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Identification of lactic acid bacteria and determination of selected biochemical properties in *emasi* and *emahewu*

Fermented foods are produced at household level for personal consumption in the Kingdom of Eswatini (formerly Swaziland). In this study, we determined the biochemical aspects, enumeration, isolation and identification of lactic acid bacteria (LAB) in *emasi* and *emahewu* – two Swazi traditional fermented foods. *Emasi* had an average pH of 4.68, titratable acidity of 0.9% and LAB count of 8.25 log CFU/mL. *Emahewu* had a pH of 3.62, titratable acidity of 0.4% and LAB count of 8.10 log CFU/mL. The LAB counts were consistent with observations for similar African fermented foods. The LAB from *emasi* and *emahewu* were identified through Gram stain, catalase reaction, sugar assimilation tests using API 50 CH test strips, and sequencing of 16S rDNA. It was found (from nine isolates) that *Lactococcus lactis* subsp. *lactis* and *Leuconostoc mesenteroides* were the common strains in *emasi*. *Lactobacillus plantarum*, *Lactobacillus paracasei* ssp. *paracasei* and *Lactobacillus brevis* were also detected. *Lb. plantarum, L. mesenteroides* ssp. *mesenteroides, Lactobacillus fermentum, Lb. brevis, Wessella confusa, Lactobacillus acidophilus* and *Lb. lactis* were found in *emahewu* (from 16 isolates). This finding was consistent with LAB found in a South African fermented milk, in which common genera were *Leuconostoc, Lactococcus* and *Lactobacillus.* Strains found in *emahewu* – mainly *Lactobacillus* spp., *Weissella* and *Enterococcus* – are similar to those found in *ting*, a South African fermented non-alcoholic beverage.

Significance:

- This study provides the first documentation of microbial and biochemical aspects of the Swazi traditional fermented foods, *emasi* and *emahewu*.

Introduction

Fermentation of food is one of the oldest forms of food preservation.[1] Several studies have shown how this technique helps in preventing food-borne illnesses, including childhood diarrhoea.[2] Consumption of fermented foods is thought to contribute to good health because of the benefit of their microflora to the human gut.[3]

Fermented foods can be grouped into four categories: alcohol, lactic acid, acetic acid and alkali fermented foods.[1,4] Several traditional African fermented cereal grain foods, such as *mahewu* (sour sorghum or maize meal non-alcoholic beverage from South Africa, Zimbabwe and Lesotho), *togwa* (thin sour maize meal porridge from Tanzania), *kenkey* (thick sour maize meal porridge from Ghana), *amasi* (spontaneously fermented milk from southern Africa) and *motoho* (thin sour sorghum porridge or beverage from Lesotho), are largely products of lactic acid fermentation.

Lactic acid bacteria (LAB) have been found to be the predominant microorganisms in most of these products.[1] However, yeasts are also important in alcoholic fermented foods[4], and may be accidental contaminants in fermented milk[5,6]. Mathara et al.[7] found that *Lactobacillus* species (*Lactobacillus plantarum*, *Lactobacillus fermentum*, *Lactobacillus paracasei* and *Lactobacillus acidophilus*) were predominant in *kule naoto*, Kenyan traditional fermented milk produced by the Maasai. Other genera isolated from *kule naoto* were *Enterococcus*, *Lactococcus* and *Leuconostoc*. Schoustra et al.[8] reported that *Lactobacillus* and *Weissella* were common genera, together with *Lactococcus*, *Streptococcus* and *Leuconostoc,* in Munkoyo and Chibwantu, traditional non-alcoholic fermented beverages popularly consumed in Zambia. *Emasi* and *emahewu* are non-alcoholic lactic acid fermented traditional foods produced by households in Eswatini.

The preparation methods of *sancta* (fermented maize meal), *incwancwa* (fermented porridge), *emasi* (fermented milk), *emahewu* (non-alcoholic cereal beverage), *umcombotsi* (alcoholic sorghum or millet beverage), *mankanjane* (malt distilled spirit), *buganu*/*marula* wine and papaya beer (fermented fruit mashes) have been previously outlined.[9] However, the microbial flora responsible for the fermentation has not been studied. The aim of this study, therefore, was to investigate the microbial diversity, to isolate potential probiotic LAB strains and to identify LAB in *emasi* and *emahewu*. Some of the biochemical properties were also investigated. This step is important in up-scaling and possible commercialisation of these products.

