

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 it 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, reducing 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

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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% in TO 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 $\text{pH} \leq 4.0$ 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 cm³) are added. Starter culture (c. 2 % of the milk volume) from previously made *Omashikwa* is introduced and mixed.

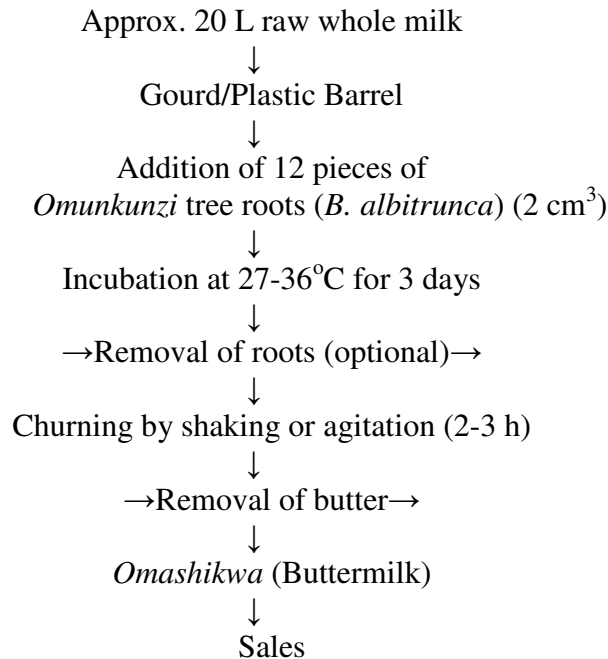


Figure 4.1 : Procedure for the production of traditional *Omashikwa* in Namibia

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 $30 \pm 1^\circ\text{C}$ for 48h. MRS agar pH 6.4 (De Man *et al.*, 1960) was used for enumeration of total LAB with anaerobic incubation at 30°C for 48h. Violet red bile agar (VRB; Oxoid, Unipath, Basingstoke, UK) was used for enumeration of coliforms at 37°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 $25 \pm 1^\circ\text{C}$ 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°C for 48 h as follows. *E. coli* was enumerated in Butterfield'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° 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°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°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 and 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 *O ماشيکوا* (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 exopolysaccharides 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 lipase enzymes present in unpasteurized milk (Walstra *et al.*, 1999).

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

Attributes	Sensory scores	
	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)
<i>Escherichia coli</i>	nd	nd
<i>Staphylococcus aureus</i>	nd	nd
<i>Clostridium</i> spp.	nd	nd
<i>Bacillus anthracis</i>	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.

4.1.5 LITERATURE CITED

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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 8.62¹ 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. thermophilus*. 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*, *Prunus africana*, *Euclea divinorum*, *Dombeya goetzenii*, *Bridella micrantha*, *Croton macrostachyus*, *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°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°C were cut into small pieces (approx. 1.5 cm³), oven dried overnight at 100 ± 1°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 *Omashikwa* 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°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 (65°C /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 *Leuconostocs*.
- 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 $25\pm 1^\circ\text{C}$ 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 $30\pm 1^\circ\text{C}$ 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 $30\pm 1^\circ\text{C}$ for 18 ± 2 h. They were centrifuged at $9800 \times g$ for 10 min. and were suspended in Active Pharmaceutical Ingredient – (API) 50 CHL (Chloramphenicol) medium (API system, bio Merieux, Sa, Marcy l'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 *Omashikwa* (2.5 Pa.s) compared to 2.9 Pa.s of laboratory *Omashikwa* (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, myracetin 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

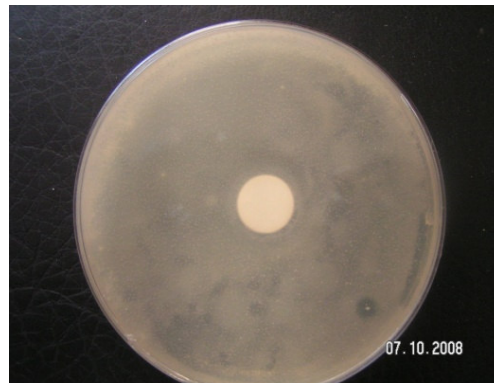


Fig. 4.2 : Bacterial inhibition effect of *B. albitrunca* root on TPC Agar (see arrow on (A) with and (B) without *B. albitrunca* root (control) respectively.

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)

