in the rural set up to improve the quality of *Omashikwa*.

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# 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$^o$ to 36$^o$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:

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

$$\mathbf{i.e.\ Lactose + 4H_3PO_4 + 4ADP \rightarrow 4Lactic\ acid + 4ATP + 3H_2O}$$

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:

$$\mathbf{Glucose + ADP + Pi \rightarrow Lactic\ acid + Ethanol + CO_2 + ATP}$$

$$\mathbf{i.e.\ Lactose + 2H_3PO_4 + 2ADP \rightarrow 2\ Lactic\ acid + 2\ Ethanol + 2CO_2 + 2ATP + H_2O}$$

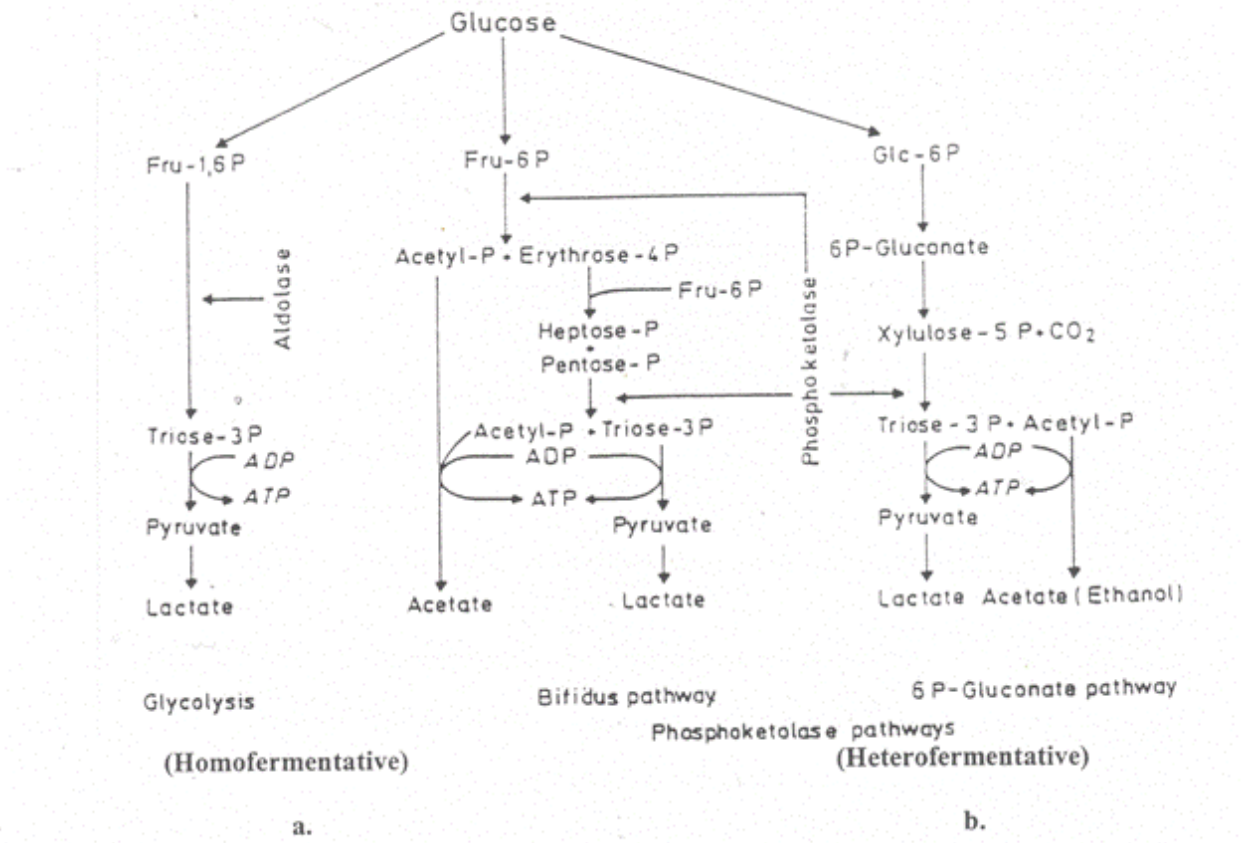


**Fig. 2.1: The pathway for glucose dissimilation by homofermenters and heterofermenters (Kandler, 1983)**

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

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:

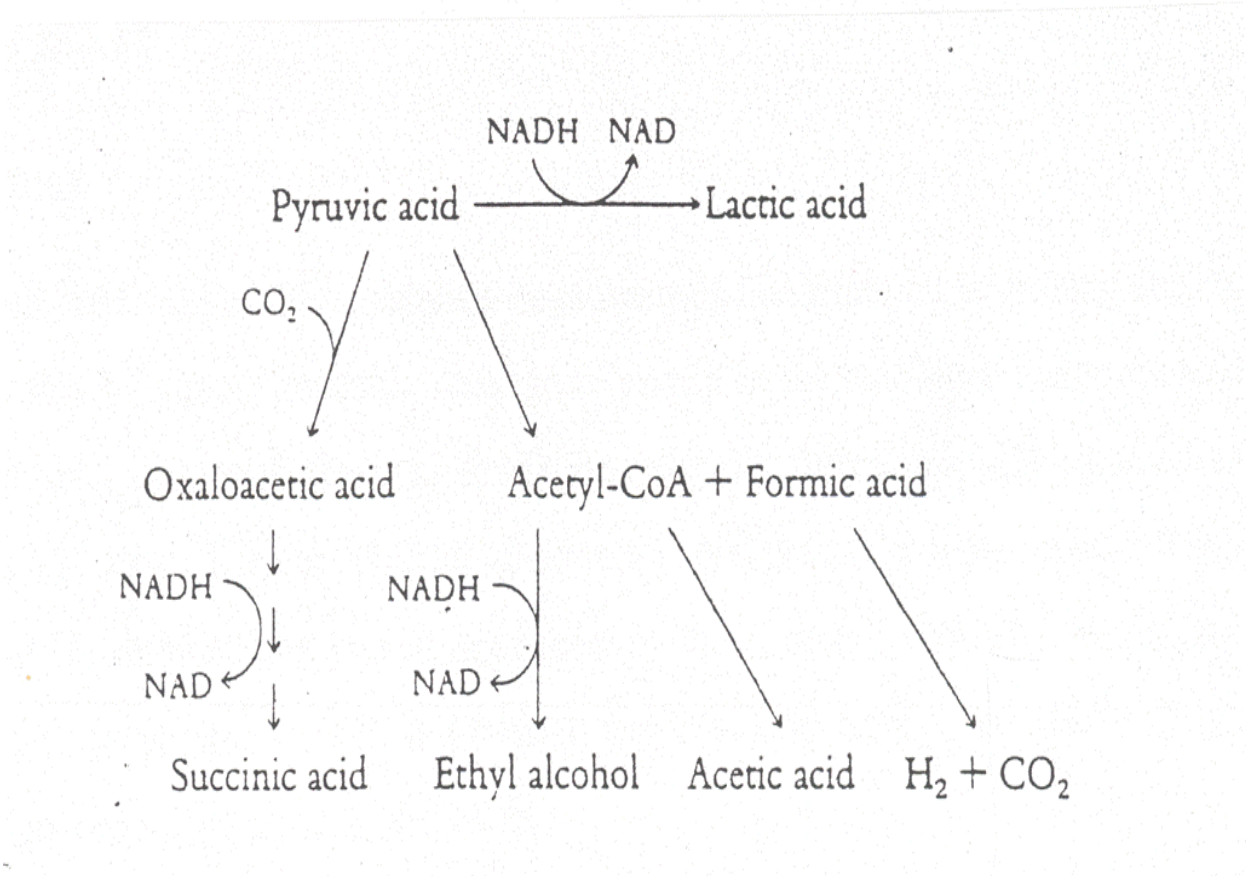


**Fig. 2.2: Mixed acid fermentation by some enteric bacteria**

### 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:

$$2H_2O_2 \rightarrow 2H_2O + O_2$$

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$^{o}$C with an optimum around 30$^{o}$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$^{o}$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 (Verhus 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 (E*h*) (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:

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

### 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:

$$\mathbf{threonine} \xrightarrow{\textbf{\textit{threonine aldolase}}} \mathbf{acetaldehyde + glycine}$$

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 syneresis 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 primarity 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

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).

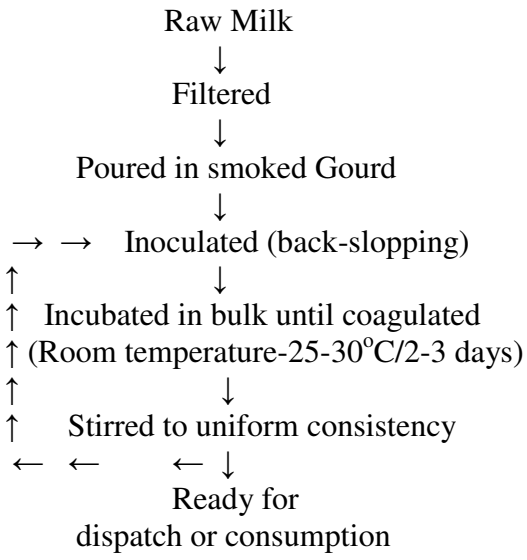


**Fig. 2. 3: Traditional procedure for making *Maziwa-lala* (Adapted from Shalo, 1987)**

*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.

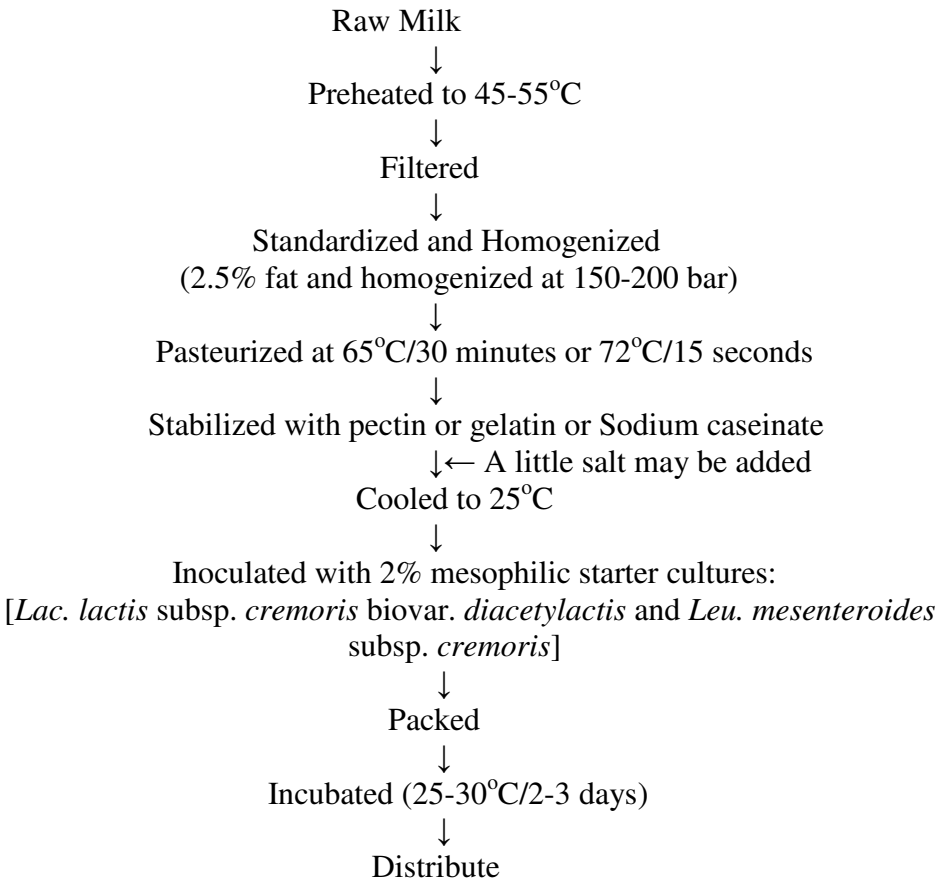


**Fig. 2.4: Flow diagram for making commercial *Mala* (Kenya Co-operative Creameries, 1976).**

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.*

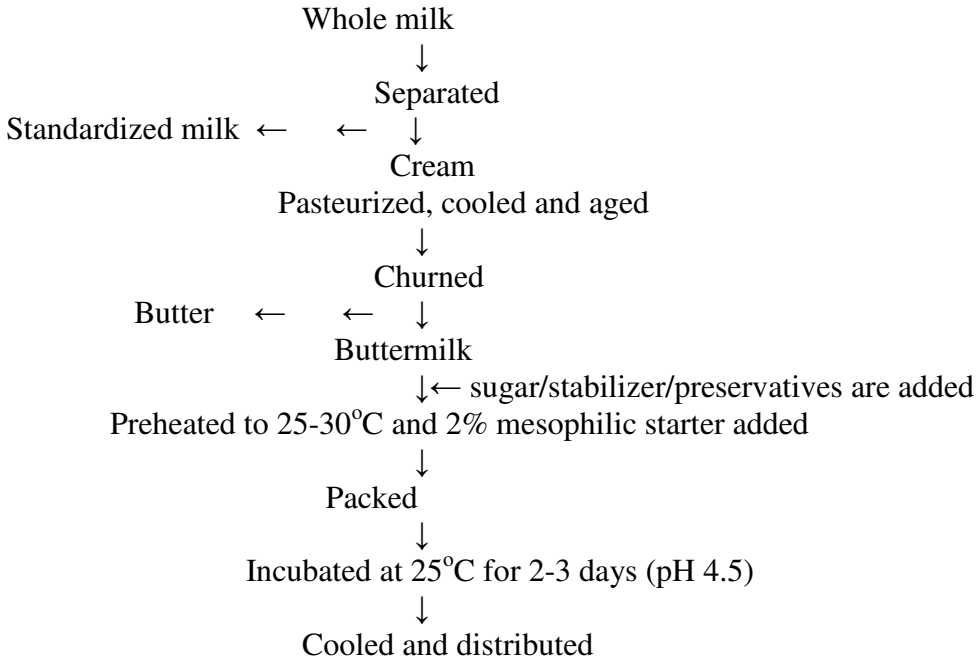


**Fig. 2. 5: Flow diagram for commercial *Omashikwa* (Namibia Dairies Ltd, 2000).**

## 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.

# 3 HYPOTHESES AND OBJECTIVES

## 3.1 HYPOTHESES

1. Processing of *Omashikwa* with *Boscia albitrunca* root may strongly increase viscosity, reduce syneresis and improve sensory characteristics of *Omashikwa* in the rural areas due to in containing mucopolysaccharides like many other African trees.

2. Processing of *Omashikwa* with *B. albitrunca* root will improve the microbiological quality of *Omashikwa* for safety and quality of the product for rural consumers due to it containing antimicrobial compounds such as phenolics

3. Application of good manufacturing practices on unit operations such as sanitation, hygiene, heat treatment and packaging will strongly improve the quality of *Omashikwa* because such processes will destroy microorganisms and preserve the product's quality by increasing its viscosity, redusing syneresis and improving its flavor as observed in industrial processed fermented milk products.

## 3.2 OBJECTIVES

a) To determine the processing technology and compositional properties of *Omashikwa* produced by traditional processors in Northern Namibia.

b) To determine the bacteriological profile of *Omashikwa* processed with the root of *B. albitrunca* tree.

c) To determine the consumer and descriptive sensory profiles of the *Omashikwa* processed with and without *B. albitrunca* root.

d) To determine consumer preference of *Omashikwa* made with and without *B. albitrunca* root.

e) To devise an improved *Omashikwa* processing method, based on the above findings, suitable for small-scale rural processing.