Materials and methods

Location of study

This study was done in the Hhohho Region of the Kingdom of Eswatini (formerly Swaziland). Hhohho Region is in the highveld of the country where the temperatures range from very cold to warm. The Region is divided into 14 local administrations called *tinkhundla*. Samples were collected from 5 *tinkhundla* that were randomly selected from the 14 *tinkhundla* using a lottery system.

Sample collection

Samples of *emasi* and *emahewu* were collected from nine locations within the five *tinkhundla*: Lobamba (coded L), Mangwaneni (M), Zone 4 (Z4), Motshane (Mot), Mbabane (Mb), Ezulwini (Ez), Mvutjini (Mv), Sitjeni (S) and

Ntfonjeni (Nt) areas in Hhohho, Swaziland. At each *inkhundla*, a list of members of the community who were known to prepare fermented foods was compiled with the assistance of community leaders, such as the *umphakatsi* or schoolteachers. Samples of fermented food were then collected from the households that were randomly selected from the list. The samples were collected in sterile screw-capped bottles and ferried in a cooler box to the laboratory at the University of Swaziland (a distance of 5–75 km) for analysis.

Preparation of emahewu and emasi

Women who prepared *emahewu* explained that the product was prepared by thoroughly mixing 1 kg maize meal with 5 L of water. The mixture was then cooked until well gelatinised into a soft porridge called *umhidvo,* then cooled (to 25–30 °C) and left to ferment at room temperature (25–30 °C; 2–5 days). Malt was not added during preparation of the product, therefore *emahewu* lacked the enzymes that come with addition of malt to trigger the start of fermentation. However, some households reported adding sugar or a peeled potato, therefore some bacterial inoculum may have originated from addition of potato and/or from bacteria that may have been present on utensils used during preparation.

Emasi was prepared by leaving raw milk to naturally ferment at room temperature (25–30 °C; 2–3 days) using plastic or metal containers or clay pots. The whey was sometimes strained to give a thick product. Back-slopping – which is inoculation using substrate from a previous fermentation – is often used during fermentation of milk to *emasi*.

Determination of pH and titratable acidity

The pH was determined using a Hanna Instruments pH meter (HI 8314, Leighton Buzzard Bedfordshire, UK) after calibrating with buffers at pH 4 and pH 7. Titratable acidity (TA) was determined using standardised 0.1 N NaOH (Rochelle Chemicals, Johannesburg, South Africa) according to the Association of Official Analytical Chemists (AOAC) method no. 947.05.[10]

Microbiological analysis

Enumeration of LAB

The fermented samples were analysed immediately upon arrival at the laboratory. LAB were enumerated on De Man, Rogosa and Sharpe (MRS) agar (Oxoid, Basingstoke, UK; CM0361) (selective agar) by spreading 0.1 mL of appropriate serial dilutions and incubating anaerobically at 30 °C for 48 h. Anaerobic conditions were created using an Oxoid anaerobic gas generating system (Oxoid, Basingstoke, UK, BR0038B) according to the manufacturer's instructions.

Isolation and selection of LAB strains

Colonies with a different appearance (based on colour, shape and size) were extracted from the MRS agar and purified by streaking on a fresh MRS agar plate. The purification process was repeated until single colonies with distinct appearance were obtained. The pure isolates were tested for Gram and catalase reactions. Cell morphology was observed under the microscope. The isolates that were Gram positive and catalase negative were taken as presumptive LAB. The LAB isolates were stored at -20 °C in MRS broth (Biolab, Wadeville, South Africa; HG000C87.500) containing 20% (v/v) glycerol until required for further tests.

Identification of LAB using Analytical Profile Index kits

The frozen LAB isolates were thawed and resuscitated by inoculating into fresh MRS broth and incubating at 30 °C for 24 h. A portion of the fresh culture was streaked onto MRS agar, which was then incubated anaerobically for 48 h. The pure colonies were extracted and inoculated onto Analytical Profile Index (API) 50 CH (bioMerieux, Marcy l'Etloile, France; Ref 50 300) test strips according to the manufacturer's instructions. The sugar fermentation profiles were then used to identify the isolates using API identification software (APIWEB™). A total of 16 LAB strains from *emahewu* and 9 LAB strains from *emasi* were identified using the API 50 CH kit. The carbohydrate profile was generated based on substrate metabolism using the API 50 CH kit. The API 50 CH approach is a well-established accurate method for manual microorganism identification for Gram-positive and Gram-negative bacteria and yeast to the species level based on extensive databases. The system offers a large and robust database accessible through the Internet-based APIWEB™ service. The method is economical to run and user-friendly.

