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EFFECT OF *BOSCIA ALBITRUNCA (OMUKUNZI)* ROOT ON THE BACTERIOLOGY AND VISCOSITY OF *OMASHIKWA*, TRADITIONAL FERMENTED BUTTERMILK FROM NAMIBIA

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ABSTRACT

The objective of this study was to determine the role of *Omukunzi* root (*Boscia albitrunca*) in the viscosity or consistency, sensory and bacteriological profile of *Omashikwa*, traditional fermented buttermilk from Namibia. *Omashikwa* is a popular traditional fermented buttermilk product made with *Omukunzi* root among the Owambo and Herero tribes living in the North and Central Namibia. It is processed by fermenting raw milk in the presence of *Omukunzi* root in a calabash and agitated to obtain butter granules. Butter granules are then removed, washed, salted and made into butter or processed into ghee (butter oil). The remaining fermented milk is *Omashikwa* or traditional fermented buttermilk. The results of this study indicated that the root of *Omukunzi* tree had low pH (4.9), exhibited bacterial inhibition properties and had high content of soluble carbohydrates (19.4%). *Omashikwa* made with *Omukunzi* root (TO) was more viscous, 2.9 Pa.s compared to 2.5 Pa.s of laboratory *Omashikwa* made without the root (LO). The total aerobic counts were 6.62 cfu/g for TO and 8.62 cfu/g for LO and lactic acid bacteria (LAB) was 6.58 cfu/g for TO and 7.87 cfu/g for LO and were lower in samples with the root. Coliforms, 2.68 cfu/g (TO) and 2.70 cfu/g (LO) and yeasts and moulds were not significantly different ($p \leq 0.05$) and were 1.57 cfu/g for TO and 1.69 cfu/g for LO. Yeasts and mould counts from the gourd swabs were high, 4.78 cfu/g. The LAB identified belonged to the genera Lactobacillus (Lb.), Leuconostoc (Leuc.), Lactococcus (Lact.) and Streptococcus (Strep.). The LAB species identified were *Lb. plantarum*, *Lb. lactis* subsp. *lactis*, *Leuc. lactis*, *Leuc. citreum*, *Lact. lactis* subsp. *lactis*, *Lact. lactis* subsp. *diacetylactis* and *Strep. thermophillus.* The results indicated that *Omukunzi* root plays a positive role in the quality of value added *Omashikwa* in terms of improving consistency, sensory quality and controlling microbial profile which could finally help to increase income, create jobs and improve food security and nutrition to the rural communities.

Key words: *Boscia albitrunca*, fermentation, buttermilk, *Omashikwa*, viscosity

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INTRODUCTION

Omashikwa, a traditional fermented buttermilk is produced by the Owambo and Herero tribes living a community life in Namibia. In the absence of cooling facilities, fermented milk products are preferred by local producers and consumers in developing countries as they have longer shelf life [1,2] and they also alleviate lactose intolerance problems to sensitive consumers due to hydrolysis of lactose by the presence of lactase enzyme (*β*-galactosidase) [2]. Processing of *Omashikwa* has been described previously by Bille *et al.* [3, 4].

In Eastern Africa, wood of some tree species is used to smoke milk and fermenting vessels such as gourd or calabash in order to preserve and improve the sensory quality of fermented milk. The method is practiced by various pastoral and agro-pastoral communities in the region [5, 6, 7, 8]. Smoke is used to curb the problems of off flavours, taste, smell and palatability. A list of plant materials that are used for smoke treatment of milk and milk containers by various communities in the three countries in Eastern Africa namely Ethiopia, Kenya and Tanzania include: *Olea Africana, O. capensis, Cassia didymobotrya, Lantana kitu, Rbus natalensis, Prumus Africana, Euclea divinorum, Dombeya goetzenii, Bridella micrantba, Croton macrostacbyus, Acacia mearnsii,* Eucalyptus spp., *Acacia gerardi, Acasia nilotica* and *Balanites aegyptica* [6,7] and in Tanzania: *Diplorhynchus candylaccarpon,* Combretun spp and *Olea Africana* [8]. 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 during the smoking process [5, 6].