# 4 RESEARCH

## 4.1. The Technology and Properties of *Omashikwa*, Traditional Fermented Buttermilk Produced by Small-holder Milk Producers in Namibia

(Published in part in the International Journal of Food Science and Technology 2007, 42. 620-624)

## ABSTRACT

The production process and quality of *Omashikwa,* traditional fermented buttermilk made with roots of the Omunkunzi tree (*Boscia albitrunca*), produced by the local farmers in Namibia, were studied. *Omashikwa* is characterized by bitter and rancid flavour, a high acidity, low pH, a rooty taste and a slimy consistency. The quality of *Omashikwa (*TO) from rural producers and laboratory *Omashikwa* (LO) produced in the laboratory were compared.

This was done to compare the quality and improve on it for income generation of rural communities in Namibia. LO had TS and SNF contents of 10.5% and 8.06% respectively while TO had 10.2% (TS) and 8.66% (SNF). LO had a higher pH 4.44 (3.25 for TO), lower acidity 0.68% (0.92% for TO), higher fat 2.44% (1.56% for TO), lower protein 3.21% (3.28% for TO), moisture was 89.5% in LO compared to 89.8% inTO and LO had no filth while TO had 7 particles of filth per 10g. LO had 4.68% lactose while TO had 4.56%. Ash content of LO was 0.77% while TO had 0.67% of TO. Higher viscosity 2.98 Pa.s (2.54 Pa.s for TO), lower syneresis 14.4/24 mL (19.6 mL for TO) and lower total microbial counts 6.72 cfu/g compared to 7.99 cfu/g. High sensory scores were also given to LO by consumer panelists. No strains of pathogenic bacteria were found in either product. Application of good manufacturing practices and the use of known lactic acid starter cultures seem to be effective methods of improving the quality of *Omashikwa.*

### 4.1.1 INTRODUCTION

*Omakshiwa* is an Owambo name for traditional fermented buttermilk produced by local farmers in Namibia. It is consumed as a refreshing drink and as a condiment for other foods like gruel and thick porridge made from maize, pearl millet and or sorghum flours. It is prepared by fermentation of milk with roots of the *Omukunzi* tree (*Boscia albitrunca*) by the Owambo and Herero communities. The fermented milk is agitated to churn it and the butter is removed. *Omashikwa* is usually thick and slimy in texture with bitter and rancid taste and a peculiar rooty flavour.

In many rural areas of northern Namibia, *Omashikwa* is sold by small-scale farmers and by vendors to consumers at the open markets, or to workers on road and building sites. The *Omashikwa* is brought to the market in 20-40 L plastic barrels and retailed in 0.5-L plastic mugs for direct consumption and in 2-5 L recycled plastic bottles for wholesale. Owing to inconsistency of flavour, viscosity and acidity, consumers tend to be selective when purchasing *Omashikwa*.

Observations by the author are that *Omashikwa* can contain high number of small flying insects and dirt particles (filth). However, growing demand for *Omashikwa* in rural Namibia gives an incentive to expand quality production. This would create a larger opportunity for small-scale Omashikwa producers for income generation and household food security.

In the production of traditional fermented milk products in Africa and elsewhere, milk is allowed to ferment spontaneously without heat treatment, or by addition of previously fermented milk as starter culture (back-slopping) described by Keller and Jordan, 1990; Walshe *et al.* 1991. Such products can have problems of off-flavours, flavour irregularities, poor hygiene and sanitation, poor shelf life, inconsistency and unattractive presentation to consumer (Nout, 1985; Olasupo and Azeez, 1992). Consumption of traditional fermented milks with a pH≤4.0 has not been a major health problem owing to the inhibition of pathogens through low pH (Nout *et al*., 1987; Aryanta *et al*., 1991), bacteriocins produced by some lactic acid bacteria (LAB) (Olsen *et al*., 1995) and low redox potential ($E_h$) ( Kim C. Hung & Brakett, 2000).

The objectives of this study were to document the traditional technology of producing *Omashikwa* and to determine its general characteristics and quality, including its potential for industrialisation, by experimental production under laboratory conditions using good manufacturing practices.

## 4.1.2 MATERIALS AND METHODS

### 4.1.2.1 Materials

Nine samples of *Omashikwa* and nine of raw fresh milk were collected in 2003 during the rainy season (January to June) from three sites in northern Namibia. The producers kept and milked indigenous mixed *Sanga* breeds of cattle (*Bos indicus*). *Omashikwa* and raw fresh milk were collected from the sites in sterile screw-cap plastic bottles and transported to the food science laboratory at the University of Namibia in Windhoek, in a portable cooler box packed with ice, as per International Dairy Federation (1985) guidelines. Chemical and microbiological analyses were performed immediately. Roots of *B. albitrunca* were collected from the same sites.

### 4.1.2.2 Production of traditional *Omashikwa*

The traditional *Omashikwa* (TO) production process is shown in Figure 4.1. Milk containers, mostly calabashes/gourd or plastic barrels, are washed, rinsed and filled with approximately 20 L of milk (3/4 full). Pieces (12-15) of *B. albitrunca* roots (each approximately 2 $cm^3$) are added. Starter culture (c. 2 % of the milk volume) from previously made *Omashikwa* is introduced and mixed.

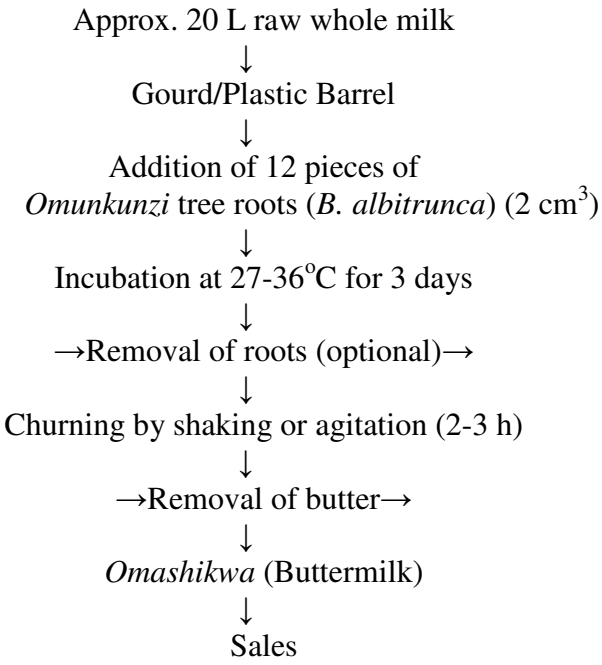


**Figure 4.1 : Procedure for the production of traditional *Omashikwa* in Namibia**

The calabash is then covered and placed at a corner of a hut for 2-3 days at 27-36°C (ambient temperature) to ferment. After fermentation, the roots are removed and the product is shaken (churned) manually, for 2-3 h until the butter separates. The butter is scooped off and washed. It is either used directly or boiled into ghee (butter oil). The buttermilk is *Omashikwa*, and is ready for sale.

#### 4.1.2.3 Production of laboratory *Omashikwa*

Laboratory *Omashikwa* (LO) was produced in a covered 5-L plastic bucket using the traditional method but following good manufacturing practices. The milk was filtered through cheese cloth to remove filth, pasteurised at 65°C for 30 min and cooled to the inoculation temperature of 30°C in a cold water bath. 2 % (based on milk volume) of TO from a previous batch (back-slopping) were added and mixed. The mixture was covered and incubated at 30°C (2-3days) until a coagulum was formed (ca. pH 4.5). The cream was carefully scooped off, accumulated and churned separately into butter, as opposed to the TO procedure. The fermented product was then gently agitated with a wooden spoon to break up the coagulum to obtain a smooth consistency. Samples for analyses were taken at this stage.

#### 4.1.2.4 Chemical analyses

The pH of the samples was measured using a pH meter. Titratable acidity, expressed as percentage of lactic acid, was determined by titration using the method of Case *et al*., (1985). Fat content was determined by the Gerber technique and total nitrogen by the Kjeldahl procedure (Egan *et al*., 1981). Crude protein was calculated by multiplying the total nitrogen by a factor of 6.38. The oven drying method was used to determine the total solids (TS) and moisture contents. Solids-not-fat (SNF) values were obtained by subtracting the fat contents from TS values. Lactose was determined by the Chloramine-T titration method described by Ceirwyn (1995). Ash was determined from the TS according to the AOAC (1995) Methods 925.23 and 945.46

#### 4.1.2.5 Microbial enumeration and isolation

Total microbial counts, LAB counts, coliform counts, yeast and mould counts were enumerated on TO and LO. Ten grams of *Omashikwa* was transferred aseptically into 90 mL sterile Ringer's solution and mixed thoroughly. Serial dilutions were made from each sample in sterile Ringer's solution and 0.1 mL of the appropriate dilutions was spread plated on selective media, as described by Harrigan (1998). Plat count agar was used for enumeration of aerobic mesophilic counts at 30 ± 1$^{o}$C for 48h. MRS agar pH 6.4 (De Man *et al*., 1960) was used for enumeration of total LAB with anaerobic incubation at 30$^{o}$ C for 48h. Violet red bile agar (VRB; Oxoid, Unipath, Basingstoke, UK) was used for enumeration of coliforms at 37$^{o}$C for 48 h. Confirmation of Coliforms was carried out in 2% brilliant green bile broth (BGBB) with Durham tubes. Positive gas and acid production were considered positive for coliforms. Rose-bengal chloramphenicol agar (RBC; Oxoid, Unipath) was used for the enumeration of yeasts and moulds at 25 ± 1 $^{o}$C for 5 days.

#### 4.1.2.6 Enumeration of common pathogens

Samples of TO and LO were analysed by Central Veterinary Laboratory in Windhoek, Namibia, for the presence of common pathogenic micro-organisms found in northern Namibia. These include *Escherichia coli*, *Staphylococcus aureus*, *Salmonella*, *Clostridium* and *Bacillus anthracis*. After serial dilutions, enumerations were carried out in the enriched broth and selective media at 37$^{o}$C for 48 h as follows. *E. coli* was enumerated in Butterfied's

Phosphate buffer (BFPB) and Laury Tryptone agar. *Staphylococcus aureus* in BFPB and Baird-Parker's medium (BPM). *Bacillus anthracis* was enumerated in buffer peptone water and blood agar. *Salmonella* was enumerated in Seline Crytine broth and brilliant green agar and *Clostridium* in reinforced *Clostridium* medium and Blood agar.

#### 4.1.2.7 Sensory evaluation

TO and LO were evaluated by a consumer panel of ten persons who were familiar with the product. The panelists were asked to score for appearance, smell, taste and consistency on a five-point Hedonic Scale, where 1 –disliked a lot, 3- liked moderately and 5-liked a lot.

#### 4.1.2.8 Viscosity

*Omashikwa* viscosity was determined using a Programmable Brookfield Rheometer (Brookfield Engineering Lab., Middlebora, MA, USA), with a spindle size-RV 2 and speed of 2 r.p.m. at $10^{o}$ C for 1 min.

#### 4.1.2.9 Syneresis

Whey separation was determined by placing samples of TO and LO (24 mL) on a wire mesh filter (350-µm opening size). The quantity of whey separating after 2 h at $10^{o}$C, was taken as a measure of syneresis, and was calculated as a percentage (Kessler, 1981).

#### 4.1.2.10 Filth

Thoroughly mixed samples of TO and LO (10 g) were spread in petri dishes and the particles of filth or dirt were enumerated with the aid of an illuminated magnifying glass.

#### 4.1.2.11 Statistical analysis

One-way analysis of variance (ANOVA) was performed on all data collected. Mean comparisons of data from both samples were carried out by Duncan's Multiple Range Test (Steel & Torrie, 1980). Significant differences were calculated at 5% significance level.

### 4.1.3 RESULTS AND DISCUSSION

The process for production of TO is shown in Fig. 4.1 . Essentially, the same procedure was used for the production of LO but without *B. albitrunca* root, milk was filtered, pasteurized, cooled to inoculation temperature, back-slopping inoculation with TO of good quality, controlled fermentation temperature (30$^{o}$C) to a pH of c. 4.5 and scooping off of cream instead of churning, followed by gentle agitation to a smooth texture.

Table 4.1 shows that there was a low pH and a high titratable acidity in both TO and LO. However, there was significant difference ($P<0.05$) between the two products in pH an acidity, with TO showing a lower pH mean of 3.25, (LO pH 4.44) and a higher titratable mean acidity of 0.92% (LO 0.68%). These differences were attributed to the fact that milk for LO was pasteurized; incubation temperature and final pH were controlled. Good manufacturing practices, hygienic and sanitary conditions were used.

Fat contents in TO and LO differed significantly ($P<0.05$) with a mean of 1.6% and 2.4% for TO and LO respectively. In the LO process, the cream was more carefully removed off the *Omashikwa* rather than churning it to butter. This was done to improve the nutritional value of the *Omashikwa* by removing less fat and to prevent it from becoming rancid. It also made it smoother, improved its viscosity and reduced production losses.

**Table 4.1 : Proximate composition and properties of *Omashikwa* (g/100 g)**

| Attributes | TO | LO |
|---|---|---|
| Crude protein | 3.28[a] (0.16) | 3.21[a] (0.08) |
| Crude fat | 1.56 [a] (0.31) | 2.44[b] (0.12) |
| Moisture | 89.8[a] (0.6) | 89.5a (0.2) |
| Lactose | 4.56[a] (0.10) | 4.68[a] (0.05) |
| Ash | 0.67[a] (0.03) | 0.77[a] (0.01) |
| Total solids | 10.2[a] (0.6) | 10.5[a] (0.1) |
| Solids-not fat | 8.66[a] (0.59) | 8.06[b] (0.16) |
| Lactic acid | 0.92[a] (0.25) | 0.68[b] (0.26) |
| pH | 3.25[a] (0.67) | 4.44[b] (0.13) |
| Filth particles [10/g] | 7.0[a] (1.2) | 0.0[b] (0.0) |
| Viscosity (Pa.s) | 2.54[a] (0.24) | 2.98[b] (0.24) |
| Syneresis | 19.6[a] (1.7) | 14.4[b] (2.2) |

**Key:** Means for same attributes followed by the same letter are not significantly different ( $P > 0.05$). Figures in brackets are standard deviation of the mean.

Syneresis was also significantly higher in TO (mean 19.6%) compared with 14.4% in LO. This was probably because of the lower pH, expelling more moisture from the TO coagulum as happens in cheese (Cogan, 1995). The churning process and the presence of gas formers such as coliforms and yeasts, allow curd to separate from whey and float (Nout *et al*., 1987). Some fermented milks in Africa such as *Amasi* of Zimbabwe (Mutukumira, 1995b) and Maass/Inkomasi of South Africa (Keller and Jordan, 1990) require removal of whey (40-50%) to maintain a uniform thick consistency. *Omashikwa* does not require whey removal as it is already thick and viscous. This is presumably because of the production of exopolysacchrides by fermenting micro-organisms. Bubb *et al*., (1997) reported a similar gummy consistency in fermented milk produced by some stains of *Streptococcus* spp. Alternatively, it could also be caused by the presence of gummy compounds released from the *B. albitrunca* roots.

The viscosity of LO was significantly higher (mean 2.98 Pa.s) than that of TO (2.54 Pa.s). Viscosities of both LO and TO were higher than that of fresh milk 1.99-2.10 Pa.s, as reported by Walstra *et al*., (1999). Higher viscosity could be caused by fermentation process (jellying) and the same reasons as for syneresis. The higher viscosity of LO could be because of the fact that LO milk was pasteurized (hence binds water better) and also less cream was removed from LO.

Traditional *Omashikwa* contained high numbers of filth particles (seven per 10 g), while LO had none. This could be attributed to poor sanitation and lack of clarification or filtration to remove filth from the raw TO milk.