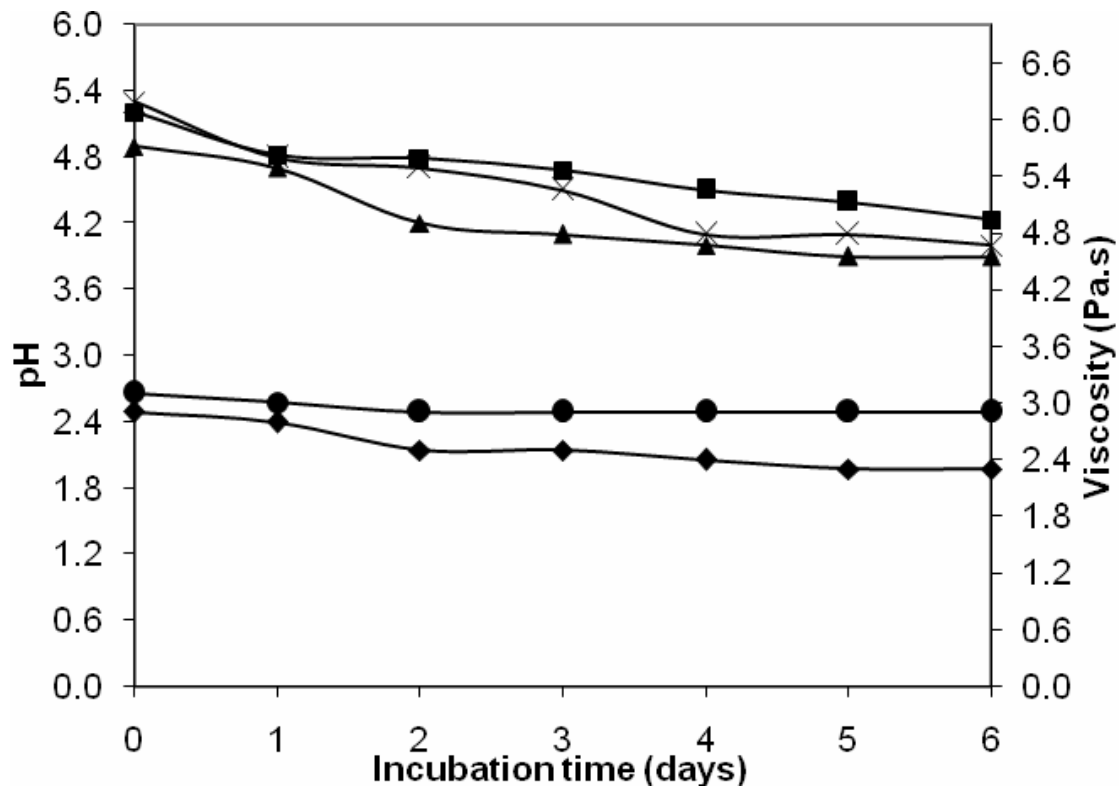


Figure 4.3 : Effect of incubation at 10°C on the pH and viscosity of *Omashikwa* with and without *B. albitrunca* root. ■- : pH of root, -▲-: pH of *Omashikwa* with root, -x-: pH of *Omashikwa* without root, -●-: viscosity of *Omashikwa* without root, -◆-: viscosity of *Omashikwa* with root.

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°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°C) and Rogosa agar (35°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°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
<i>Lactobacilli</i> and <i>Leuconostocs</i>	8.46 – 8.97	7.41 – 7.91	8.66±0.23 ^a	7.60±0.22 ^b
<i>Lactobacilli</i> and <i>Streptococcus</i>	7.40 – 7.92	6.40 – 6.88	7.62±0.22 ^a	6.40±0.20 ^b
<i>Lactobacilli</i> spp	7.43 – 7.98	6.46 – 6.99	7.60±0.25 ^a	6.70±0.23 ^b
<i>Lactococcus</i> 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 significantly 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
<i>Lactobacillus</i>	36	20	-	56	38	11	-	49
<i>Streptococcus</i>	-	13	-	13	-	6	-	6
<i>Leuconostoc</i>	17	-	-	17	23	-	-	23
<i>Lactococcus</i>	-	-	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 & 35⁰C), M17 (30⁰C) and Rogosa agar (35⁰C) 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
<i>Lactobacillus</i> species	<i>Lb. plantarius</i>	5 (25%)	4 (20%)
	<i>Lb. lactis</i> subsp. <i>Lactis</i>	3 (15%)	2 (10%)
<i>Leuconostoc</i> species	<i>Leuc. lactis</i>	4 (20%)	3 (15%)
	<i>Leuc. dextranicum</i>	2 (10%)	3 (15%)
	<i>Leuc. citreum</i>	-	1 (5%)
<i>Lactococcus</i> species	<i>Lact. lactis</i> subsp. <i>lactis</i>	3 (15%)	4 (20%)
	<i>Lact.lactis/diacetylatis</i>	1 (5%)	2 (10%)
<i>Streptococcus</i> species	<i>Strep. thermophilus</i>	2 (10%)	1 (5%)
Totals (n=3)		20	20

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 in 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). Acceptability 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 cm³) 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°C in a water-bath and inoculated with 2% *Omashikwa* culture. At the same time 4 pieces of *B. albitrunca* root (approx. 2 cm³ 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 *O ماشيکوا*

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
<u>Bitterness</u>	<u>Taste similar to that of quinine</u>

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 $7\pm 1^{\circ}\text{C}$ 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 Styrofoam 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 DISCUSSION

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 thermophilic 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°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 proteolytic 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 <i>Omashikwa</i>	Laboratory <i>Omashikwa</i>
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 different ($p < 0.05$).

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 preferred 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:	Overall 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 <i>Omashikwa</i> (TO)	Laboratory <i>Omashikwa</i>	% 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 proteins, 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 network 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 flavour 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 improve the sensory attributes and consumer preference of *Omashikwa*.

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