In Namibia with *Omashikwa*, a different approach to tackle the problems of flavour, palatability, taste, syneresis and viscosity is used. *Omashikwa* is the most common traditional fermented buttermilk in Northern and Central Namibia made from indigenous cow's (*Bos indicus*) milk and the root of *Boscia abitrunca* (*Omukunzi*) tree [3,4]. The *Omukunzi* root is used traditionally in processing *Omashikwa* in order to musk the local milk flavours and improve its consistency by imparting root taste and smell and by reducing syneresis and thickening of the product, as it binds water, thus improving the viscosity or consistency of *Omashikwa*.

The author also observed that other less common plant roots and leaves are used for the same purpose in Namibia namely, *Pavonia senegalensis* (root), *Acacia mellifera* (root), *Acacia senegalensis* (root), *Crotolaria spp* (root) and *Loncocarpus nelsii* (leaves).

The objectives of this study were, therefore, to determine the role of *Omukunzi* root on the microbiology, sensory and viscosity of *Omashikwa,* a traditional fermented buttermilk from Namibia, as there is a big difference in consistency and flavour between *Omashikwa* and other traditional fermented milks in the region (4).

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MATERIALS AND METHODS

Raw materials

Samples of *Omashikwa,* and *Omukunzi* root were obtained from individual households in the rural areas of Northern Namibia. Skim milk samples were obtained from Neudamm Campus Dairy Farm in Windhoek, Namibia. Milk samples were collected and delivered in sterile containers, capped and stored at 4-5 $^{\circ}$C overnight before processing into *Omashikwa.*

Preparation of the *Omukunzi* root for analyses

Parts of the fresh *Omukunzi* roots obtained from individual households in Northern Namibia and stored overnight at 5-7 $^{\circ}$C were cut into small pieces, oven dried overnight at 100 ± 1°C and ground into fine flour-like product for proximate analysis and for determination of soluble carbohydrate or hydrocolloids.

Processing of traditional *Omashikwa* (TO)

Three liters of raw skim milk were processed in triplicate into O*mashikwa* with fresh *Omukunzi* root by back-slopping with *Omashikwa* culture, using the traditional household method described by Bille *et al.*, [4]. When *Omashikwa* fermented to pH of 4.5, it was removed from the incubator and stored overnight at 4-5^{o}C. Samples for pH, viscosity and microbiological determinations were taken at this stage for analyses.

Processing of *Omashikwa* without *Omukunzi* root (LO)

Similarly, laboratory *Omashikwa* without the root was made from pasteurized (65^{o}C/30 min) skim milk to bind water, following the procedure described above.

pH

The pH of *Omukunzi* root samples was monitored daily over a period of 7 days after suspending 10 g of dry ground *Omukunzi* root in 90 ml distilled water. The suspension was stirred and allowed to stand for 15 min, shaken for 20 min and filtered. The filtrate obtained was subjected to pH determination. Similarly, the pH of *Omashikwa* samples prepared with and without the root of *Omukunzi* tree was monitored over the same period at 10^{o}C.

Proximate analysis

Moisture, dry matter, crude fiber, 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 [9]. Total nitrogen was estimated by Kjeldahl method of Egan *et al.*, [10] 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 fiber was determined by the Weende's method [9].

Soluble carbohydrate was determined by the phenol-sulphuric acid method described by Dubois *et al.*, [11]. Zero-60 µg of sucrose and ground *Omukunzi* root were serially

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diluted in 1 liter of distilled water, respectively. Two ml of each solution was mixed with 1 ml of 5% solution of phenol and 5 ml of concentrated sulfuric acid. The triplicate solutions were transferred into cuvettes and soluble carbohydrates were determined by Spectrophotometer, type Varian-Cary 50 Probe (LabWrench, Midland, ON, Canada). Sucrose was used as standard.