LO was given higher sensory scores than TO by the consumer panelists (Table 4.2). Comments of the panelists were that LO was thicker and tasted less harsh. They also stated that TO had a soapy flavour, was watery and thinner and had a very harsh flavour. These can be interpreted as higher viscosity (thicker), mild acidity (less harsh flavour), lower viscosity (watery or thinner) and rancid flavor (soapy). These comments can be attributed to good manufacturing practices used in LO production. The rancid flavour in TO was presumed to be caused by the long process of churning of *Omashikwa* to make butter. This process would rupture the membrane of the fat globules, allowing liquid fat to escape and be hydrolysed by the natural Iipase enzymes present in unpasteurized milk (Walstra *et al*., 1999).

**Table 4.2 : Sensory evaluation of traditional (TO) and laboratory (LO) *Omashikwa***

| | Sensory scores | |
|---|---|---|
| **Attributes** | TO | LO |
| Appearance | 2.8a *(0.2) | 4.0 b (0.3) |
| Smell | 2.6a (0.1) | 3.7 b (0.3) |
| Taste | 2.6a (0.2) | 4.06 b (0.3) |
| Consistency | 2.7a (0.2) | 3.7 b (0.5) |

*Means for the same attributes followed by the same letter are not significantly different (P>0.05). Five-point Hedonic Scale was used: 1 – disliked a lot, 3 – liked moderately, 5 –liked a lot. Figures in brackets are the standard deviation of the mean. Comments on TO and LO by panelists: appearance-thick (LO), thin/watery (TO); consistency – slimy (LO); taste – harsh and soapy (TO).

Table 4.3 shows that total viable cell counts were on the average lower (log 6.72 cfu/g) in LO than log 7.99 cfu/g in TO. LO had lower counts probably because of pasteurization of milk prior to fermentation, controlled incubation temperature and pH and maintenance of good manufacturing practices. According to Kurmann *et al*., (1992) such total cell counts are typical of traditional milk products at the end of fermentation.

Lactic acid bacteria were the dominant micro-organisms in *Omashikwa,* with the same mean count level, log 7.99 and log 7.97 cfu/g in TO and LO, respectively. The dominance of LAB in the *Omashikwa* is presumably because of the acidic environment, which is LAB habitat (Teuber & Geis, 1981).

Coliforms, yeasts and moulds count (Table 4.3 ) were low and were at the same level in both TO and LO. Mean coliforms counts in TO was log 2.68 cfu/g (LO log 2.62 cfu/g) and yeasts and moulds were also low and were at the same level, mean log 1.69 and log 1.56 cfu/g in TO and LO, respectively. The presence of coliforms, yeast and moulds in LO were probably

caused by back-slopping contamination with TO used as a starter culture. The use of known cultures of LAB could improve further the quality of *Omashikwa*.

Pathogenic bacteria were not found in either product. This could be due to low pH, low redox potential ($E_h$) and production of bacteriocins antagonistic to the pathogens as reported earlier. Despite the fact that LO had a relatively higher pH level, it did not contain pathogens. This can be attributed to pasteurization of the milk, good manufacturing practices, sanitation and hygiene.

**Table 4.3 : Mean total microbial numbers (log. cfu/g) of traditional (TO) and laboratory (LO) *Omashikwa***

| Attributes | TO | LO |
|---|---|---|
| Total viable cell counts | 7.99a* (0.04) | 6.72b (0.14) |
| Lactic acid bacteria | 7.99a* (0.06) | 7.97b (0.03) |
| Coliforms | 2.68a (0.09) | 2.62b (0.16) |
| Yeasts/moulds | 1.69a (0.17) | 1.56b (0.26) |
| *Escherichia coli* | nd | nd |
| *Stayphylococcus aureus* | nd | nd |
| *Clostridium* spp. | nd | nd |
| *Bacillus anthracis* | nd | nd |

*Means for same attributes followed by the same letter are not significantly different (P>0.05). nd – not detected. Figures in brackets are the standard deviation of the mean.

### 4.1.4 CONCLUSIONS

The presence of coliforms, yeasts and moulds, high total bacterial counts and high content of filth, low pH, rancid flavour, high syneresis and low viscosity of TO, clearly indicate that the traditional process requires some improvement for extended quality production. Basic good manufacturing procedures such as filtration, use of clean and proper equipment, pasteurization of raw milk and the use of known lactic acid starter culture under controlled incubation temperature with proper packaging, could produce a better quality *Omashikwa*. Characterization of LAB isolated from *Omashikwa* for developing a suitable starter for use by the small-holder milk producers and identification of possible gummy compounds in the *Omunkunzi* root, should be the subject for further research.

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## 4.2 Effect of *Boscia albitrunca* (*Omukunzi*) root on the bacteriology and viscosity of Omashikwa, traditional fermented buttermilk from Namibia

**ABSTRACT**

The role of *Boscia albitrunca* (*Omukunzi)* root in *Omashikwa,* traditional fermented buttermilk made with and without the root from Namibia was studied. *B. albitrunca* root had a low pH (4.7), exhibited bacterial inhibition properties and had high content of soluble carbohydrates (19.4%). Traditional *Omashikwa* (TO) processed with the root was slightly less viscous (2.5 Pa.s) compared to 2.9 Pa.s of LO. The total aerobic counts were 6.62 log cfu/g for TO and 8.62[1] log cfu/g for LO and lactic acid bacteria (LAB) were 6.58 log cfu/g for TO and 7.87 log cfu/g for LO and the counts were significantly lower in samples with the root. Coliforms were 2.68 log cfu/g (TO) and 2.70 log cfu/g (LO) and yeasts and moulds were 1.57 log cfu/g for TO and 1.69 log cfu/g for LO and were not significantly different ($p>0.05$). Most of the LAB identified belonged to the genera *Lactobacillus (Lb), Leuconostoc (Leu), Lactococcus (L.)* and *Streptococcus (Str).* The LAB species identified were *Lb. plantarum, Lb. lactis* subsp. *lactis, Leu. lactis, Leu. citreum, L. lactis* subsp. *lactis, L. lactis* subsp. *diacetylactis* and *S. thermophillus.* The results indicate that *B. albitrunca* root slightly increases viscosity, reduces syneresis and controls microbiological quality of *Omashikwa.*

### 4.2.1 INTRODUCTION

*Omashikwa,* traditional fermented buttermilk is produced by the Owambo and Herero tribes living a communal life in Namibia. It is preferred by local producers and consumers. Such fermented dairy products may have benefits. For example, alleviation of lactose intolerance problems in sensitive milk consumers due to hydrolysis of lactose by lactase enzyme, (*β*-galactosidase) (Daly and Davis, 1998; Soomro *et al.,* 2002). The processing of *Omashikwa* was discussed in the previous chapter (4.1).

In Eastern Africa, use of smoke from wood of some tree species is practiced as a method of preserving and improving the quality of fermented milk, (Shalo and Hansen, 1973; Kimonye and Robinson, 1991; Kurwijila, 1989). It is used to curb the problems of offflavours, taste, smell and palatability. Many plant materials are used for smoke treatment of milk and milk containers by various communities in Africa. In Ethiopia, Kenya and Tanzania; *Olea africana, O. capensis, Cassia didymobotrya, Lantana kitu, Rhus natalensis, Prumus africana, Euclea divinorum, Dombeya goetzenii, Bridella micrantba, Croton macrostacbyus, Acacia mearnsii, Eucalyptus* spp., *Acacia gerardi, Acacia nilotica* and *Balanites aegyptica*, *Diplorhynchus candylaccarpon, Combretum* spp *and O. africana* are used in the processing of traditional fermented milks (Shalo and Hansen, 1973; Kimonye and Robinson, 1991; Kurwijila, 1989). The treatment has the functions of imparting smoke flavour and colour to the fermented milks and to disinfect or sterilize the containers with antibacterial compounds such as phenols, formaldehyde, formic acid, acetic acid, alcohol, carbonyls and hydrocarbons, which are present in the smoke and are deposited in the containers (Pearson and Tauber, 1985).

In *Omashikwa*, a different approach is used. The *Boscia albitrunca* [(Burch) Gilg and Benedict] root is used traditionally in processing traditional buttermilk (*Omashikwa).* The author also observed that other less common plant roots and leaves are used for the same purpose in processing traditional fermented buttermilk namely, *Pavonia senegalensis* [(Cav.) Leistner] (root), *Acacia mellifera* [(Vahl) Benth] (root), *Acacia senegalensis* (L Vazquez-Chavez) (root), *Crotolaria* subsp. (Rattlepods-JCU) (root) and *Loncocarpus nelsii* [(Schinz) Heerind and Grimme] (leaves). The objectives of this study were therefore to determine the

role of *B. albitrunca* root on the microbial profile and viscosity of traditional fermented buttermilk from Namibia.

## 4.2.2 MATERIALS AND METHODS

### 4.2.2.1 Collection of *Omashikwa, B. albitrunca* root and skim milk samples

Nine samples of *Omashikwa* and *B. albitrunca* root were collected from households in Northern Namibia from November, 2005 to January, 2006 in a cool box and transported to the Faculty of Agriculture and Natural Resources' laboratory for experimentation. Skim milk samples were obtained from Neudamm Agricultural College Dairy Farm in Windhoek, Namibia. Milk samples were collected in sterile containers, capped and stored at 4-5$^{\circ}$C overnight before processing into *Omashikwa.*

### 4.2.2.2 Preparation of the *B. albitrunca* root for analysis

Fresh pieces of *B. albitrunca* root obtained from households in northern Namibia and stored overnight at 5-7$^{\circ}$C were cut into small pieces (approx. 1.5 cm$^3$), oven dried overnight at 100 ± 1$^{\circ}$C and ground using mortar and pestle into fine flour-like product for proximate analysis and for determination of soluble carbohydrates.

### 4.2.2.3 Processing of traditional *Omashikwa* (TO)

Three litre samples of the raw skim milk were processed in triplicate into O*mashikwa* using cultures from traditional *Omashikwa* (back-slopping) and fresh *B. albitrunca* root using the traditional household method described in the previous chapter ( 4.1). After fermentation to pH of 4.5, *Omashikwa* was removed from the incubator and stored overnight at 4-5$^{o}$C. Samples for pH, viscosity and lactic acid bacteria determinations were taken after cooling and analyzed.

#### 4.2.2.4 Processing of *Omashikwa* without *B. albitrunca* root (LO)

Similarly, *Omashikwa* without the root was made from pasteurized skim milk (65ºC /30 min and cooled to 25°C), inoculated with 2% traditional *Omashikwa* as starter culture (back-slopping), incubated, cooled and analyzed following the procedure described above.

#### 4.2.2.5 pH of *B. albitrunca* root and *Omashikwa*

The pH of *B. albitrunca* root samples was monitored daily over a period of 7 days after suspending 10 g of dry milled *B. albitrunca* root in 90 ml distilled water. The suspension was stirred and allowed to stand for 15 min, shaken for 20 min and filtered. The pH of the filtrate was determined. Similarly, the pH of *Omashikwa* samples prepared with and without the root of *B. albitrunca* tree was monitored over the same period.

#### 4.2.2.6 Proximate analysis of *B. albitrunca* root

Moisture, dry matter, crude fibre, ash, crude protein, fat, and carbohydrate were determined using standard procedures. Moisture, dry matter and ash were determined by oven drying and muffle furnace methods (Association of Official Analytical Chemists - AOAC, 1995). Total nitrogen was estimated by Kjeldahl method (Egan *et al.,* 1981) and crude protein was calculated by multiplying nitrogen content by a factor of 6.25. Soxhlet petroleum ether extraction procedure was used to determine fat content, carbohydrate was determined by difference and crude fibre was determined by the Weende method (Association of Official Analytical Chemists, 1995). Soluble carbohydrate was determined by the phenol-sulphuric acid method described by Dubois *et al.* (1956). Sucrose was used as standard.

#### 4.2.2.7 Viscosity of *Omashikwa*

Viscosity of two samples of *Omashikwa* (TO and LO) with three replicates was determined using a Programmable Brookfield Rheometer (Brookfield Engineering Laboratory, Middleboro, MA), with a spindle size-RV 2 and speed of 2 rpm at 10°C for 60 seconds.

#### 4.2.2.8 Enumeration and identification of microorganisms

Ten mL samples of *Omashikwa* with and without *Omunkunzi* root were aseptically added to 90 mL of sterile buffered peptone water (Oxoid, L 37) and mixed with a stomacher (Interscience St. Nom, France) for 5 min. Serial dilutions were made and 1 mL portions of the appropriate dilutions were pour-plated in triplicate plates per sample on the following media:

a) Plate count agar plates (Oxoid, Basingstoke, UK) were incubated at 30°C for 72± h for enumeration of total aerobic mesophilic bacteria. Total colony count was determined as described in the International Dairy Federation (1991) reference method (IDF 100 B: 1991).

b) MRS (De Man, Rogosa and Sharpe) agar plates (De Man *et al.,* 1960) (Oxoid CM 361) were incubated in anaerobic jars (Anaerobic system, Oxoid, Basingstoke, England) with gas generating kit (Oxoid) for 48±2 h at 42±1°C for enumeration of thermophilic *Lactobacilli* and *Streptococci.* MRS agar was also incubated aerobically at 35±1 °C for 48±2 h for enumeration of mesophilic *Lactobacilli* and *Leuconostoc*s.

c) M17 agar plates (Therzaghi and Sandine, 1975) (Oxoid CM 785) were incubated aerobically at 30±°C for 48±2 h for enumeration of *Lactococci.*

d) Rogosa agar plates (Rogosa *et al*., 1951) were incubated anaerobically at 35±1°C for 48±2 h for enumeration of *Lactobacilli.*

e) Violet red bile agar plates (VRB; Oxoid) were incubated at 37±1°C for 48 h. for enumeration of coliforms.

f) Rose-bengal chloramphenicol agar (RBC; Oxoid) was incubated at 25±1°C for 5 days for the enumeration of yeasts and moulds from *Omashikwa* samples.

Twenty five isolates were picked randomly from plates containing between 30 and 300 colonies of MRS (35°C), MRS (42°C), M17 (30°C) and Rogosa (35°C). Isolates (100 each) from samples with and without *B. albitrunca* root were sub-cultured and purified using MRS agar five times. Pure strains, as judged by microscopic observations for homogeneity of cellular morphology were tested for Gram reaction and catalase production. The pure isolates were cultivated in MRS broth at 30±1°C for 18±2 h for identification.

Gram-positive, catalase-negative, isolates from MRS agar (35°C and 42°C), Rogosa agar and M17 (Merck) agar were assigned to a genus level on the basis of key characteristics and tests described by Harrigan and McCance (1976). Morphological and arrangement of cells were examined according to Gram-stain preparations (Gerhardt *et al.,* 1981). Gas production from glucose was assessed in sugar basal medium (SBM) broth containing 2% (w/v) glucose dispensed in test tubes containing inverted Durham tubes. The inoculated tubes were examined for the production of gas after 3 day's incubation. Growth at 10, 15 and 45°C in MRS broth was determined by visual turbidity after 72±2 h incubation. Arginine deamination was detected in sugar basal medium (SBM) supplemented with 1% (w/v) arginine monochloride, 0.3% (w/v) Bacto-agar and 0.01% phenol red, pH 7.2. After inoculation the medium was incubated in anaerobic jars for 3 days. Arginine hydrolysis was observed by the culture turning yellow. The salt tolerance test was done using MRS broth containing 6.5 % (w/v) NaCl with incubation time of 4 days at 37 °C.