Identification of LAB by sequencing 16S rDNA

Identification of LAB was performed at Inqaba Biotec Industries (Pretoria, South Africa). Briefly, DNA was extracted using ZR Fungal/Bacteria DNA™ kit (Zymo Research, Irvine, CA, USA). The 16S rDNA target region was amplified using DreamTaq™ DNA polymerase (Thermo Scientific™, Waltham, MA, USA) and the primers 16S-27F (sequence 5′-AGAGTTTGATCMTGGCTCAG-3′) and 16S-1492R (sequence 5′-CGG-TTACCTTGTTACGACTT-3′). Polymerase chain reaction (PCR) products were gel extracted (Zymo Research, Zymoclean™ Gel DNA Recovery kit), and sequenced in the forward and reverse directions on the ABI PRISM™ 3500 x l Genetic Analyser. Purified sequencing products (Zymo Research, ZR-96 DNA Sequencing Clean-up™ kit) were analysed using CLC Main Workbench 7 followed by a BLAST search on the database of the US National Center for Biotechnology Information.[11] Of the 16 LAB strains initially identified from *emahewu* using the API 50 CH kit, 9 were identified using the 16S rDNA method.

Statistical analysis

Mean (± standard deviation) was calculated for the pH, TA and microbial counts for the samples in the different categories using Microsoft Excel. The statistical significance ($p<0.05$) of the data sets was evaluated using Statistical Package for Social Science (SPSS) software.

Results and discussion

Emasi

pH and TA

The average pH of *emasi* was 4.68±0.25, and TA was 0.9±0.08% (Table 1), which corresponds well with values obtained in other studies for naturally fermented milk. For example, Kebede et al.[12] reported that *sethemi*, South African naturally fermented milk similar to *emasi,* had pH values of about 4.1–4.3. Beukes et al.[13] also reported that the pH of indigenous fermented milks from South Africa and Namibia ranged from 4.0 to 5.4, with an average of 4.6. *Amasi* produced at household level in Zimbabwe was found to have a mean pH of 3.98 and 1.0% TA.[14] Gran et al.[15] found that the pH of naturally fermented *amasi* produced by smallholder producers in Zimbabwe was about 4.2 after 48 h fermentation. *Nunu* is a Ghanaian spontaneously fermented milk with the consistency of yoghurt and a pH of about 3.4 after 48 h of fermentation.[16] However, the reported TA of 4.5% for *nunu* was uncharacteristically high compared with the values recorded for *emasi*, *amasi* and other similar products in southern Africa. In comparison, Moyane and Jideani[17] found that the pH of commercially produced *amasi* in Venda, South Africa, ranged from 4.22 to 4.34, with an average TA of 0.8%, which is close to what was recorded for spontaneously fermented *emasi*.

Table 1: The pH, titratable acidity and lactic acid bacteria (LAB) count of *emasi*, a Swazi naturally fermented milk

Sample code	pH*	Titratable acidity (% lactic acid)	LAB count (log CFU/mL)
MOT-emasi	4.31±0.01	1.0±0.03	8.34
Nt-emasi-1	4.57±0.01	1.0±0.04	8.69
Nt-emasi	4.52±0.51	0.8±0.03	8.82
L-emasi	5.03±0.07	0.8±0.07	7.30
Mb-emasi-1	4.98±0.11	0.9±0.04	7.78
Mb-emasi-2	4.87±0.03	0.9±0.01	8.24
Mb-emasi-3	4.62±0.1	0.9±0.03	8.36
Mb-emasi-4	4.55±0.03	0.9±0.01	8.45
Average	4.68±0.25	0.9±0.08	8.25±0.49

MOT, Motshane; Nt, Ntfonjeni; L, Lobamba; Mb, Mbabane (locations from where samples were collected)
**There were no significant differences in the column (p>0.05).*

The pH range for *emasi* (Table 1) was 4.31–5.03. Although there were variations in pH of the samples, the deviations were not significant ($p>0.05$). The differences in varying values of pH in Table 1 may be attributed to the variations in the amount of available substrate for LAB to ferment, the type and quantity of predominant fermenting LAB (*emasi* production often involves back-slopping), and the duration of fermentation.