Viscosity of *Omashikwa*

Viscosity of two samples of *Omashikwa* in triplicates (TO and LO) was determined by placing samples in the Programmable Brookfield Rheometer apparatus containing a shearing spindle, size-RV2 and speed of 2 rpm at 10 °C for 60 seconds. The results were read from the computer monitor print out (Brookfield Engineering Laboratory, Middleboro, MA, USA).

Enumeration and isolation of microorganisms

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

a) Plate count agar plates (Oxoid, Basingstoke, Hampshire, 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 reference method IDF 100 B [12].

b) MRS agar plates [13] (Oxoid CM 361) were incubated in anaerobic jars (Anaerobic system-Oxoid Ltd, Basingstoke, Hampshire, England) with gas generating kit (Oxoid Ltd, Basingstoke, UK) 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 [14] (Oxoid CM 785) were incubated aerobically at 30± °C for 48±2 h for enumeration of Lactococci.

d) Rogosa agar plates [15] were incubated anaerobically at 35±1 °C for 48±2 h for enumeration of Lactobacilli.

e) Violet red bile agar plates (VRB; Oxoid, Unipath, Basingsstoke, UK) [16] were incubated at 37±1 °C for 48 h for enumeration of enteric bacteria (Coliforms).

f) Rose-Bengal chloramphenicol agar (RBC; Oxoid, Unipath) was incubated at 25±1 °C for 5 days for the enumeration of yeasts and moulds from *Omashikwa* and swabs samples from gourds or calabashes (Fig.1).

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Figure.1: Traditional fermented milk calabash/gourd

Twenty five colonies 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) from samples with and without *Omukunzi* root were cultivated in MRS broth. Purity was checked by streaking severally on MRS agar. The pure isolates were cultivated in MRS broth at 30±1°C for 18±2 h for identification.

Bacterial inhibition test by *Boscia albitrunca* (*Omukunzi)* root extract

Bacterial inhibition ring test using *Omukunzi* root extract was carried out from the previous plate count agar preparations to determine the effect of the root on bacterial growth. Microbiological disc papers soaked and un-soaked (control) in *Omukunzi* root extract after the root was submerged into boiling water for a second to eliminate yeasts and moulds were placed on the plate count agar plates as they started to solidify. The plates were then incubated as described above and the results were observed after 48 h of incubation. Photograph of the result was taken as shown on Fig. 3**.**

Identification of lactic acid bacteria to genus level

Gram-positive, catalase-negative, isolates from MRS agar (35°C and 42°C), Rogosa agar and M17 agar were assigned to a genus level on the basis of key characteristics and tests described by Harrigan and McCance, [16]. Morphological and arrangement of cells were examined according to Gram-stain preparations [17]. 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 SBM supplemented with 1 % (w/v) phenol red at 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

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tolerance test was done using MRS broth containing 6.5 % (w/v) NaCl with incubation time of 4 days at 37°C.

Identification of lactic acid bacteria to the species level

Twenty represented isolates, five from each previously isolated plate of MRS (35°C, 42°C), M17 (30°C) and Rogosa (35°C) agars with and without the root were selected randomly for identification to species level using the API 50 CH galleries. The pure isolates were distributed aseptically using sterile pipette into API 50 CHL medium (bio Merieux Sa, France) containing dye and different carbohydrates. The galleries were incubated at 30±1°C for 24 and 48±2 h to ferment. Changes in colour after fermentation were recorded on the API 50 data sheet as positive, negative or doubtful. The APILAB PLUS database (bio Merieux Sa, France) was used to interpret the result of fermentation on colour change in different sugars [11].

Statistical analysis

Experiments were done in triplicate. Data were analyzed by calculating mean and standard deviation. The *t-test* (SPSS Ver. 14) was used to test the role of *Omukunzi* root (*Boscia albitrunca*) on the viscosity and the LAB profile of *Omashikwa* made with and without the root ($P \geq 0.05$).

RESULTS

Proximate composition of *B. albitrunca*

The proximate composition of *Boscia albitrunca* (*Omukunzi)* root (Table 1) had 19.8g/100g of total carbohydrates (CHO) and 19.4g/100g of soluble carbohydrates. Moisture content was 68g/100g, dry matter 32g/100g, ash 1.8g/100g, protein 6.5g/100g, fat 0.3g/100g and fiber 3.6g/100g.