Twenty five isolates were picked randomly from plates containing between 30 and 300 colonies of MRS (35°C), MRS (42°C), M17 (30 °C) and Rogosa (35°C). Isolates (100 each) from samples with and without *Omukunzi* root were sub-cultured and purified using MRS agar five times. Pure strains, as judged by microscopic observations for homogeneity of cellular morphology were tested for Gram reaction and catalase production. The pure isolates were cultivated in MRS broth at 30±1°C for 18±2 h. They were centrifuged at 9800 x g for 10 min. and were suspended in Active Pharmaceuticol Ingredient – (API) 50 CHL (Chloramphenicol) medium (API system, bio Merieux, Sa, Marcy I'Etoile-France). Using sterile PSIpette, homogenized suspension of the cells in the medium, with subsequent vortex

mixing, were transferred into each of the 50 well of the API 50 CH strips, overlaid with sterile paraffin oil to affect anaerobiosis and incubated at 30°C for up to 2 days to monitor colour change. Changes in colour after fermentation were recorded on the API 50 data sheet as positive, negative or doubtful. Tests were performed according to the manufacturer's instructions. The APILAB PLUS database (BioMerieux Sa, France) was used to interpret the results.

#### 4.2.2.9 Bacterial inhibition test by *Boscia albitrunca* root

Bacterial inhibition ring test using *B. albitrunca* root extract was carried out to determine the effect of the root on bacterial growth. A microbiological disc paper was soaked in *Omukunzi* root extract after the root was submerged into boiling water for 5-10 seconds to eliminate yeasts and moulds and was placed on the Total Plate Count (TPC) agar plates just before solidification. The plates were then incubated as described above and the results were observed after 48 h of incubation. Photographs of the results were taken as shown on Fig. 3.2.1.

#### 4.2.2.10 Statistical analyses

One-way analysis of variance (ANOVA) was performed on all data collected. Mean comparisons of data from both samples were carried out by Duncan's Multiple Range Test (Steel & Torrie, 1980). Significant differences were calculated at 5% significance level.

## 4.2.3 RESULTS AND DISCUSSION

### 4.2.3.1 Proximate composition

The proximate composition of *B. albitrunca* root was 19.8% total carbohydrates and 19.4 g/100 g soluble carbohydrates (Table 4.4). The high content of soluble carbohydrates (hydrocolloids) (Whistler & BeMiller, 1997) in *B. albitrunca* root may explain the reason for slightly increase in viscosity of traditional O*mashikwa* (2.5 Pa.s) compared to 2.9 Pa.s of laboratory O*mashikwa* (Fig.4.1). Soluble carbohydrates may bind water, reduce syneresis and improve the viscous consistency of the TO due to their gummy nature. Lower viscosity of TO compared to LO could have been attributed to the poor hygiene, sanitation, technology and fermentation process with whey separation and rendered TO less viscous, a phenomenon observed previously by researchers dealing with other types of traditional fermented milks (Mutukumira, 1995a ; Feresu & Muzondo, 1989). The slightly higher viscosity of LO is due to pasteurization of milk and good manufacturing practices of *Omashikwa*. Pasteurization could denature some of the whey proteins and combine with caseins and thus bind more water as is done with yoghurt (Parnell-Clunies *et al.,* 1986). It may also be due to better hygiene, sanitation and controlled fermentation process despite back-slopping with TO cultures containing coliforms, yeasts and moulds.

**Table 4.4 : Proximate composition of *B. albitrunca root* (g/100 g)**

| **Attributes** | |
|---|---|
| Moisture | 68.0 ± 2.0 |
| Dry matter (by difference) | 32.0 ± 2.0 |
| Ash | 1.8 ± 0.1 |
| Protein (N x 6.25) | 6.5 ± 0.3 |
| Fat | 0.3 ± 0.0 |
| Crude fibre | 3.6 ± 0.2 |
| Carbohydrates by difference | 19.8 ± 2.0 |
| Soluble carbohydrates | 19.4 ± 2.1 |

± – Standard deviation of the means. (n=3).

#### 4.2.3.2 Bacterial inhibition properties

The presence of *Boscia albitrunca* root in traditional fermented milk (*Omashikwa)* appears to inhibit some microorganisms as shown in Fig. 4.2. The root may contain phenolic compounds that inhibit the growth of some sensitive microorganisms. These compounds may include phaseolin, phaseolin isoflavin, kientone, myraccetin etc. as described by Makoi and Ndakidmi (2007) and Megharaj *et al*. (1992. Thus the presence of *B. albitrunca* root in traditional *Omashikwa* may have played a role in improving the quality of *Omashikwa* by inhibiting some of the microorganisms and stabilizing fermentation process. Ring test shows a clear zone with the arrow around the disc pad A, soaked in an extract of *B*. *albitrunca* root, as an indication of bacterial growth inhibition by the root. Photo B is without the root.

**A** **B**

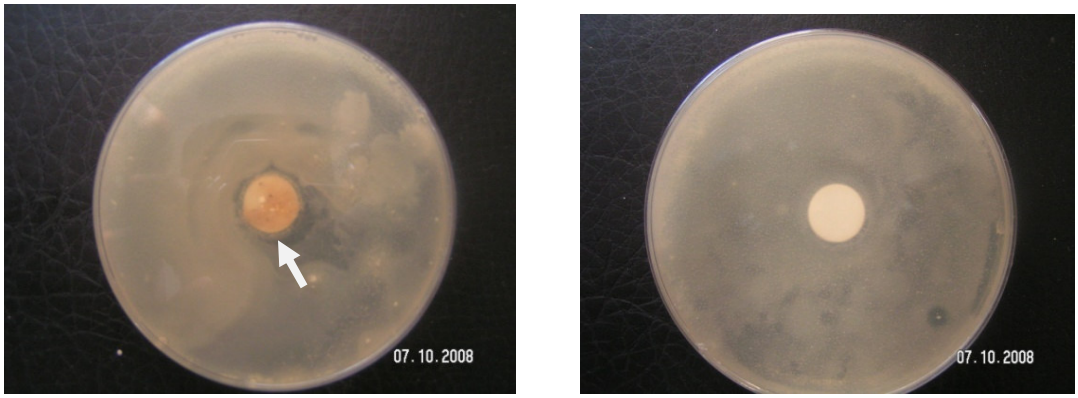


**Fig. 4.2 : Bacterial inhibition effect of *B. albitrunca* root on TPC Agar (see arrow on (A) with and (B) without *B. albitrunca* root (control) respectively.**

#### 4.2.3.3 pH of *B. albitrunca* root and *Omashikwa* samples

The pH of *B. albitrunca* root was low (pH 4.3). The pH of TO and LO were also low (4.2 for TO and 4.5 for LO) and were not significantly different ($p \geq 0.05$) (Fig. 4.3). The low pH of the root and *Omashikwa* samples may have increased the viscosity of the products by gelation and also reduced the initial bacterial counts, as low pH may have discouraged the growth of non-acid and spoilage microorganisms and encouraged the growth of LAB as their habitat (Sharpe, 1981). The growth of LAB may also increase the viscosity of *Omashikwa* as some of them like *Lb. bulgaricus, Strep. Thermophilus* and others produce polysaccharide responsible viscous consistency in fermented milk products (Nakajima *et al.,* 1990). Low pH ($p < 4.5$) of *Omashikwa* may also render the products safe for human consumption due to inhibition of spoilage bacteria like *Pseudomonas* spp. and *Listeria monocytogens* etc. and pathogenic bacteria as reported by Kosikowski (1982); Schaack and Marth (1988); Feresu and Nyati (1990); Kimonye and Robinson (1991). Phenolic compounds in *B. albitrunca* root have several hydroxyl groups which can form H bonds with carbonyl groups of proteins. They can also form hydrophobic interaction with proline residues or other hydrophobic side chain amino acids. The phenols can then interact with the milk proteins, casein micelles and cross link them to form a net work and cause an increase in viscoity compared to other traditional fermented milk in the region (Megharaj *et al.,* 1991). *B. albitrunca* root also has a high content of soluble carbohydrates that may increase viscosity and reduce syneresis (Table 4.4)

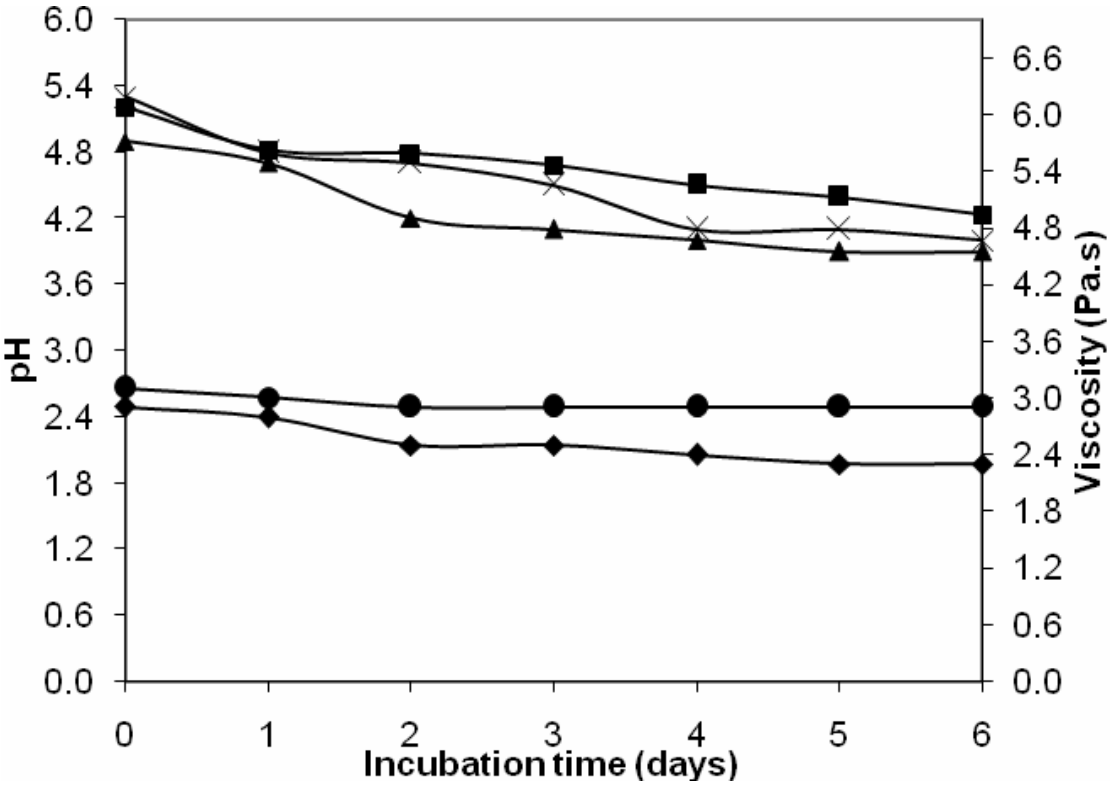


**Figure 4.3 : Effect of incubation at 10°C on the pH and viscosity of *Omashikwa* with and without *B. albitrunca* root. ■-: pH of root, –▲–: pH of *Omashikwa* with root, –x–: pH of *Omashikwa* without root, –●–: viscosity of *Omashikwa* without root, –♦–: viscosity of *Omashikwa* with root.**

#### 4.2.3.4 Total microbial composition of *Omashikwa* with and without *B. albitrunca* root

Table 4.5 summarizes the total aerobic and lactic acid bacteria counts obtained from *Omashikwa* with and without *B. albitrunca* root. The presence of coliforms in TO of 2.68 log cfu/g and 2.70 log cfu/g in LO, yeasts and moulds counts of 1.57 log cfu/g in TO and 1.69 log cfu/g in LO were not significantly different though LO milk was pasteurized. This could be explained by the back-slopping contamination of LO milk with traditional starter cultures from TO with rapid growth of microorganisms in LO due to lack of competition.

The lactic acid bacteria counts on MRS agar (35$^{o}$C) from TO and LO were 7.6 log cfu/g and 8.66 log cfu/g and were not significantly different from the total plate counts (6.62 and 8.62 log cfu/g respectively) indicating the predominance of LAB in the total microflora. The results also indicate that the LAB count was significantly higher in LO due to lack of competition. The thermophilic counts on MRS agar (42$^{o}$C) and Rogosa agar (35$^{o}$C) were also high, 7.62 and 7.60 log cfu/g respectively. The higher counts of thermophilic LAB in MRS, M17 and on Rogosa agars in both *Omashikwa* samples may be explained by the fact that the samples were collected in summer during the hot season, at the temperatures ranging between 37 and 43$^{o}$C (November/January) in northern Namibia, at which the fermentation process of TO may have taken place, and probably favoured the proliferation of thermophilic bacteria. It is also worth noting that *Omashikwa* samples processed with *B. albitrunca* root (TO) showed slightly lower mesophilic counts in most of the agar media. This may be explained by the acidic nature of the root and *Omashikwa* with the root, and probably the presence of inhibitory compounds (phenolics) in the root controlling the growth of some sensitive bacteria (Figure 4.5 ), thus reducing their numbers.

**Table 4.5: Effect of *B. albitrunca* root on the total counts of aerobic microbes and lactic acid bacteria counts (cfu/g) of samples of *Omashikwa* (TO and LO) from Namibia. (n=3)**

| Species | Range of counts (log cfu/g) | | Mean counts (log cfu/g) | |
|---|---|---|---|---|
| | Without root | With root | Without root | With root |
| Total aerobic mesophiles | 8.41 – 8.88 | 6.43 – 6.92 | 8.62±0.20[a] | 6.62±0.22[b] |
| *Lactobacilli* and *Leuconostocs* | 8.46 – 8.97 | 7.41 – 7.91 | 8.66±0.23[a] | 7.60±0.22[b] |
| *Lactobacilli* and *Streptococcus* | 7.40 – 7.92 | 6.40 – 6.88 | 7.62±0.22[a] | 6.40±0.20[b] |
| *Lactobacilli* spp | 7.43 – 7.98 | 6.46 – 6.99 | 7.60±0.25[a] | 6.70±0.23[b] |
| *Lactoccocus* spp | 7.40 – 7.89 | 5.31 – 5.94 | 7.60±0.20[a] | 5.60±0.27[b] |
| Yeasts/Moulds | 1.38 - 1.76 | 1.52 – 1.87 | 1.57±0.18[a] | 1.69±0.20[b] |

Key: Mean counts with different superscripts on the same row were significantlty different ($p<0.05$) from each other. Figures ± is Standard deviation of the mean. (n=3).

#### 4.2.3.5 Identification of LAB to genus level

The root seemed to promote the proliferation of the thermophilic bacteria with 56% of the genus *Lactobacillus* in TO and 49% in LO respectively, as shown in Table 4.6 . *Streptococci* were 13 in TO and only 6 were identified in LO. The mesophilic *Leuconostocs* and *Lactococci* were, however, lower in both the TO and LO and were 17 and 14 in TO and 23 and 22 in LO indicating their growth inhibition due probably to high summer temperatures. The higher numbers in LO (23 and 22) compared to TO (11 and 14) could be attributed to lack of microbial competition in LO. The species of LAB identified in both *Omashikwa* samples were normal cultures used in milk fermentation except that between 20 and 25% of the *Lactobacilli* belonged to *Lb. plantarum* species which are usually found in plant materials. This could be attributed to the use of gourds, *B. albitrunca* root and other contaminating plant materials such as grass and splinters from the environment during milk handling. The high LAB counts compares closely with findings of other studies on fermented milks by other workers in South Africa (Beukes *et al*., 2001), Zimbabwe (Mutukumira, 1995a), Northern Tanzania (Isono *et al.,* 1994), Cameroon (Jiwoua and Milliere, 1990) and Africa in general (Olasupo & Azeez, 1992 ).