Enumeration of LAB

The LAB counts in *emasi* ranged from 7.30 to 8.82 log CFU/mL (translating to an average of 8.25±0.49 log CFU/mL) (Table 1). The LAB counts were very comparable to those of similar African naturally fermented milk products. For instance, the presumptive LAB counts in indigenous spontaneously fermented *amasi* from South Africa were about 7.7 x 10^8 CFU/mL (8.89 log CFU/mL).[13] Zimbabwean *amasi* had a LAB ranging from 8.29 to 9.88 log CFU/g[14], while a Nigerian fermented milk, *nono*, was found to have LAB counts of about 9.8 x 10^6 CFU/mL (6.99 log CFU/mL). In addition, Egyptian traditional fermented milk, Laban Zeer, had LAB counts of up to 7.4 log CFU/g. The Ghanaian *nunu* was also reported to have LAB counts of up to 9 log CFU/mL after 48 h fermentation.[16] In contrast, Matsheka et al.[18] reported a much lower value of 5.3 log CFU/mL LAB in *madila*, Botswanan spontaneously fermented milk.

Other studies on non-African fermented milks showed similar trends for LAB counts. Traditional naturally fermented goat's milk collected from households in the Haixi Region of China had LAB counts of 2.5 x 10^8–3.0 x 10^9 CFU/mL (8.4–9.5 log CFU/m).[19]

There was a relationship amongst the pH, TA and LAB of *emasi*. The LAB fermented the lactose in raw milk that led to production of organic acids. The organic acids lowered the pH and increased the TA. As the acidity in *emasi* increased over the processing time, it inhibited the growth of low tolerant LAB. The amount of fermentable lactose in raw milk therefore had an influence on pH and TA.

Emahewu

pH and TA

The average pH of *emahewu* was 3.61±0.55, ranging from 2.95 to 4.51. The TA was 0.42±0.17% (Table 2). A similar product prepared in Zimbabwe, which is also called *mahewu*, had a final pH of 3.0.[20] This product had a TA of about 0.9% after 48 h fermentation, which is higher than that observed for *emahewu*. The Zimbabwean *mahewu* is made with maize meal and sorghum malt flour, which is probably the reason for production of higher amounts of organic acids. Sorghum malt is not added during preparation of *emahewu*.[9] The pH in *bushera,* a non-alcoholic sorghum-based beverage from Uganda, was found to range from 3.7 to 4.5,[21] which is close to the values obtained for *emahewu*. The TA of this product was 0.5%, which tallies with the results of the current study and the pH values obtained.

Table 2: The pH, titratable acidity and lactic acid bacteria (LAB) count of *emahewu*, a Swazi non-alcoholic fermented beverage

Sample code	pH*	Titratable acidity (% lactic acid)	LAB count (log CFU/mL)
L-emah-1	4.34±0.2	0.2±0.03	6.91
L-emah-2	4.17±0.1	0.4±0.03	7.78
L-emah	3.28±0.04	0.5±0.06	9.30
L-emah-3	3.86±0.06	0.5±0.04	8.75
Nt-emah	3.84±0.06	0.5±0.03	8.14
Z4-emah-1	4.51±0.08	0.8±0.03	6.88
L-emah-20	2.95±0.13	0.4±0.04	8.11
L-emah-21	3.09±0.1	0.4±0.03	8.67
L-emah-22	3.15±0.21	0.3±0.04	8.43
Ez-emah	3.30±0.21	0.2±0.01	7.74
Mv-emah	3.24±0.2	0.4±0.06	8.41
Mean	3.61±0.55	0.42±0.17	8.10±0.74

L, Lobamba; Nt, Ntfonjeni; Z4, Zone 4; Ez, Ezulwini; Mv, Mvutjini (locations from where samples were collected)
**There were no significant differences in the column ($p>0.05$).*

The pH range for *emahewu* (Table 2) was 3.09–4.51. Although there were variations in pH of the samples, the deviations were not significant ($p>0.05$). The differences in varying values of pH in Table 2 may be attributed to the variations in the amount of available substrate for LAB to ferment, the type and quantity of predominant fermenting LAB, and the duration of fermentation.