The pH of *Omukunzi* root was low (pH 4.9). The pH of TO and LO were also low (4.7 for TO and 4.5 for LO, respectively) and were not significantly different ($p \geq 0.05$).

Viscosity of *Omashikwa* with the root was 2.9 Pa.s and that without the root was 2.5 Pa.s despite the fact that LO was pasteurized at a temperature of 65°C/30 min.

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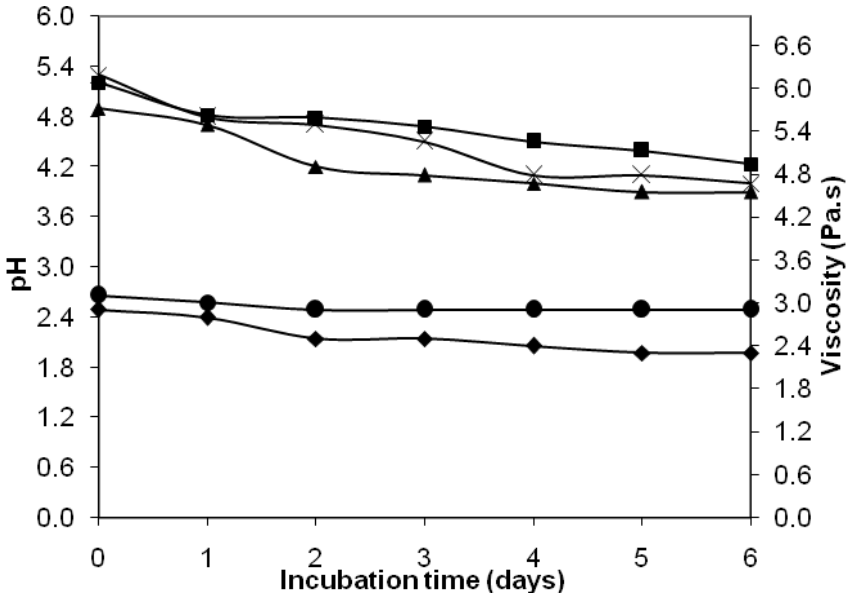

Figure 2: Effect of incubation at 10°C on the pH and viscosity of *Omashikwa* with and without *Omunkunzi* root.–■–■–: pH of root, −▲−: pH of *Omashikwa* with root, −x−: pH of *Omashikwa* without root, −●−: viscosity of *Omashikwa* without root, −♦−: viscosity of *Omashikwa* with root

B. albitrunca root showed bacterial inhibition properties as shown in Fig. 3 ring test with and without the root, a significant quality for improving fermentation and the consistency of *Omashikwa.*

A B

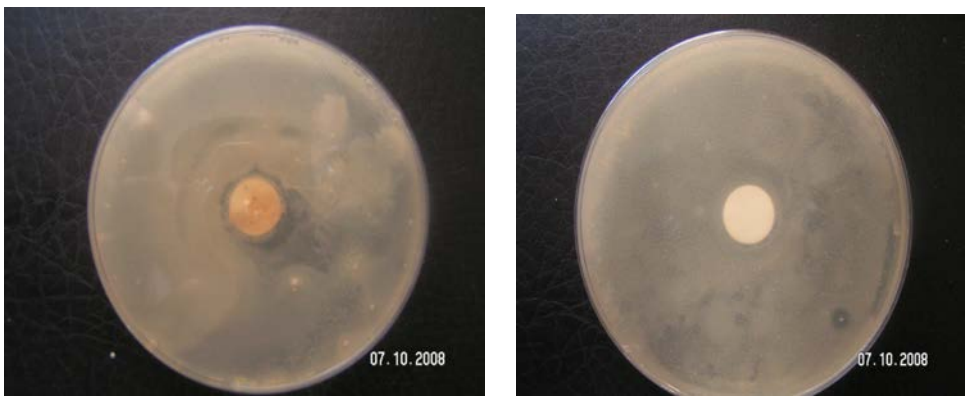

Figure 3: Bacterial inhibition effect of *B. albitrunca* root on TPC Agar (A) and (B) without *B. albitrunca* root (control), respectively.