**Table 4.6 : Effect of *B albitrunca* root on the distribution of 100 dominant lactic acid bacteria isolated from *Omashikwa* samples with and without the *B. albitrunca* root in northern Namibia**

| % Isolates | TO with root | | | | LO without root | | | |
|---|---|---|---|---|---|---|---|---|
| | Thermoph. | | Mesoph. | | Thermoph | | Mesoph. | |
| | 35°C | 42°C | 30°C | **Total** | 35°C | 42°C | 30°C | **Total** |
| *Lactobacillus* | 36 | 20 | - | 56 | 38 | 11 | - | 49 |
| *Streptococcus* | - | 13 | - | 13 | - | 6 | - | 6 |
| *Leuconostoc* | 17 | - | - | 17 | 23 | - | - | 23 |
| *Lactococcus* | - | - | 14 | 14 | - | - | 22 | 22 |
| **Totals** | | | | **100** | | | | **100** |

**Key:** Thermoph.=Thermophilic, Mesoph.=Mesophilic, TO=Traditional *Omashikwa*, LO=Laboratory *Omashikwa*

- = NO growth

#### 4.2.3.6 Identification of LAB to species levels

From the twenty lactic acid bacteria isolated from TO and LO and cultured in four media namely, MRS agar (42 & 35$^0$C), M17 (30$^0$C) and Rogosa agar (35$^0$C) and identified with API 50 CH identification system; five belonged to *Lb. plantarum* and three to *Lb. lactis* subsp. *lactis* in TO while four and two respectively were isolated from LO (Table 4.7). Four belonged to *Leuconostoc lactis* and two to *Leu. mesenteroides* subsp. *dextranicum* in TO while three to *Leu. lactis,* three to *Leu. mesenteroides* subsp. *dextranicum* and one to *Leu. citreum* in LO. Three *Lactococcus* species belonged to *L. lactis* subsp. *lactis* and one to *L. lactis* subsp. *diacetylactis* in TO while four belonged to *L. lactis* subsp. *lactis* and two to *L. lactis* subsp. *diacetylactis* in LO. Only a small number of *Streptococcus* species were isolated and identified. Two *Streptococcus thermophilus* were isolated and identified in TO and one in LO respectively. In general, there were no significant differences between the two products in terms of bacterial genus and species identified as they originated from back-slopping with TO. Only the counts and species numbers were different due to the presence of the root in TO, lack of competition in pasteurized milk and controlled fermentation in LO.

The species identified in this work (Table 4.7) were generally in good agreement with other similar studies. *Lactobacillus plantarum*, *Lactobacillus lactis* subsp. *lactis*, *Lactobacillus delbrueckii* subsp. *lactis, Leuconostoc lactis* and *Leuconostoc citreum* were identified in South African traditional fermented milks (Beukes *et al.,* 2001). *Lactobacillus lactis* subsp. *lactis*, *Lactobacillus plantarum* and *Lactobacillus delbrueckii* subsp. *lactis* were identified in Zimbabwe fermented milk (Feresu and Muzondo, 1989). *Lactobacillus plantarum, Lactobacillus lactis* subsp. *lactis* and *Weissella confusa* (former *Lactobacillus confusus)* were identified in Maasai fermented milk in Northern Tanzania (Isono *et al.,* 1994) and *Lactobacillus lactis* subsp. *lactis, Lactobacillus lactis* subsp. *lactis* biovar. *diacetylactis, Weissella confusa, Lactobacillus plantarum, Lactobacillus delbrueckii* subsp. *lactis, Leuconostoc citreum* and *Leuconostoc lactis* were identified in Burkina Faso fermented milk (Savadogo *et al.,*2004). Most of these species cited were also identified in fermented *Omashikwa* in Northern Namibia. This fact explains the diversity of lactic acid bacteria species in *Omashikwa.*

**Table 4.7: Identification of lactic acid bacteria isolated from *Omashikwa* with and without *B. albitrunca* root to species level by API 50 CH method**

| Genus LAB | Species identified | Numbers | |
|---|---|---|---|
| | | With root | Without root |
| *Lactobacillus* species | *Lb. plantarious* | 5 (25%) | 4 (20%) |
| | *Lb. lactis* subsp. *Lactis* | 3 (15%) | 2 (10%) |
| *Leuconostoc* species | *Leuc. lactis* | 4 (20%) | 3 (15%) |
| | *Leuc. dextranicum* | 2 (10%) | 3 (15%) |
| | *Leuc. citreum* | - | 1 (5%) |
| *Lactococcus* species | *Lact. lactis* subsp. *lactis* | 3 (15%) | 4 (20%) |
| | *Lact.lactis/diacetylatis* | 1 (5%) | 2 (10%) |
| *Streptococcus* species | *Strep. thermophilus* | 2 (10%) | 1 (5%) |
| Totals | | 20 | 20 |

(n=3)

It is known that aseptically drawn milk contains no *Lactobacilli* when it leaves the udder, but contamination with these organisms occurs rapidly from dairy utensils, dust and feedstuffs (Sharpe, 1981). Since TO samples were used as starter culture to inoculate samples of pasteurized skim milk with and without the root of *B. albitrunca* tree in this study, it can be assumed that the isolates originated from back-slopping contamination with starter culture.

All these species identified can be used and contribute to the quality of *Omashikwa* or any other traditional fermented milk products it terms of acid, flavour, consistency, syneresis and aroma production, if the spoilage microorganisms, non-acid producers, coliforms and yeasts and moulds are eliminated.

### 4.2.4 CONCLUSIONS

Based on the results obtained by investigating the role of *B. albitrunca* root in *Omashikwa*, it can be concluded that low pH, antibacterial property and high levels of soluble carbohydrates (hydrocolloids) in the root may control microbial profile, reduce syneresis and increase viscosity of *Omashikwa.* Thus, the use of *B. albitrunca* root in processing traditional fermented buttermilk in rural Namibia is justifiable. Although *B. albitrunca* root appears to increase viscosity, reduce syneresis and control some of the microorganisms in *Omashikwa*, appropriate lactic starter cultures, good hygiene and sanitation and application of good manufacturing practices on unit operations including packaging seem to be the effective methods to further improve and stabilize the quality of *Omashikwa*.

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## 4.3 Descriptive sensory evaluation of *Omashikwa,* traditional fermented buttermilk from Namibia

**ABSTRACT**

The sensory properties of *Omashikwa* were examined by descriptive sensory analyses and consumer preference test. This was done to determine the quality attributes and preference between traditional and laboratory made *Omashikwa* in Namibia for improvement and marketing. Descriptive sensory analysis of *Omashikwa* samples was conducted by eight panelists, four males and four females aged between 19 and 36 years using a 5-point hedonic scale 1 (very slight perception) to 5 (very intense). LO scored 3.8 on viscosity (TO 2.5), syneresis 2.9 (3.4 for TO), filth 1.8 (3.0), flavour 4.2 (2.6), rancidity 1.8 (3.4 for TO), Acidity 2.6 (4.5 for TO) and bitterness 2.5 against 4.2 for TO. The consumer preference analysis was conducted by forty five panelists who were familiar with *Omashikwa*. Samples were significantly different ($p<0.05$) with traditional *Omashikwa* showing higher intensity scores for acidity 4.5 (2.6 for LO), rancidity 3.6 (2.0), bitterness 4.4 (2.6), syneresis 4.2 (2.9) and filth 3.9 against 1.8 for LO and lower viscosity 2.5 (3.9). Acceptabilty score was 2.3 for TO and 4.6 for LO. These differences may explain the 80 percent consumer preference, (36 against 9) (n=45) for laboratory made *Omashikwa*. The application of good manufacturing practices on unit operations, particularly heat treatment and filtration of milk prior to fermentation, contributed significantly to the quality of laboratory made *Omashikwa*.

### 4.3.1 INTRODUCTION

Traditional fermented milk products play an important socio-economic role in developing countries as well as making a major contribution to the nutrients requirement of rural populations (Achi, 2005). Milk fermentation is regarded as one of the oldest ways of food processing and preservation (Feresu and Nyati, 1990; Kimonye and Robinson, 1991). In traditional fermented products, microorganisms are used spontaneously to prepare and preserve the products, adding to their nutritive value, the flavour and other qualities associated with edibility (Pederson, 1971). However, according to Nout (1985), most of the traditionally processed fermented milk products lack the appeal due to poor sensory attributes caused by poor hygiene and sanitation, crude handling and processing techniques employed, lack of shelf life, poor homogeneity and unattractive presentation. Inadequate presentations inhibit consumer to develop regular purchasing attitudes (Achi, 2005). Sensory properties of fermented milks are influenced by milk quality and the end products of microbial metabolites (Imhof *et al.,* 1994). Knowing the sensory characteristics of traditional fermented milks among competitors is a key priority in producing quality product for competitive business (Stone *et al.,* 1974). The sensory evaluation also presents the ideal knowledge as it provides detailed information, reliable and consistent results for processing of competitive product for the competitive market (Rodrigue *et al*., 2000).

*Omashikwa,* traditional fermented buttermilk in Namibia is a popular rural product processed in the northern and central Namibia by the Owambo and Herero tribes respectively. This fact has prompted Namibia Dairies to rename industrial buttermilk *Omashikwa* due to its popularity among the largest ethnic groups in Namibia for commercial and marketing purposes. Processing of traditional *Omashikwa,* is based on household traditional technology as described in chapter 4.1 (Fig. 4.1) is processed for the purpose of quenching thirst, an accompaniment for use with other foodstuffs, for creating employment and for income generation. However, due to inconsistency of its quality, consumers tend to be selective in purchasing *Omashikwa.*

The objective of the present study was to compare the sensory attributes of traditional and laboratory made *Omashikwa* by descriptive and consumer preference analyses in order to

assess the reasons for the differences and preference of the products and to design methods for improving *Omashikwa* for competitive market in Namibia.

## 4.3.2 MATERIALS AND METHODS

### 4.3.2.1 Fresh raw milk, *Omashikwa* and *Omukunzi* samples

*Omashikwa* and *Boscia albitrunca* root obtained from northern Namibia were used to produce traditional *Omashikwa* for descriptive and consumer sensory evaluation and preference testing. Both samples were delivered to the pilot plant of the Department of Food Science and Technology, University of Namibia under cold storage for experimentation. Prior to utilization, *B. albitrunca* roots were chopped into small sizes (approx. 2 $cm^3$) as indicated in chapter 4.1, section 4.1.2.2 for addition into the milk during *Omashikwa* processing and fermentation. Samples of *Omashikwa* were used as starter cultures in milk fermentation into *Omashikwa.*

### 4.3.2.2 Processing of *Omashikwa*

*Omashikwa* samples for analysis were made from Friesian cow's milk in the pilot plant of the Department of Food Science and Technology, University of Namibia, using method described in section 4.1.2.2. Traditional *Omashikwa* was made with the root of *B.albitrunca* root and laboratory *Omashikwa* without the root (control) in the same way. Raw milk was placed in a 5 L plastic container; temperature was raised to 25$^{o}$C in a water-bath and inoculated with 2% *Omashikwa* culture. At the same time 4 pieces of *B. albitrunca* root (approx. 2 $cm^3$ each) were added per 5 L milk and allowed to ferment to a pH of 4.5 for 2-3 days. The sour milk was then agitated by manual shaking until butter separated out, scooped off and washed with cold water to remove buttermilk. The remaining fermented milk after churning was buttermilk or *Omashikwa.* Samples were taken at this stage for descriptive and consumer sensory analyses and consumer's preference testing. Laboratory *Omashikwa* was processed using traditional method described above but without the root and applying good manufacturing practices to all unit operations (Chapter 4.1). Milk was filtered, pasteurized at

65°C for 30 min and cooled to inoculation temperature of 25 °C in ice water-bath, inoculated with 2% *Omashikwa* (back slopping) as above. When a pH of 4.5 was reached, cream was scooped off instead of churning into butter as in the traditional method. The remaining fermented milk was gently stirred to mix. Samples were taken for analyses as above.

#### 4.3.2.3 Descriptive Sensory Analysis

The sensory properties of *Omashikwa* were examined by descriptive sensory analysis (Gacula, 1997). Eight trained panelists comprising four males and four females aged between 19 and 36 years and familiar with *Omashikwa,* analyzed the samples, using sensory attributes. The panelists were students and lecturers at the University of Namibia, Department of Food Science and Technology. The selected panelists had 14 sessions of 2 hrs each of intensive training during which they were familiarized with the products and samples in terms of flavours (buttery or diacetyl, quinine and rancid or soapy cream), generated descriptors and agreed on attributes definitions and assessment criteria. The sensory attributes and definitions reached by consensus were used and included descriptors which described and differentiated between the *Omashikwa* samples. The attributes included seven sensory terms: intensities of aroma flavour, rancidity, acid taste, bitterness, viscosity, syneresis and the presence of filth (Table 4.8 ).

**Table 4.8 : Descriptors and definitions for sensory attributes of *Omashikwa***

| Sensory attributes | Definitions |
|---|---|
| Viscosity | Difficult or easy to flow or thickness or thinness etc. |
| Syneresis | Separation of liquid part on the surface of the product |
| Filthiness | Presence of unwanted objects like insects, splinters, grass, hair etc. |
| Aroma Flavour | Aromatic taste and flavour associated with butter (diacetyl) |
| Rancidity | Spoiled fat with soapy taste. |
| Acid taste | intensity of sourness as in traditional fermented milks |
| Bitterness | Taste similar to that of quinine |

**Key:** Commercial butter (diacetyl). Quinine and rancid or soapy cream were used for familiarization of above tastes

Samples were presented to the panelists in individual Styrofoam cups (50 mL) stored in water-bath with ice to keep the *Omashikwa* temperature low and uniform during testing. The *Omashikwa* temperature during testing was 7±1ºC and evaluation was done on 3 days old *Omashikwa* samples that had been kept under refrigeration after the incubation period. Testing was conducted under fluorescent illuminated room light conditions. Each panelist evaluated both samples of the *Omashikwa* in triplicate at a rate of one session per sample set conducted over three days. On each test day samples were presented in 3-digit coded Styrofoam cups and served to the panelists at a randomized order with a sampling plastic spoon. The attributes were scored using a scale ranging from 1 (very slight perception) to 5 (very intense) anchored for each of the tested attributes.

#### 4.3.2.4 Consumer preference test

Consumer preference test was conducted by a panel of 45 consumers familiar with *Omashikwa* and consisted of students from other departments and non-teaching staff members from Neudamm Campus, Faculty of Agriculture and Natural Resources of the University of Namibia. The consumer panelists aged between 19-58 years were served with samples in 3-digit Styroform coded cups in a randomized order. The panelists were asked to choose and indicate which of the two *Omashikwa* samples they preferred or liked based on their experience with *Omashikwa.*

#### 4.3.2.5 Statistical analysis

Descriptive sensory analyses were done in three sessions and means and standard deviations were subjected to analysis of variance (ANOVA) test. Duncan's multiple range tests were applied to determine the differences between the attributes of traditional Omashikwa and those of laboratory made Omashikwa (Lea *et al.,* 1997). Consumer preferences of traditional Omashikwa were also compared to that of laboratory made Omashikwa. Significant differences were calculated at 5% significance level.

### 4.3.3 RESULTS AND DISCUSION

#### 4.3.3.1 Mean scores for descriptive sensory analysis

The mean score results for descriptive sensory analysis of *Omashikwa* are shown in Table 4.9. The attributes of syneresis, filth, acidity, rancidity and bitterness differed significantly ($p<0.05$) between the traditional and laboratory made *Omashikwa* samples. The traditional *Omashikwa* had higher scores on the intensity of filth, syneresis, rancidity, acid flavour and bitterness. Whereas, laboratory made *Omashikwa* had lower scores on these attributes but higher scores in aroma flavour and viscosity.