Enumeration of LAB

The LAB counts in *emahewu* ranged from 6.88 to 9.30 log CFU/mL (translating to an average of 8.10±0.74 log CFU/mL) (Table 2). The LAB counts were within the range expected when compared to those of other studies. Muyanja et al.[21], in their study of *bushera*, found that the LAB counts varied between 7.1 and 9.4 log CFU/mL. LAB counts in homemade *mahewu* from Zimbabwe increased from 2.0 to 8.0 log CFU/mL after 72 h of fermentation.[20] *Ting* is a non-alcoholic beverage prepared in Botswana and is made from sorghum meal and malt. The LAB counts of *ting* were found to range between 8.08 and 10.1 log CFU/g.[22]

As with *emasi*, there was a relationship amongst the pH, TA and LAB of *emhewu*. The LAB fermented the carbohydrates (starch and some sugars) in maize meal used to make *emahewu* that led to production of organic acids. The organic acids lowered the pH and increased the TA. As the acidity in *emahewu* increased over the processing time, it inhibited the growth of low tolerant LAB. The amount of fermentable carbohydrates in maize meal therefore had an influence on pH and TA.

Identification of LAB

The isolates were initially screened as presumptive LAB using the Gram stain, catalase test and microscopic examination. The Gram-positive, catalase-negative isolates were identified to species level using API 50 CH test strips and by sequencing the 16S rDNA as shown in Table 3 and Table 4 and carbohydrate profile of LAB was as shown in Table 5.

Emasi

Among the 9 *emasi* isolates identified using the API 50 CH kits, 4 were identified as *Leuconostoc mesenteroides* ssp. *mesenteroides*/*dextranicum*, 2 as *Lactococcus lactis*, 1 as *Lb. plantarum* and the other 2 as *Lactobacillus brevis* (Table 3). The four *Leuconostoc* isolates were characterised by sequencing the 16S rDNA and were identified as *Leuconostoc pseudomesenteroides* (Table 4), which was in close agreement with the API identification. The small difference in the identification of LAB between API and sequencing 16S rDNA methods is because the latter method is much more accurate than API.

In a study on South African naturally fermented milk, Beukes et al.[13] reported that the genera *Leuconostoc*, *Lactococcus* and *Lactobacillus* were the main flora. The dominant lactococci species in the South African product was *Lactococcus lactis* subsp. *lactis*, while most of the *Leuconostoc* isolates were identified as *Leuconostoc mesenteroides* subsp. *dextranicum,* similarly to the findings for Swazi *emasi*. Other species identified in that study include *Leuconostoc citreum*, *Leuconostoc lactis*, *Lactobacillus delbrueckii* subsp. *lactis* and *Lb. plantarum*.

Mutukumira[14] observed that *Lactococcus lactis* subsp. *lactis* was the predominant strain isolated from *amasi,* spontaneously fermented milk produced in Zimbabwe. The Zimbabwean *amasi* is produced in a similar way to *emasi*, which may explain the similarity in microbial ecology. Slight differences that may be found in the microbial diversity can be attributed to different types of containers used, as well as the environment under which the fermentation is done. Clay pots, metal containers, calabashes and gourds are often used and have been found to impact the microbial diversity.[12] The current observations also agree with recent work by Osvik et al.[23] who studied the bacterial diversity of *amasi* from the EkuPindiseni community of KwaZulu-Natal in South Africa using 16S rRNA and denaturing gradient gel electrophoresis for identification. The majority of the strains found were in the genus *Lactococcus*, as well as *Lactobacillus*, *Leuconostoc* and *Enterococcus*. However, a study by Mathara et al.[7] showed that the genus *Lactobacillus* was predominant in *kule naoto*, Kenyan traditional fermented milk produced by the Maasai, in which the major *Lactobacillus* species was *Lb. plantarum*, followed by *Lb. fermentum*, *Lb. paracasei* and *Lb. acidophilus*. Other genera that were isolated in *kule naoto* were *Enterococcus*, *Lactococcus* and *Leuconostoc*.