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Bacteriological characteristics

Table 2 and 3 summarize the total aerobic and lactic acid bacteria counts obtained from 100 samples of *Omashikwa* with and without the *Omukunzi* root. The undesirable presence of coliforms in TO of 2.68 cfu/g and in LO of 2.70 cfu/g, yeasts and moulds counts of 1.57 cfu/g in TO and 1.69 cfu/g in LO were not significantly different at $p \geq 0.05$, although the LO milk was pasteurized.

The mean counts on MRS agar (35^{o}C) of TO and LO were 7.6 cfu/g and 8.66 cfu/g and were higher than the total plate counts (6.62 and 8.62 cfu/g), indicating higher counts of LAB. The higher counts of microbes in LO could be due to freedom from competition with other microbes as the milk was pasteurized prior to inoculation. The mean counts on MRS agar (42^{o}C) and Rogosa agar (35^{o}C) were also high, 7.62 in LO compared to 6.40 cfu/g of TO and 7.60 cfu/g in LO compared to 6.70 in TO, respectively.

Table 4 summarizes the results of the 20 isolated lactic acid bacteria. From the 20 lactic acid bacteria isolated from TO and LO and cultured in 4 media types namely, MRS agar (42 and 35^{o}C), M17 (30^{o}C) and Rogosa agar (35^{o}C) and identified with API 50 CH identification system, 5 belonged to *Lb. plantarum* and 3 to *Lb. lactis* subsp. *lactis* in TO while 4 and 2 belonged to LO, respectively. Four belonged to *Leuconostoc lactis*, 2 to *Leuconostoc mesenteroides* subsp *dextranicum* in TO while 3 to *Leuconostoc lactis*, 3 to *Leuconostoc mesenteroides* subsp. *dextranicum* and 1 to *Leuconostoc citreum* in LO. Three *Lactococcus* species belonged to *Lact. lactis* subsp. *lactis* and 1 to *Lact. lactis* subsp. *diacetylactis* in TO while 4 belonged to *Lact. lactis* subsp. *lactis* and 2 to *Lact. lactis* subsp. *diacetylactis* in LO. Only a few Streptococcus species (*thermophiles)* were found and identified. Two were found in TO and 1 in LO, respectively.

DISCUSSION

High content of soluble carbohydrate in *Omukunzi* root may explain the reason for improved viscosity or thickness of TO 2.9 Pa.s compared to 2.4 Pa.s of LO. The presence of soluble carbohydrates or hydrocolloids may bind water, reduce syneresis and improve the viscous consistency of TO due to their gummy nature. The poor viscosity of LO may be due to low pasteurization temperature (65^{o}C/30 min) which could not denature whey proteins and bind water as is usually done with milk for Yoghurt making [18, 19]. It may as well be that back-slopping with TO containing non-acid and spoilage microorganisms including coliforms, yeasts and moulds may have resulted in poor fermentation process and rendered LO less viscous as was also observed by researchers in other types of fermented milk products [20, 21]. In addition, the presence of *Omukunzi* root in TO might have played a significant role in improving the viscosity of *Omashikwa* due probably to its ability in controlling some of the non-acid and spoilage microbes as shown in the inhibitory ring test (Fig.3).

The low pH of the root and *Omashikwa* samples might have affected the initial bacterial counts, as the high acidity might have discouraged the growth of non-acid

producers and spoilage microorganisms and encouraged the growth of LAB as their habitat [22] (Table 4). The low pH (4-5) of fermented milk may also render the products safe for human consumption as reported by Feresu and Nyati [23] and Kimonye and Robinson [6].