Higher level of syneresis in traditional *Omashikwa* may be due to high acid content or low pH as observed during cheese making (Early, 1992; Cogan, 1995). In addition, poor fermentation process, uncontrolled incubation temperatures and time may also cause syneresis. Natural microorganisms especially the gas formers such as coliforms and yeasts may also allow curd to separate and float in whey (Nout *et al.,* 1987). Higher viscosity of laboratory made *Omashikwa* (3.8 Pa.s as opposed to 2.5 Pa.s of TO) may be caused by good manufacturing practices on unit operations, especially heat treatment of milk which controls spoilages caused by microorganisms and enzymes. The heating process may also denature whey proteins and combine with casein micelles to form a product with better ability to bind water. This process thickens and increased the viscosity of the laboratory made *Omashikwa* as it influences its flow properties like with yoghurt as described by Parnell-Clunies, *et al.* (1986).

The presence of higher level of filth is attributed to lack of proper hygiene and sanitation, poor milking and handling conditions and lack of filtration during production and processing of traditional *Omashikwa* in the rural setup. Higher acid content scores in traditional *Omashikwa* may be due to wild micro flora present in raw milk, including uncontrolled time and temperatures of incubation during fermentation process. The thermophillic group of microorganisms such as Lactobacilli and Streptococcus species may dominate and cause

higher acid flavour in traditional *Omashikwa* as the temperatures of incubation during fermentation and storage are high (37-42$^{o}$C) in northern Namibia.

The churning process of raw milk may be the reason for higher intensity of rancid flavour in traditional *Omashikwa* as compared to the laboratory made *Omashikwa.* The presence of natural milk and microbial lipase enzymes in the traditional *Omashikwa* may hydrolyze membrane-free milk fat globules during churning process causing rancid flavour (Walstra, *et al.,* 1999). In the laboratory made *Omashikwa,* the lipase enzymes were inactivated during milk pasteurization. In addition, cream was scooped off to prevent development of rancid flavour defects in laboratory made *Omashikwa*, since the fat globule membrane remained intact. However, the presence of mild rancid flavour observed in laboratory made *Omashikwa* may have been caused by back-slopping with the traditional *Omashikwa* as starter culture, which was already rancid.

The intensity of bitter flavour perceived in the traditional *Omashikwa* may be due to the addition of *B. albitrunca* root in traditional *Omashikwa* to improve sensory properties and viscosity, which is naturally bitter. In addition, the bitterness may be caused by the presence of natural enzymes in raw milk such as lipase and other proteolitic enzymes including those that are produced by microorganisms. These enzymes split fats and proteins into bitter fatty acids such as butyric, caproic, caprylic, capric and lauric acids and bitter amino acids, like tryptophan and tyrosine as described in other types of fermented milk products by Forss (1973); Belitz and Grosch (1987); Combes *et al.,* (2002) and Gular (2005).

**Table 4.9 : Mean scores for descriptive sensory attributes of *Omashikwa***

| Sensory attributes | Traditional *Omashikwa* | Laboratory *Omashikwa* |
|---|---|---|
| Viscosity | 2.5±0.71[a] | 3.8±0.79[b] |
| Syneresis | 3.4±0.70[a] | 2.9±1.29[b] |
| Filth | 3.0±1.05[a] | 1.8±1.03[b] |
| Aroma flavour | 2.6±0.70[a] | 4.2±0.42[b] |
| Rancid | 3.4±0.84[a] | 1.8±0.92[b] |
| Acid taste | 4.5±0.72[a] | 2.6±0.68[b] |
| Bitterness | 4.2±0.79[a] | 2.5±0.71[b] |

**Key:** Scores were obtained with structural scale ranging from 1(very slight perception) to 5 (very intense perception). Mean scores with different superscripts on the same row were significantly diffrenr ($p<0.05$).

### 4.3.3.2 Consumer acceptability results

The consumer acceptability ratings showed that LO was more acceptable due to its mild acid taste, low rancid flavour, bitterness, syneresis and filth compared to TO. LO had a higher viscosity and lower syneresis compared to TO (Table 4.10).Therefore, the overall preference was then given to LO with 80 percent of the consumers preferring laboratory made *Omashikwa* (Table 4.11). This was based on higher intensity of aroma flavour and thickness or higher viscosity. Other attributes namely, syneresis, filth, rancidity, acidity and bitterness had very low perception in laboratory made *Omashikwa.* The consumer preference for laboratory made *Omashikwa* may have been attributed to good manufacturing practices on unit operations, particularly heat treatment on κ-casein of milk prior to processing and controlled fermentation (Walstra, *et al.,* 1999 ; Bylund, 1995). The use of unit operation may have contributed to these quality attributes as 39 consumer panelists out of 45 of the age group ranging between 19 and 39 years, preferred laboratory made *Omashikwa.* Only 20 per

cent of consumers or 9 consumers of the older generation, aged between 40 and 58 years, preferred traditional *Omashikwa.* Heat treatment of milk tends to precipitate whey proteins and during acid fermentation, casein micelles combine with these whey proteins to form a network with ability to bind more water and increase viscosity. This process tends to be preferred by consumers as it thickens the product and improves its mouth feel as described by Walstra and Jenness (1984) and Dannenberg and Kessler (1988b). In addition, heat treatment and controlled fermentation processes create good environment for production of aromatic compounds from citrate such as diacetyl, acetoin, and acetate which improved flavours of laboratory made *Omashikwa* and prefereed by consumers (Cogan, 1987 and 1995).

**Table 4.10 : Consumer acceptance results: Mean rating of acceptability and attributes of two *Omashikwa* samples (TO & LO)**

| **Products:** | **Overal Attributes:** | | | | | | |
|---|---|---|---|---|---|---|---|
| | **Acceptability** | **Acid** | **Rancid** | **Bitter** | **Viscosity** | **Syneresis** | **Fith** |
| TO | $2.3^{*}$ | $4.5^{a}$ | $3.6^{a}$ | $4.4^{a}$ | $2.5^{a}$ | $4.2^{a}$ | $3.9^{a}$ |
| LO | $4.6^{*}$ | $2.6^{b}$ | $2.0^{b}$ | $2.6^{b}$ | $3.9^{b}$ | $2.9^{b}$ | $1.8^{b}$ |

**Key:** Mean scores with different superscripts on the horizontal row were significantly different ($p<0.05$). TO = traditional *Omashikwa* and LO = Laboratory *Omashikwa*. 1* = Disliked extremely; 5* = Liked extremely. $1^{a}$ = extremely low; 5 = extremely high.

**Table 4.11 : Consumer Preference taste results**

| Panelists | Traditional *Omashikwa* (TO) | Laboratory *Omashikwa* | % Pref. LO |
|---|---|---|---|
| 45 | 9 Preferred TO | 36 Preferred LO | 80 |

**Key:** N=45; 36 = Age 19-39 yrs; 9= Age 40-58 yrs. 36 preferred LO and 9 Preferred TO in the preference test.

### 4.3.3 CONCLUSIONS

The attributes of syneresis, filth, acidity, rancidity, aroma flavour, viscosity and bitterness contributed significantly to the differences in quality between the traditional and laboratory made *Omashikwa.* However, the observed improvement in quality and consistency of traditional *Omashikwa* compared to other similar traditional fermented milks in the region has been due to the incorporation of *B. albitrunca* root in *Omashikwa.* The addition of *B. albitrunca* root reduced syneresis and increased viscosity due to the presence of high content of soluble carbohydrates (Chapter 4.2). Probably *B. albitrunca* root also contains phenolic compounds that could be responsible for increased viscosity of *Omashikwa*. It is known that phenolics have several hydroxyl groups and can form H bonds with carbonyl groups of proteins. It can also form hydrophobic interaction with proline residues or other hydrophobic side chain amino acids. The phenolic can then interact with milk proteis, casein micelles and it is also known that they can interact with more than one micelles at a time and can then cross link them to form a net work and cause an increase in viscosity (O'Connell & Fox, 2001; Rawel *et al.,* 2001a). *B. albitrunca* root could also mask undesirable cowish and barny smell or flavous caused by poor milking conditions, poor hygiene, sanitation and fermentation processes in the rural set up, as *B. albitrunca* has a typical strong natural root smell and taste. Despite these good attributes of *B. albitrunca* root in *Omashikwa* with advantages over other traditional fermented milks in Africa as described in chapter 2 section 2.6, it can still be concluded that good manufacturing practices on unit operations, particularly filtration, heat treatment and controlled fermentation, could further improved the sensory attributes and consumer preference of *Omashikwa*.

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# 5. TRAINING OF MILK PRODUCERS/PROCESSORS

## 5.1 Background

Namibia is located on the south-western coast of Africa and is largely a semi-arid country with a surface area of 824.5 thousands of square kilometers and an overall estimated population of 1.8 million (Population and Housing Census, 2001). Agriculture, despite its low Gross Domestic Product (GDP) share, is of major importance to the economy in terms employment and export. The agriculture sector is divided into a commercial farming subsector, where farmers operate on freehold title land, and a communal farming subsector, where farmers operate on land under a communal tenure system. The two farming subsectors are separated by a Veterinary Cordon Fence (VCF), which is used in Namibia to control the spread of livestock disease from the communal areas in the north to the commercial areas south of the fence.

The communal subsector directly support 95 percent of the national farming population, but occupies only 48 percent of the total agricultural land and is characterized by low levels of agricultural productivity, high incidences of poverty, food insecurity, lack of appropriate farming methods and high unemployment levels. Farmers in communal areas mainly engage in subsistence rain fed crop and livestock production, and this makes the sector very vulnerable to changing climatic conditions, which is reflected in the high variability of output from one year to another. The sector is further constrained by poor marketing initiatives.

The livestock sector is an important subsector within the Namibian agriculture, as it accounts for 10 percent of the GDP and more than 25 percent of export earnings. The national cattle herds fluctuate from 1.8 to 2.5 million, the majority of which is found in the communal areas. In 1999, there were an estimated 1.46 million cattle, 267,146 sheep and 1.256 million goats in the communal farming areas of Namibia. At any single moment, a good portion of these animals are lactating, but dependence on rain fed agriculture results in a high variability of milk quantities between the seasons. Despite the large number of animals, people in the communal areas do not engage in economic activities with their wealth of livestock. Studies on rural livelihoods seem to suggest that, although communal farming is an important direct provider of staple foods for many rural households, there is extremely low or no cash income from the ownership of livestock in the communal areas.

This is especially the case in the northern part of the country where lack of cash income and economic hardship lead to poverty and cause under nutrition among children of less than five years old. Some farmers try to get cash income from their livestock by milking them, but do not know about proper technologies for adding value to their milk to increase returns, with the milk being wasted away due to spoilage or lack of market access. However, on-farm milk processing of butter and sour buttermilk does exist, mainly for household consumption. A very small part of these raw milk products is sold in recycled plastic containers by middlemen along the road for N$ 2.50 per liter. Namibian Dairies, the main dairy company in the country, supplies dairy products to the urban areas from its location in Windhoek. The major consumer market apart from the urban areas lies in Ovamboland (North Namibia), but since these products retail at high prices, they are beyond the reach of most consumers.

The country continues to import a large volume of food products, and developing the agricultural sector is one of the options available to the Government if food security and improved nutritional status are to be realized. The Government of Namibia formulated the First National Development Plan (NDP1) in 1996-2000, which has assigned high priority for the eradication of poverty and achievement of food security and nutritional standards. Some of the projects and progammes initiated under the NDP1 are continued under NDP2 to meet the challenges facing the Government of Namibia, namely to reduce the gap between the wealthy commercial farmers and the communal farmers through increased productivity and commercialization of the latter, together with the necessary support services such as extension, credit facilities and marketing. One of the programme areas identified is dairy development in communal areas. This is to improve livelihoods through organizing small-scale milk collection and processing facilities, training farmers in proper dairy management, organizing farmers into dairy cooperatives and improving marketing infrastructure. At the same time, the programme will promote generation of complementary on and off-farm income and employment opportunities.

Currenty, there is very little knowledge of proper milk handling on communal farms. With the milk still being processed in the traditional way, hygienic and safety measures are not exercised when handling the milk. Although no official data are available on current and

potential milk production and marketing of dairy products in the communal areas, it was observed that Namibia has the potential to produce enough milk to meet demand by mobilizing the small-scale dairy sector, but major problems are holding up progress. There is a general consumer perception that although locally processed milk and milk products retail at lower prices, they are unhygienic and unsafe. If affordable and efficient collection, processing and marketing systems are put in place, the quantity of locally produced milk available to processors and consumers could be increased significantly and the risk from zoonotic diseases, such as tuberculosis, virtually eliminated.

There is an urgent need therefore, to provide technical know-how and practical skills for those involved in getting milk and milk products from the farm to the consumer. This type of practical vocational training does not exist in Namibia and the Government has thus requested United Nations Food and Agriculture Organization (FAO) assistance to look into the potential and constraints of developing the milk production subsector in the communal areas and establishing a dairy demonstration and training centre in the northern communal areas. FAO experience indicates that small farmers can increase their net earnings from milk by up to 50 percent when they are directly involved in processing and marketing their own milk – milk that is surplus to their domestic needs. FAO studies (1985) in Africa and Asia indicate that up to six off-farm jobs can be created for each 100 L of milk collected, processed and marketed by small-scale sector.

The training will therefore demonstrate improved, low-cost, practical technologies for small-scale milk collection and on farm processing and marketing of butter and buttermilk. Butter production from cream, rather than from whole milk, will increase the yield by 50 percent. Proper milking, handling, collection and processing of pasteurized milk and fermented milk products will be demonstrated. These technologies allow relatively small volumes of milk and dairy products to be marketed cheaply and safely.

The main beneficiaries will be resource-poor milk producing households, in particular women, who are mainly responsible for milking and processing. Providing hands-on training will have an immediate and catalytic effect on mobilizing the small-scale dairy sector through increased and a more stable income from processed milk and dairy products. This in turn will

stimulate the adoption of technologies to increase milk production. Urban and peri-urban consumers and school children will indirectly benefit from safer and better quality products as more milk becomes available at affordable prices. Furthermore, many off-farm jobs will be created. It is estimated one job for each 20 liters of milk collected, processed and marketed. In addition, the Ministry of Agriculture, Water and Forestry (MAWF) will have gained valuable experience in the detailed design of its long term programme of dairy development strategy through this project.

### 5.2 Objective of training programme

The main objective of this programme is to improve the income and livelihoods of milk producers in the northern regions of Namibia throught training of the milk producers/processors, extension officers and technicians of the Ministry of Agriculture, Water and Forestry and other state holders in improved milk production, handling, collection, processing and marketing of value added milk products.

### 5.3 Training needs assessment

After visits and going through the traditional milking, milk handling, processing, packaging and marketing process and interviewing the farmers, a list of training needs was established by the author covering milking, milk handling, animal health, co-operative venture, milk composition and laboratory analyses, hygiene, sanitation, processing, packaging and marketing as indicated below:

1. Training on animal health and husbandry (local diseases and feeding)
2. Training on formation of a small-scale milk producer organization (dairy co-operative society)
3. Training in hygienic milking practices and sanitation
4. Small-scale milk collection, transportation and preservation

5. Training on milk composition and simple quality control tests such as organoleptic, clot-on-boiling, acidity alcohol test, sediment, resazurin, fat and density tests, total solids of milk and solids-not-fat tests.

6. Training on small-scale milk processing into value added products such as butter, *Omashikwa, Omaere* (sour milk) and quality tests of the finished products.

7. Training in small-scale group dairy business management including record keeping and finally

8. Marketing of dairy products.

9.

### 5.3 Training

A large group of communal farmers comprising over 50 males and females (Fig. 5.1) were divided into smaller groups and were assigned training sessions according to the above needs. The trainers came from the Departments of Agric-economics, Animal Science and Food Science and Technology of the University of Namibia (UNAM) and the Cooperative Division of the Ministry of Agriculture. Training lasted for two months at Ogongo Agricultural College (UNAM Campus) where FAO donated processing facilities were installed as part of National Dairy Training Center for communal farmers in the country.