Laban Zeer produced in Egypt seems to have similar flora to that of *emasi*. Saleh[24] identified the LAB species in Laban Zeer as *Leuconostoc mesenteroides* subsp. *cremoris*, *Lb*. *rhamnosus*, *Lb. plantarum*, *Lb. paracasei* subsp. *paracasei*, *Lb. delbruekii* subsp. *bulgaricus*, *Lb. curvatus* subsp. *curvatus* and *Lb. acidophilus*. The most frequently isolated LAB species were found to be *Leuconostoc mesenteroides* subsp. *cremoris* and *Lb. rhamnosus*.

The Swazi fermented milk's microflora is therefore similar to that in other naturally fermented products from southern Africa, in particular *amasi* from South Africa and Zimbabwe, in which the dominant genera are *Leuconostoc, Lactobacillus and Lactococcus.*

Emahewu

Of the 16 isolates from *emahewu* identified using the API 50 CH test kit, 6 were identified as *Lb. plantarum*, 3 as *Leuconostoc mesenteroides* ssp*. mesenteroides*, 2 as *Lb. fermentum*, 2 as *Lb. brevis*, and 1 as *Lb. collinoides* (Table 3).

Table 3: Identification of lactic acid bacteria isolated from Swazi traditional fermented *emasi* and *emahewu* using API 50CH kit

	Isolate code	Identity
		Emasi
1	L-emasi-1	*Leuconostoc mesenteroides* ssp. *mesenteroides/dextranicum*
2	L-emasi-5	*Leuconostoc mesenteroides* ssp. *mesenteroides*
3	L-emasi-7	*Leuconostoc mesenteroides* ssp. *mesenteroides/*dextranicum
4	L-emasi-8	*Leuconostoc mesenteroides* ssp. *mesenteroides/dextranicum*
5	Mot-emasi-7	*Lactococcus lactis* ssp. *lactis*
6	Nt-emas-2	*Lactococcus lactis* ssp*. lactis*
7	Nt-emas2-6	*Lactobacillus paracasei* ssp. *paracasei*
8	Nt-emas-5	*Lactobacillus plantarum*
9	Nt-emas-6	*Lactobacillus brevis*
		Emahewu
10	L-emah-1	*Leuconostoc mesenteroides* ssp. *mesenteroides/dextranicum*
11	L-emah-3	*Lactobacillus plantarum*
12	L-emah-5	*Lactobacillus brevis*
13	L-emah-6	*Lactobacillus brevis*
14	L-emah-7	*Lactobacillus collinoides / Lb. fermentum*
15	L-emah-8	*Leuconostoc mesenteroides* ssp. *mesenteroides/dextranicum*
16	L-emah-9	*Lactobacillus plantarum*
17	L-emah-13	*Leuconostoc mesenteroides* ssp. *mesenteroides/dextranicum*
18	L-emah-16	*Lactobacillus plantarum*
19	L-emah-18	*Lactobacillus plantarum*
20	Mot-emah-4	*Lactobacillus fermentum*
21	Mot-emah-6	*Lactobacillus collinoides*
22	Nt-emah-2	*Lactobacillus fermentum*
23	Nt-emah-6	*Lactobacillus paracasei* ssp. *paracasei*
24	S-emah	*Lactobacillus plantarum*
25	S-emah-5	*Lactobacillus plantarum*

L, Lobamba; MOT, Motshane; Nt, Ntfonjeni; S, Sitjeni (locations from where samples were collected)

The predominant isolates were therefore *Lb. plantarum* strains. Of the 9 isolates further characterised by sequencing the 16S rDNA, 4 were confirmed as *Lb. plantarum*, while the others were identified as *Leuconostoc lactis*, *Weissella confusa*, *Lactobacillus acidophilus* and *Lactococcus lactis* (Table 4).