Higher counts of MRS and M17 agar compared to count on Rogosa agar may be explained by the fact that MRS and M17 agars are elective while Rogosa agar is selective as described by Reuter [24]. The higher counts of thermophilic bacteria may be explained by the fact that *Omashikwa* samples were collected during the hot season, at the ambient temperatures ranging between 37 and 43^{o}C (November/January) in Northern and Central 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 *Omukunzi* root (TO) showed lower mesophilic counts in most of the agar media. This may be explained by the acidic nature of the root, high ambient temperature and probably the presence of inhibitory compounds in the root controlling the growth of non-acid producers and spoilage microorganisms. The root seemed to promote the proliferation of the thermophilic group of bacteria with 56% of the Genus Lactobacillus in TO and 34% in LO respectively, as shown in Table 3. The counts however, compared closely with findings of other studies on fermented milks by other workers in Zimbabwe [21], South Africa [25], Northern Tanzania [26], Cameroon [27], Nigeria [28] and Burkina Faso [29].

These differences in microbial counts may be explained by the high temperatures of processing and collection, low pH and probably the presence of inhibitory compounds in the root of *Boscia albitrunca* tree as reported earlier. In general, there were no significant differences between the two products in terms of the genus and species found as they originated from back-slopping with TO. Only the counts and species numbers were different due probably to the presence of the root in TO and controlled fermentation in LO.

It is known that aseptically drawn milk contains no Lactobacilli when it leaves the udder, but contamination with these organisms rapidly occurs from dairy utensils, dust and feedstuffs [22]. Since unpasteurized *Omashikwa* samples were used as starter culture to inoculate samples of pasteurized skim milk with and without the root of *Omukunzi (Boscia 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 contribute to the quality of *Omashikwa* or any other traditional fermented milk products in terms of acid, flavour, consistency, syneresis and aroma production if properly handled, except for the presence of spoilage microorganisms, non-acid producers, coliforms and yeasts and moulds.

In this study on fermented buttermilk in Northern and Central Namibia, it was established that the use of *Omukunzi (Boscia albitrunca)* root in *Omashikwa* is justifiable. This can be explained by low pH and high levels of soluble carbohydrates

in the root which may control syneresis and improve viscosity of the fermented buttermilk. *Omukunzi* root contains compounds, some of which may be inhibitory to microbial growth and may control proliferation of non-acid and spoilage microorganisms and may promote the growth of lactic acid bacteria especially of the thermophilic group; Lactobacilli, as shown in Tables 2, 3, and 4, which were high in both samples of *Omashikwa.* Poor sanitation and hygiene, the use of the root and traditional fermenting containers such as gourds or wood containers appear to have stimulated the growth of *Lactobacillus plantarum* in *Omashikwa*, since TO contained high numbers of this species commonly found in plants materials, as reported earlier [22]. The lactic acid bacteria occurring in relatively high numbers were identified as representatives of Lactobacilli, Leuconostoc, Lactococci and the least Streptococci. This may be attributed to high environmental temperatures during processing and probably the use of *Omukunzi* root. Most of the lactic acid bacteria were of the genus *Lactobacilli/Weissella*; 56% in TO and 34% in LO, respectively. The identified species of LAB from *Omashikwa* can be isolated and be used as starters for small-scale dairy industries. Although *Omukunzi* root appears to play a role in the quality of *Omashikwa*, appropriate starter cultures, hygiene, sanitation and application of good manufacturing practices on unit operations seem to be the effective methods to improve and stabilize the quality of *Omashikwa*. Future studies will focus on the identification of possible inhibitory compounds in *B. albitrunca* root in order to study their role in controlling the proliferation of the non-acid and spoilage microorganisms in fermented milk products.

Generally, the species identified in the present study (Table 4), were 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 [25]. *Lactobacillus lactis* subsp *lactis*. *Lactobacillus plantarum* and *Lactobacillus delbrueckii* subsp *lactis* were identified in Zimbabwe fermented milk [21], *Lactobacillus plantarum, Lactobacillus lactis* subsp *lactis* and *Weissella confusa* (former *Lactobacillus confusus)* were identified in Maasai fermented milk in Northern Tanzania [26] 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 [29]. Most of these species cited were also identified in fermented buttermilk *(Omashikwa*) in Northern Namibia. This fact explains the diversity of lactic acid bacteria species in *Omashikwa.*