**Figure:5.1 Communal farmers who participated in the training**

### 5.3.1 Dairy training

Dairy training covered hygienic milking, safe milk handling, preservation and collection, transportation to the processing facilities, value addition, packaging, storage and distribution. In addition to this, the course also touched on milk composition, hygiene and sanitation, microbiology and laboratory analyses to ensure milk quality, shelf life and safety of consumers.

Besides traditional milk processing of *Omashikwa, Omaere* (sour milk), butter and ghee, the farmers were also introduced to yoghurt and cheese making in case they would like to venture into processing of modern products in the future. Using the FAO donated facilities shown below (Figs. 5.2, 5.3 & 5.4). They were taught to process Set and Stirred Yoghurts and Feta and Cottage type of cheeses, which do not need curing as such.

**Fig 5.2 : Milk-Pro processing facilities at Ogongo used for training**

**Figure5.3: Cream separation for butter and ghee making**

**Fig. 5.4: Laboratory for milk analysis and quality control –Ogongo Campus**

Farmers practiced making *Omashikwa* by following the laboratory method described in chapter 4.1.This made them aware of the improvements in the products quality that can be attained through the application of the technology. They were happy as the products were consistent and of good quality every time they were made, and this increased saleability at a better price.

At the end of the course, farmers were awarded with free milk cans donated by FAO and certificates of course attendance, which they were awarded by the Regional Agricultural Officer of the region. After training, the farmers were then left in the hands of trained Agriculture extension officers to proceed with their usual business of making the products on their farms. However, this time following the instructions given during the course of training so that they can produce quality and marketable products.

# 6 GENERAL DISCUSSION

The discussion in this chapter starts by critiquing the way key methods were used in this study with the objective of revealing strengths and weaknesses in the applications, as well as making suggestions for applying the methods better in future. It then compares the characteristics and quality of *Omashikwa* processed under traditional and laboratory conditions. Furthermore, it examines the effects of the *B. albitrunca* root on the microbiology and viscosity of *Omashikwa* and it examines sensory characteristics of the products for acceptability. A summary of the advantages and disadvantages of each of the two processing methods used are given, and recommendations for a more effective product processing technology designed for small-scale rural processing of *Omashikwa* are elaborated. Lastly, recommendations for further research work are given.

## 6.1. Methodologies: a critical review

As stated in the previous chapters, *Omashikwa* samples were obtained at random from households of *Omashikwa* processors in northern Namibia during the summer season. These were used for this study. Samples obtained from households were meant to represent all *Omashikwa* processors in the area in order to provide a wide variety of quality characteristics. However, the collection included only *Omashikwa* which was processed in summer and no samples were obtained in winter season. Sampling of *Omashikwa* in winter could give a broader presentation of microflora and sensory characteristics of the products processed in the two seasons. The reason for this shortcoming is that most of the producers who provided *Omashikwa* samples in summer did not have milk in winter due to seasonality of production and movement of livestock from vicinity to the cattle post for pasture and water. More complete data on the genera and growth pattern of microorganisms (thermophiles/mesophiles) from both seasons would have been generated if samples were available from the same source in winter (Feresu and Muzondo, 1990; Fantuzi *et al.,* 1992 and Beukes *et al.,* 2001). It has also been reported that traditional fermented milk products coming from regions with cold temperatures contain mesophilic bacteria such as *Lactococcus* and *Leuconostoc* species, while thermophilic bacteria, which include *Lactobacillus* and

*Streptococcus*, prevail in regions with hot tropical or subtropical climates (Thomas, 1985; Tamime and Robinson, 1988; Kurmann, 1994).

As stated in chapter 4.1, *Omashikwa* was processed using traditional technology methods similar to other traditional methods described by Walshe (1990) and Walshe *et al.* (1991). Milk was unfiltered and contained filth, was unpasteurized and cultures used were natural from traditional *Omashikwa* (back-slopping). Cleaned pieces of *B. albitrunca* root were simply added. The incubation temperatures and time were not controlled and butter removal was done by shaking or agitation of raw fermented milk containing natural enzymes including lipase. The use of raw untreated milk, back-slopping with natural culture and the process of agitation to remove butter were the main reasons for traditional *Omashikwa* to be accepted by only a low percentage (20%) of consumer panelists (Chapter 4.3). Traditional *Omashikwa* was too acid with bitter taste and rancid flavour caused by hydrolysis of fat. Rancidity and bitterness are attributed to churning of raw fermented milk to obtain butter in the presence of natural enzymes, including lipase (Deeth & Fitz-Gerald, 1995). It also contained a lot of extraneous dirt or filth and had significantly more whey separation due to poor fermentation process caused by poor hygiene, sanitation and uncontrolled fermentation temperature and time (Chapter 4.2).

Milk for laboratory *Omashikwa* on the other hand, was filtered and pasteurized at low temperature (65 $^{o}$C/30 min) as opposed to high temperature designed for yoghurt making (85-90 $^{o}$C/30 min). Bovine milk contains approximately 3% casein, which comprises 80% of the total proteins (Walstra and Jenness, 1984). Casein contains a number of protein fractions of which the most important are $\alpha_{s1}$- and $\alpha_{s2}$-casein, β- and κ-casein (Bylund, 1995 ). Casein is present in the form of spherical particles as micelles which are casein calcium-phosphate-complexes including small amounts of magnesium and citrate (Bylund, 1995 ). The micelles exhibit great heat stability in milk, but susceptible to changes in milk composition related to ions, salts concentration, processing and in particular to changes of pH (hydrogen ion concentration) (Walstra and Jenness, 1984; Lewis, 1986).

Serum or whey proteins in a native state exhibit a different behaviour as they are affected considerably during heat treatment of milk. These proteins start to denature at temperatures

above 65 $^{o}$C (Walstra, 1990). A significant effect of denaturation may be observed by a considerable decrease in serum protein solubility under acidic conditions and complete coagulation in denatured form at pH 4.6-4.7, which is important for the technology of fermented milk (Walstra and Jenness, 1984).

Milk for this study was processed with a low temperature for the purpose of saving on firewood as a source of energy in the rural areas and also for comparing the role of the root in *Omashikwa.* This low temperature may not have been sufficient to denature all whey proteins (α-lactalbumins and β-lactoglobulin) and bind to casein micelles in order to absorb water and thicken the products, as it is done with yoghurt (Dannenberg & Kessler, 1988a, b). Pasteurization was used to simply control microorganisms from competing with *Omashikwa* starter cultures and to inactivate natural enzymes especially lipase in raw milk in order to prevent rancidity, poor fermentation process and sensory quality. The weakness of this study was that milk for laboratory *Omashikwa* was pasteurized at low temperature. Despite, the laboratory made *Omashikwa* was accepted by a high percentage (80%) of consumer panelists. In order to produce *Omashikwa* with a higher viscosity and lower syneresis, higher pasteurization temperatures ranging from 85–90 $^{o}$C for 30 min could be used to denature whey protens and bind casein, as described by Dannenberg and Kessler (1988a & b).

Viscosity in this study was determined as an important tool for assessing textural quality of *Omashikwa*, just like other fermented milks described by Corredig and Dalgleish, (1996). The instrument used to determine viscosity of the product samples was a rheometer, a device with a shearing and thinning effect on the product (Bylund, 1995; Walstra *et al.,* 1999 ). A weakness in this study was that the Brookfield Rheometer used was not necessarily the right one for the product as it has a thinning effect due to shearing on the texture of the product, as the spindle rotates. It would have been better to use Bostwick Consistometer that does not shear the product.

This instrument measures the consistency of a sample by its resistance to flow under specific conditions and for a specified time, and not by shearing effect as is done by Brookfield Rheometer. As such, some degree of variability in the ability to resist the flow may have resulted due to thinning effect, thus influencing the viscosity data of the two samples of

*Omashikwa* differently from other methods. However, while this may be perceived as a weakness in the study, it was the only instrument available at the time.

The role of *B. albitrunca* root in *Omashikwa* was determined in order to assess its effect on the microorganisms, viscosity and sensory attributes of *Omashikwa.* A weakness in this study was that, although it was found that there was slight bacterial inhibition activity, very slightly higher viscosity compared to other traditional fermented milks and improved taste and aroma of *Omashikwa* with addition of *B. albitrunca* root, the study did not determine the real compounds in the root that were responsible for these improved qualities.

Although compounds in *B albitrunca* root were not determined, Makoi and Ndakidemi (2007) and Okolo *et al.,* (2007) reported that some phenolic compounds in plants may control the growth of microorganisms. Phenolic compounds are some of the most widespread molecules among plant secondary metabolites and are of great significance in plant development (Makoi and Ndakidemi, 2007). Their toxicity to microbes has also been reported by Mort and Dean-Ross (1994) and Bukowska and Kowalska (2003). Phenolic compounds are membrane damaging microbiocides and their overall toxicity is caused by distinct and complex mechanisms such as narcosis, the inhibition of growth and the uncoupling of adenosine trisphosphate synthesis (Choi and Gu, 2001). High concentrations of phenols have been shown to be toxic even for species capable of using it as a growth substrate (Santos *et al*., 2001). The activities of micro-organisms have also been observed to be inhibited by nitrophenols and some other phenolic compounds such as phaseolin, phaseolin isoflavin, kientone, quercetinglucoside, myraccetin quercetin, tannins, syringic, phaseolin, p-hydroxybenzoic, ferulic, caffeic and chlorogenic acids and pisatin (Megharaj *et al.,* 1991, 1992; Makoi and Ndakidemi, 2007; Ravin, *et al.,* 1989). Some of these could be the phenolic compounds in the root of *B. albitrunca* tree that played a role in controlling the growth of microorganisms in the production of *Omashikwa*.

Soluble carbohydrates were found in *Omukunzi* root, and are known to assist Newtonian products to resist flow due to their gummy nature. However, as stated a weakness in this study was that the types of carbohydrates responsible for viscosity were not determined. The soluble carbohydrates responsible for thickening aqueous solutions and controlling rheological properties have, however, been described previously by Whistler and BeMiller

(1997) to be hydrocolloid compounds such as gums. The determination of the type of hydrocolloid present in the *Omunkunzi* root is important for future development as they could be extracted and processed into stabilizers, such as pectin, for commercial use.

Polysaccharides consist of monosaccharides bound to each other by glycosidic linkages (Belitz and Grosch, 1987). Their acidic hydrolysis yields monosaccharides. Polysaccharides can consist of one type of sugar structural unit (homoglycans) or of several types of sugar units (heteroglycans). The monosaccharides may be joined in a linear pattern, as in cellulose and amylose, or in a branched fashion (amylopectin, glycogen and guaran). The frequency of branching sites and the length of side chains can vary greatly (glycogen, guaran). The monosaccharide residue sequence may be periodic, one period containing one or several alternating structural units (cellulose, amylose or hyaluronic acid), the sequence may contain shorter or longer segments with periodically-arranged residues separated by non-periodic segments (alginate, carrageenans, pectin), or the sequence may be non-periodic all along the chain, as in the case of carbohydrate components in glycoproteins.

Polysaccharides are widely and abundantly distributed in nature, fulfilling roles as: structure-forming skeletal substances (cellulose, hemicellulose and pectin), assimilative reserve substances (starch, dextrins and inulin) and water-binding substances or hydrocolloid or gel (agar, pectin and alginate) in plants (Whistler and BeMiller, 1997). This could be the cause of viscous consistency of *Omashikwa* when *B. albitrunca (Omunkunzi)* root containing 19.4% of soluble carbohydrates is used in the processing of *Omashikwa,* traditional fermented buttermilk in Namibia (Chapter 4.1).

The natural taste, smell and flavour of compounds in *B. albitrunca* root could have been used by the traditional processors to mask and improved the poor sensory attributes of *Omashikwa* caused by poor milking, storage and processing environment. These were not determined. *B. albitrunca* root has a bitter taste and strong aromatic smell typical of the root. Though this appears to be a weakness of this study, it was not one of the objectives of the paper. However, previous studies on plant flavours have shown that simple phenolic compounds from plant materials can be responsible for imparting taste and smell to food products, including *Omashikwa* which is processed with the *B. albitrunca* root. In addition to the wide spectrum of functions narrated by Makoi and Ndakidemi, (2007), phenolic compounds are

also responsible for astringency, bitterness, colour, flavours and odours of plant products. The real compounds responsible for taste and aroma in *B. albitrunca* root can still be identified in future research work.

## 6.2 Enumeration of microorganisms from *Omashikwa*

The study of microorganisms in *Omashikwa* was aimed at enumeration, isolation and identification to species level. The study revealed that significant differences existed in microorganism counts on traditional *Omashikwa* compared to laboratory *Omashikwa,* with traditional *Omashikwa* showing lower numbers due to the addition of the *B. albitrunca* root. However, the growth of coliforms, yeast and moulds were insignificantly affected. The result of *Omashikwa* samples with added root had lower bacterial counts but it contained coliforms, yeasts and moulds just like laboratory *Omashikwa.* The presence of coliforms, yeast and moulds in laboratory *Omashikwa* were probably caused by back-slopping contamination with traditional *Omashikwa* cultures as described in other similar products by Shalo and Hansen (1973).

Identification of LAB to species level was carried out using the API 50 CH identification system, which is based on carbohydrates (sugars) fermentation in association with physiological and chemical tests such as morphology, Gram and catalase reactions. In general, phenotypic methods are cheaper compared to genotypic methods, which have stimulated the popular use of commercially available miniaturized identification systems such as API (BioMerieux). Although the application of phenotypic techniques has proven to be useful for certain LAB, there is a general awareness that similar phenotypes displayed by strains do not always correspond to similar or even closely related genotypes. Additional weaknesses of phenotypic methods include poor reproducibility, ambiguity in some techniques, extensive logistics for large-scale investigations and poor discriminatory power (Corsetti *et al.,* 2001). The API method was not able to identify some of the species because some identification pattern was not in the database. Phenotypic characterization based on sugar fermentation pattern, may not always provide sufficient basis for the reliable identification of LAB to species level, as reported by other researchers (Nigatu, 2000; Corsetti *et al.,* 2001; Muyana *et al.,* 2003), although it is a useful tool for classification. Sugar

fermentation pattern should be combined with conventional phenotypic properties or with genotypic techniques to be more accurate in identification of LAB to species level. However, the API method was simple to run and probably more effective compared to other methods usually carried out such as biochemical assay. There has been a shift towards the use of genotypic characterization methods in order to provide a more robust classification and differentiation (McCartney, 2002).

The most predominant species of microorganisms found in both products were *Lactobacillus plantarum* and *lactis; Leuconostoc lactis*, *Leu. dextranicum* and *Leu. citreum*; *Lactococcus lactis* and *diacetylactis* and *Streptococcus thermophilus* species. A weakness in this study was that a more efficient method of identification could have been used with better results. The molecular method using gene (DNA) sequencing such as 16S rRNA Sequencing method (Marziali, A. & Akeson, M., 2001 ), the DNA Checkerboard Hybridization method (Socransky *et al.,* 1994) and Genomic DNA Probes method (Siqueira *et al.,* 2002), to identify the microorganisms to species level could have been used with better results but neither the department nor the faculty has the equipment to do it. The genotypic characterization techniques are also not without limitations such as cost of equipment and databases etc. and thus a polyphasic or combined approach is preferred (McCartney, 2002).