Table 4: Identification of lactic acid bacterial isolates from Swazi traditional fermented *emasi* and *emahewu* using API 50 CH kit and by sequencing 16S rDNA

	Isolate code	Identity using API 50 CH kit	Identity using 16S rDNA†
		Emasi	
1	L-emasi-1	*Leuconostoc mesenteroides* ssp. *mesenteroides/dextranicum*	*Leuconostoc pseudomesenteroides*
2	L-emasi-5	*Leuconostoc mesenteroides* ssp. *mesenteroides*	*Leuconostoc pseudomesenteroides*
3	L-emasi-7	*Leuconostoc mesenteroides* ssp. *mesenteroides/dextranicum*	*Leuconostoc pseudomesenteroides*
4	L-emasi-8	*Leuconostoc mesenteroides* ssp. *mesenteroides/dextranicum*	Not identified
5	Mot-emasi-7	*Lactococcus lactis* ssp. *lactis*	Not identified
6	Nt-emas-2	*Lactococcus lactis* ssp. *lactis*	Not identified
7	Nt-emas2-6	*Lactobacillus paracasei* ssp. *paracasei*	Not identified
8	Nt-emas-5	*Lactobacillus plantarum*	Not identified
9	Nt-emas-6	*Lactobacillus brevis*	Not identified
10	L-emasi-13		*Leuconostoc pseudomesenteroides*
		Emahewu	
11	L-emah-1	*Leuconostoc mesenteroides* ssp. *mesenteroides/dextranicum*	Not identified
12	L-emah-3	*Lactobacillus plantarum*	*Lactobacillus plantarum*
13	L-emah-5	*Lactobacillus brevis*	Not identified
14	L-emah-6	*Lactobacillus brevis*	Not identified
15	L-emah-7	*Lactobacillus collinoides / Lb. fermentum*	*Weissella confusa*
16	L-emah-8	*Leuconostoc mesenteroides* ssp. *mesenteroides/dextranicum*	Not identified
17	L-emah-9	*Lactobacillus plantarum*	*Lactobacillus plantarum*
18	L-emah-13	*Leuconostoc mesenteroides* ssp. *mesenteroides/dextranicum*	Not identified
19	L-emah-16	*Lactobacillus plantarum*	*Lactobacillus plantarum*
20	L-emah-18	*Lactobacillus plantarum*	*Leuconostoc lactis*
22	L-emah-19		*Lactobacillus plantarum*
23	Mot-emah-4	*Lactobacillus fermentum*	*Lactococcus lactis*
24	Mot-emah-6	*Lactobacillus collinoides*	*Lactobacillus acidophilus*
25	Nt-emah-2	*Lactobacillus fermentum*	Not identified
26	Nt-emah-6	*Lactobacillus paracasei* ssp. *paracasei*	*Leuconostoc pseudomesenteroides*
27	S-emah	*Lactobacillus plantarum*	Not identified
28	S-emah-5	*Lactobacillus plantarum*	Not identified

L, Lobamba; MOT, Motshane; Nt, Ntfonjeni; S, Sitjeni (locations from where samples were collected)
†Only representative strains were further identified by molecular method by sequencing 16S rDNA.

In comparison, the main LAB in *ogi*, a Nigerian fermented cereal beverage, were found to be *Lb. plantarum*, *Lb. casei*, *Lb. brevis, Lb. fermentum*, *Lb. delbrueckii*, *Lb. acidophilus*, *Leuconostoc mesenteroides* and *Pediococcus acidilacti*.[25] In a separate study, Madoroba et al.[26] isolated and identified LAB in *ting*, a South African spontaneously fermented sorghum non-alcoholic beverage, and found that the predominant LAB were *Lb. plantarum, Lactococcus lactis*, *Lactobacillus fermentum*, *Lactobacillus rhamnosus*, *Weissella cibaria* and *Enterococcus faecalis*. Some Enterobacteriaceae were also isolated. The Swazi *emahewu* samples were prepared from maize meal. The predominant microorganisms in *koko*, a Ghanaian spontaneously fermented porridge from millet, were identified as *Weissella confusa* and *Lactobacillus fermentum*[27], while Yousif et al.[28] found that *Lactobacillus fermentum* and *Pediococcus acidilacti* were

Table 5: Carbohydrate fermentation (+ = positive reaction, - = negative reaction) by lactic acid bacteria species isolated from *emasi* and *emahewu*