CONCLUSION

In this study on fermented buttermilk in Northern and Central Namibia, it was established that the use of *Boscia albitrunca* root in traditional *Omashikwa* is justifiable in the absence of modern technology in order to musk off flavours by imparting root taste, control fermentation and improve its viscosity. The root has low pH which may inhibit the growth of spoilage and pathogenic bacteria and stimulate the initial growth of lactic acid bacteria in the product. It contains high levels of

soluble carbohydrates that may play a role in increasing the viscosity of traditional *Omashikwa*. The *Omukunzi* root also appears to contain some inhibitory compounds controlling the proliferation of some non-acid and spoilage microorganisms as indicated on a ring test.

The quality of fermented milk is characterized by its aroma and taste, texture and shelf-life. These properties are dependent on the microflora responsible for the fermentation and their metabolites, temperatures, hygiene and sanitation. However, the presence of coliforms, yeast and moulds and observed variable consistency of the product, clearly indicate that the current process needs some improvement. Although *Omukunzi* root appears to play a role in the quality of *Omashikwa*, it is recommended that basic technology procedures (GMPs & HACCP) such as heat treatment, use of appropriate starter cultures, hygiene, sanitation and application of good manufacturing practices on unit operations, including proper packaging, seem to be the effective methods to improve and stabilize the quality of *Omashikwa* for income generation, employment, food security and nutrition of the rural communities in developing countries.

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Table 1: Proximate composition of *Omukunzi* 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 fiber	3.6 ± 0.2
Carbohydrates by difference	19.8 ± 2.0
Soluble carbohydrates	19.4 ± 2.1
± – Standard deviation of the means. (n=3).	

Table 2: Total counts of anaerobic and lactic acid bacteria counts (log_{10} cfu/gCFU g-1) of samples of TO and LO from Namibia. (n = 3)

Medium	**Range counts (log_{10} cfu/g**		**Mean counts (log_{10} 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}
Lactobacillus & Leuconostocs	8.46 – 8.97	7.41 – 7.91	8.66 ± 0.23^{a}	7.60 ± 0.22^{b}
Lactobacillus & Streptococcus	7.40 – 7.92	6.40 – 6.88	7.62 ± 0.22^{a}	6.40 ± 0.20^{b}
Lactobacillus 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}
Coliforms	2.39 – 2.92	2.28 – 2.87	2.70 ± 0.10^{a}	2.68 ± 0.15^{a}
Yeast/ Moulds	1.38 – 1.76	1.52 – 1.87	1.57±0.18a	1.69±0.26b
Yeast: Gourd swabs	-	3.32 - 6.23	-	4.78 ±0.46

Key words: mean counts with different superscripts on the same row were significantly different (P≤0.05) from each other. Figure ± is standard deviation of the mean. (n = 3)

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Table 3: Identification of lactic acid bacteria isolated from *Omashikwa* with and without the root of *Omukunzi* tree to species level by the API 50 CH method

% Isolates	With root					Without root				
	Thermoph.		Mesoph.	Thermoph.		Thermo.		Meso.	Thermo	
	MRS		M17	Rogosa		MRS		M17	Rogosa	
	35°C	**42°C**	**30°C**	**35°C**	**Totals**	**35°C**	**42°C**	**30°C**	**35°C**	**Totals**
Lactobacillus	11	20		25	56	21	11		17	49
Streptococcus		13			13		6			6
Leuconostocs	17				17	23				23
Lactococcus			14		14			22		22
Totals					**100**					**100**

Key: Thermo. = Thermophilic, Mesoph. = mesophilic

Table 4: Identification of lactic acid bacteria isolated from Omashikwa with and without the root of Omukunzi tree to species level by API 50 CH method

Genus LAB	Species identified	Numbers	
		With root	**Without root**
Lactobacillus species	*Lb. plantarum*	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 sp lactis*	3 (15%)	4 (20%)
	Lactic.lactis/diacetylatis	1 (5%)	2 (10%)
Streptococcus species	*Strep.thermophilus*	2 (10%)	1 (5%)
Totals		**20**	**20**

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