The viscosity of *Omashikwa* processed with the addition of *B. albitrunca* root was slightly higher than that of laboratory *Omashikwa* processed without the root. Besides *B. albitrunca* root, viscosity could also be attributed to lactic acid bacteria that are responsible for production of viscous polysaccharide compounds. Examples of such bacteria include *Streptococcus thermophilus, Lactobacillus delbrueckii* subsp. *bulgaricus,* including some species of *Lactococcus lactis* subsp. *cremories* and subsp. *lactis* (Nakajima *et al.,* 1990; Cerning *et al.,* 1992). These viscous compounds include polysaccharides such as exopolymers that have been described by Nakajima *et al.* (1990); Cerning *et al.* (1990, 1992) and Bubb *et al.* (1997). A layer of these polysaccharides built of sugar residues can envelop the bacteria cell and form capsules known as glycocalix or can be excreted into the medium in the form of slime known as exopolysaccharides or homopolysaccharides (dextrans) which are responsible for viscous consistency. This study found that lactic acid bacteria that are responsible for production of polysaccharides were present in *Omashikwa.*

### 6.3 Effect of pH on viscosity of fermented milks

The fundamental structure of fermented milk is composed of casein network. The formation of a gel during the manufacture of fermented milk is basically due to quiescent acidification caused by bacterial fermentation. During the acidification of milk by fermentation, colloidal calcium-phosphate, which binds the casein micelles together, is leached out into the serum. As the pH reaches 5.2, β-casein also leaches out of the micelles into the serum. The micelles disintegrate into sub-micelles that aggregate forming a network. Serum protein and fat globules are trapped in the casein network (Driessen and Puhan, 1988).

As the pH decreases during bacterial acidification, the viscosity of the gel increases dramatically as the pH drops to below 5.2, which is also around the pH value where network formation starts. At pH below 5.2, β-casein seems to reaggregate into the protein network with the other caseins. The viscosity reaches a maximum at a pH *ca* 4.6-4.7. The on-set of gelation and the rheological properties of the acid gel are, however, influenced by heat treatment of milk, the acidification temperature and the composition of the milk (Heertje *et al.,* 1985; Parnell-Clunies *et al.,* 1986; Dannenberg and Kessler, 1988a, b; Vliet van and Keetels, 1995).

### 6.4 Descriptive and consumer sensory analyses

Einstein (1991) defined descriptive sensory evaluation as “the identification, description and quantification of the sensory attributes of a food material or product using human subjects who have been specifically trained for this purpose”. As such, the success of the descriptive sensory analysis exercise relies primarily on the collective ability of the descriptive panel to reliably and precisely grade and differentiate the products from given attributes. The panelists found traditional *Omashikwa* to be bitter in taste due to rancidity and with a strong aromatic smell originating from the *B. albitrunca* root, besides lactic acid taste, whereas the flavour and aroma of laboratory *Omashikwa* were that of lactic acid and aroma compounds originating from lactic acid fermentation such as diacetyl. According to the *Omashikwa* processors, the flavours and aroma from *B. albitrunca* root are used to mask and modify the poor taste and smell of traditional *Omashikwa,* which are acquired from the raw milk and picked up from the environment during milking, handling and processing (Chaper 4.1). These

off-flavours and odours are collected from the unsanitary and unhygienic milking environment; urine, cows, water, milk handlers and milking equipments, as described in other similar traditional fermented milks by Kimonye & Robinson (1991); Marshall (1992); Olasupo & Azeez (1992). Since the *Omunkunzi* root flavour and smell are distinctive and strong, they can easily be detected from the product, as was the case in the sensory analysis exercise carried out by the descriptive panelists (Chapter 4.3).

Rancidity was probably caused by the hydrolysis of fat in the presence of lipase enzyme during agitation of raw fermented milk to obtain butter, which was also reported in other fermented milks by Walstra *et al.* (1999). Bitter favours in *Omashikwa* were probably caused by hydrolysis of fat and proteins or acquired from *B. albitrunca* root, as it is naturally bitter as detected by the descriptive sensory panelists (Chapter 4.3). Filth content was high in traditional *Omashikwa* and was probably caused by poor milk handling, hygiene, unsanitary conditions and lack of filtration of milk prior to fermentation in the rural areas as reported in other similar products by Cousins and Bramley (1981) and this may explain the 80% of consumer's preference for laboratory *Omashikwa.*

## 6.5 Use of the knowledge gained to improve the quality of *Omashikwa*

It has been confirmed by this study that the quality of traditional fermented milk, *Omashikwa* can be improved in terms of increased viscosity, reduced syneresis and improved flavor and smell by the use of *B. albitrunca* root. However, the quality has been variable and poor in consistency due to poor hygiene, sanitation, fermentation and processing technology. There is a need therefore to improve the quality of *Omashikwa* for rural community so that its acceptability and marketability can be improved and sustained so that it can be expanded to reach a wider spectrum of consumers as described in other milk products by O'Mahony and Peters (1987); Tamime and Robinson (1988). This may generate more jobs and income to farmers for food security and alleviate poverty for the community concerned. The first approach would be to improve the hygiene and sanitation standards of milking by using clean environment, clean water and udder, clean and healthy milking personnel and handlers and by using clean and proper containers such as aluminium or plastic containers instead of

gourds. The second stage would be to ensure that the milk is filtered using clean cloth filters and cream is separated using hand separator or milk should be heat treated or pasteurized before fermentation. This should be done before shaking or agitating to obtain butter.

Shaking of raw fermented milk would rupture the membrane of the fat globules, allowing liquid fat to escape and be hydrolyzed by the natural lipase enzymes present in milk to rancid flavour, if not pasteurized (Walstra *et al.,* 1999). Pasteurization of milk to at least 85-90 $^{o}$C/30 minutes or boiled in order to kill all the unwanted microorganisms and inactivate enzymes for quality product and to render it safe for human consumption is the critical point. This temperature time combination or boiling would also denature whey proteins and and combine with caseins to make the end product more viscous by binding water, as reported by Dannenberg & Kessler (1988a and b); Mottar *et al.* (1989) and Corredig & Dalgleish (1996). The milk should then be cooled to ambient temperatures of 25-30$^{o}$C in a water bath or at room temperature before inoculation with good quality *Omashikwa* cultures (back-slopping) and incubated at 25-30$^{o}$C for 2-3 days or at room temperature until fermented (Fig. 2.1). At this stage, if *Omunkunzi* root is still needed, it can be added for flavour, smell and viscosity but after thorough cleaning in hot clean water to remove dirt and destroy yeasts and moulds. The product should then be covered to prevent contamination during incubation period. After fermentation, the product can then be cooled to below 10$^{o}$C, if facilities are available, and gently agitated to mix to homogenous and smooth consistency for packaging.

Alternatively, immediately after inoculation, the product can be packed in appropriate, cheap plastic bags, sealed and incubated in the packets to avoid post-pasteurization contamination. The *Omashikwa* can then be distributed for sale in sealed plastic packets instead of dirty recycled bottles as indicated and shown in Chapter 1.

The cost here will only include the purchase of a hand separator, plastic pouches and a sealer. Others will include boiling pots, storage milk cans, filter cloth and firewood. A cooperative venture would be an ideal approach here so that some of these items could be donated by agencies like Food and Agriculture Organization of the United Nations (FAO), farmers associations and the government through extension services, when approached for assistance.

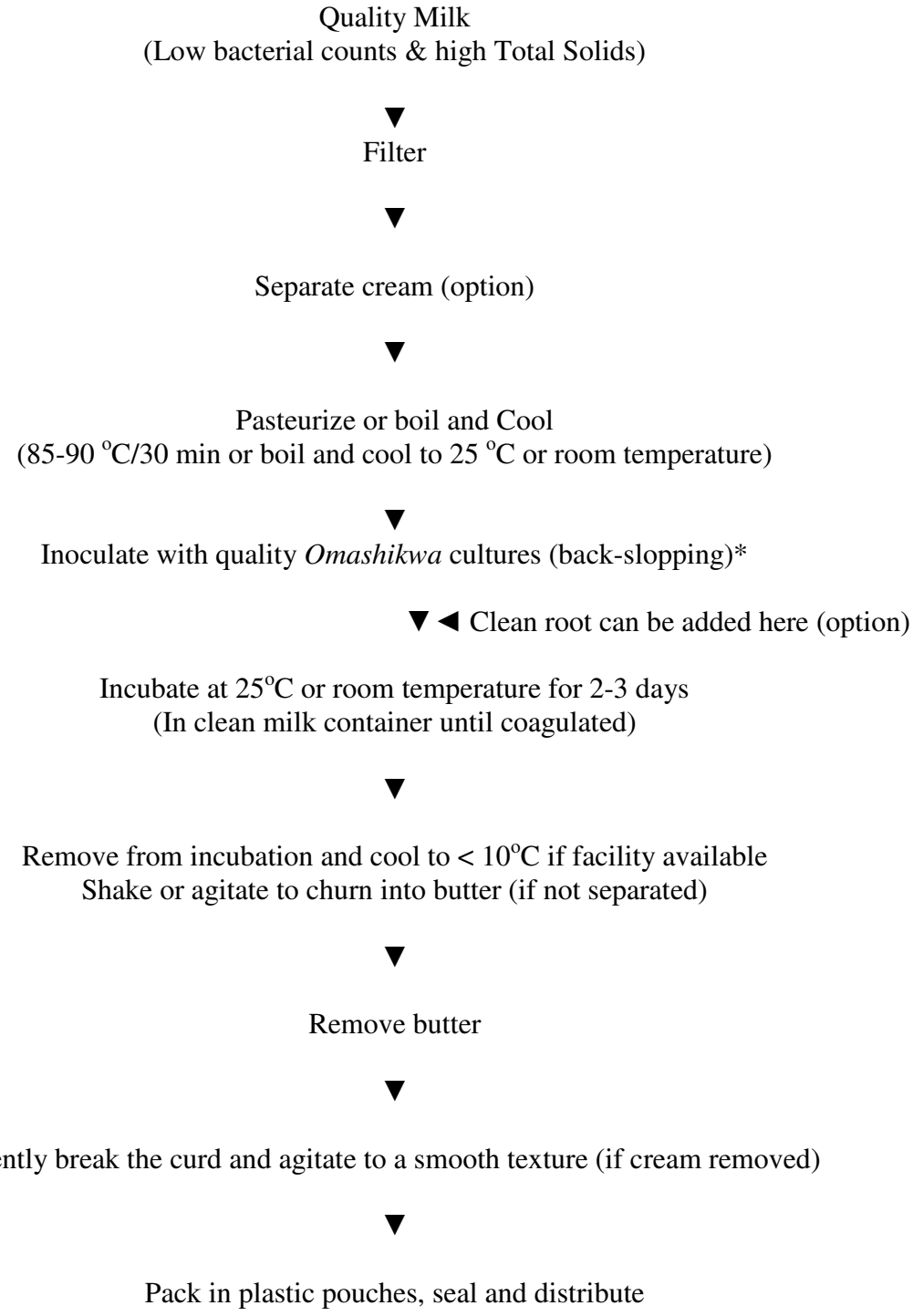


**Figure: 6.1 Flow-chart of the proposed small-scale *Omashikwa* processing**

**Note:**

*Alternatively, it can be packaged and sealed after inoculation and incubated in packets. It is safer to ferment in packets to avoid recontamination during packaging in the rural set up.

## 6.6 Recommendation for future research

As a follow up of *Omashikwa* studies, it would be desirable to collect milk during the winter period to see if the microflora would be different from those of summer season and whether this will have effects on the quality of *Omashikwa* due to mesophilic and thermophilic lactic acid bacteria as reported by other researchers (Thomas, 1985; Tamime and Robinson, 1988; Kurmann, 1994).

It is also important to test other methods of microbial identification like for example, identification of LAB to species level using molecular biology or DNA sequencing method (genotype) and comparing it to API CH system (phenotype) which is sugar fermentation based, or others methods in order to provide a better picture and recommendations for their use in future research.

The bacterial inhibition compounds in the *B. albitrunca* root is another area which could be looked into. These compounds need to be determined to ascertain the types that are responsible for controlling microbes in *Omashikwa.* If they are effective, they could in future be extracted and processed for commercial purpose.

The taste and aroma of compounds from the root are traditionally used by *Omashikwa* processors to mask the poor flavour and odours originating from poor surrounding environment of milk production. These flavours and aromatic compounds need to be identified to know what they are and whether they could be extracted and processed for commercial purpose, just like other food flavourants in the market.

The soluble carbohydrates (hydrocolloids) in *B. albitrunca* root responsible for viscosity of *Omashikwa* also need to be identified and extracted for commercial purposes. The soluble carbohydrates can be made into products like stabilizers such as pectins etc. found in the market. It will be more convenient to use the processed products instead of fetching the roots every time *Omashikwa* is processed.

# 7 CONCLUSIONS AND RECOMMENDATIONS

During the course of this study, it was observed that *Boscia albitrunca (Omukunzi)* root plays a role in slightly improving the viscosity of *Omashikwa*, slightly controls microorganisms and also improves the sensory attributes and acceptability of *Omashikwa.* However, poor handling of milk and processing technology results in poor product quality. These therefore need to be addressed in order to control, stabilize and improve the current variable quality of *Omashikwa* in general. The problems are in the physico-chemical and sensory characteristics with high syneresis, filth, bitterness, rancid flavour and low viscosity and acceptability by the consumers. Addressing the issues will enable the product quality to improve and be distributed to a larger area and to a wider spectrum of consumers in Namibia, thus creating more jobs and generating more income for food security and general welfare of the community concerned. Good manufacturing practices including hygiene and sanitation, use of proper lactic acid starter cultures in a controlled environment and distribution of the product in proper and affordable containers will enable the community to succeed in their endeavors.

Training of rural milk producers and processors is the prerequisite and important recommendation issue for the exercise to work. The training should cover areas of hygiene, sanitation, processing, preservation, packaging and marketing of their commodity. Training should be emphasized if communities dealing with the product need to come out of the vicious circle. It is very important to empower them especially the women with knowledge and skills of milk processing technologies to enable them to appreciate the quality and produce acceptable product for general consumers. The idea is to market their product to a wider area and generate more income. This will liberate the farmers from poverty and allow them to forge ahead with milk business in order to alleviate the problems of food security and hunger.

Since traditional microorganisms in *Omashikwa* is one of the culprits of poor fermentation process, it is further recommended that the veterinary service centres or such institutions like Ogongo Campus of the University of Namibia, should propagate and store quality lactic acid fermenting cultures in order to supply to the farmers when needed. Cultures, such as *Lactobacillus plantarum, lactis* and *cremoris; Lb. delbruckii* subsp. *bulgaricus*; *Lactococcus.*

*lactis* and *cremoris*; *Leuconostoc lactis* and *cremoris* and *Streptococcus thermophilus*, could be isolated from traditional fermented milk products and made into mother cultures and stored chilled or frozen for farmers use. The cultures can then be distributed to *Omashikwa* processors individually or through their Cooperative Society for distribution in the rural areas when needed. This should be handled in the same manner they handle veterinary medicines, vaccine and seamen for farmers. This will enable *Omashikwa* processors to access quality cultures and to use the knowledge gained during training sessions to process better products.

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# 9 APPENDIX

## Publications and presentation from this work

### Scientific papers

Bille, P.G., Ozuuko, A.T.R. and Ngwira, T. 2002. Sensory properties of traditionally fermented buttermilk (*Omashikwa)* processed in Namibia. *Journal of Food Technology in Africa*, **7,** 52-54.

Bille, P.G., Buys E. and Taylor, J.R.N. 2007. The technology and properties of *Omashikwa*, traditional fermented buttermilk produced by small-holder milk producers in Namibia. *International Journal of Food Technology*, **42**, 620-624.

## Conference papers

Bille, P.G., Ozuuko, A.T.R and Ngwira, T. 2002. Sensory properties of traditionally-fermented buttermilk (*Omashikwa)* processed in Namibia. Paper presented at the National Annual Agriculture Research Reporting Conference. 19-23 October, 2002. Ministry of Fisheries Conference Hall, Swakopmund, Namibia.

Bille, P.G. and Nakajima, H. 2004. Traditional processing of fermented buttermilk in Namibia. Paper presented at a workshop on traditional milk fermentations at Obihiro University of Agriculture and Veterinary Medicine. 2-5th February, 2004. Obihiro, Japan.