Substrate	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
Control	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Glycerol	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Erythritol	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
D-arabinose	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
L-arabinose	-	-	+	+	-	-	-	+	-	-	-	+	+	-	-	-	+	-	-	-	-	-	-	+	+
Ribose	+	+	+	+	+	+	+	+	+	-	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+
D-xylose	+	+	-	-	+	+	-	-	-	+	-	+	+	+	+	-	+	-	-	-	+	-	-	-	-
L-xylose	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Adonitol	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
B-methyl-xyloside	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-
Galactose	+	+	+	+	+	+	+	+	+	-	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+
D-glucose	+	+	+	+	+	+	+	+	+	+	+	-	-	+	+	+	+	+	+	+	+	+	+	+	+
D-fructose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
D-mannose	+	+	+	+	+	+	+	+	+	+	+	-	-	+	+	+	+	+	+	+	+	+	+	+	+
L-sorbose	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Rhamnose	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+
Dulcitol	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Inositol	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Mannitol	-	-	-	-	-	+	+	+	+	-	+	-	-	-	-	+	-	+	+	-	-	-	+	+	+
Sorbitol	-	-	-	-	-	-	-	+	-	-	+	-	-	-	-	+	-	+	+	-	-	-	-	+	+
α-Methyl-D-mannoside	-	-	-	-	-	-	-	+	-	-	+	-	-	-	-	+	-	+	+	-	-	-	-	+	+
α-Methyl-D-glucoside	+	+	+	+	-	-	-	-	-	+	-	-	-	-	+	-	+	-	-	-	-	-	-	-	-
N-acetylglucosamide	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+
Amygdaline	-	-	-	-	-	+	+	+	+	-	+	-	-	-	-	+	-	+	+	-	-	-	+	+	+
Arbutine	-	-	-	-	-	+	+	+	+	+	+	-	-	-	-	+	-	-	-	-	-	-	+	+	+
Esculine	-	-	+	+	+	+	+	+	+	+	+	-	+	-	+	+	+	+	+	-	+	-	+	+	+
Salicine	-	-	-	-	-	+	+	+	+	+	+	-	-	-	+	+	-	+	+	-	-	-	+	+	+
Cellobiose	+	+	+	+	+	+	+	+	+	-	+	-	-	-	-	+	+	+	+	-	+	-	+	+	+
Maltose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lactose	+	+	+	+	-	+	+	+	+	-	+	-	-	-	-	+	+	+	+	+	-	+	+	+	+
Melibiose	+	+	+	+	-	-	-	+	+	+	+	+	+	-	+	+	+	+	+	+	-	+	-	+	+
Saccharose	+	+	+	+	+	-	+	+	+	+	+	-	-	+	+	+	+	+	+	+	+	+	+	+	+
Trehalose	+	+	+	+	-	+	+	+	+	+	+	-	-	-	+	+	+	+	+	-	-	-	+	+	+
Inuline	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Melizitose	-	-	-	-	-	-	-	+	-	-	+	-	-	-	-	+	-	+	+	-	-	-	-	+	+
D-raffinose	-	+	+	-	-	-	-	+	+	+	+	-	-	-	+	+	+	+	+	+	-	+	-	+	+
Starch	-	-	-	-	-	+	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-
Glycogen	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Xylitol	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
B-gentiobiose	+	+	+	+	+	+	+	+	+	-	+	-	-	-	-	+	+	+	+	+	+	-	+	+	+
D-turanose	+	+	+	+	-	-	-	+	-	+	-	-	-	-	+	-	+	-	-	-	-	-	-	+	+
D-lyxose	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
D-tagatose	-	-	-	-	-	-	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-
D-fucose	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
L-fucose	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
D-arabitol	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-	+	+
L-arabitol	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Gluconate	-	+	+	+	+	+	+	+	+	-	+	+	+	+	-	+	-	+	+	+	+	+	+	+	+
2-Ketogluconate	+	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-
5-Ketogluconate	-	+	+	-	-	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-

Key: *1:* Leuconostoc mesenteroides *ssp.* mesenteroides/dextranicum *(L-emasi-1); 2:* L. mesenteroides *ssp.* mesenteroides *(L-emasi-5); 3:* L. mesenteroides *ssp.* mesenteroides/dextranicum *(L-emasi-7); 4:* L. mesenteroides *ssp.* mesenteroides/dextranicum *(L-emasi-8); 5:* Lactococcus lactis *ssp.* lactis *(Mot-emasi-7); 6:* Lc. lactis *ssp.* lactis *(Nt-emas-2); 7:* Lactobacillus paracasei *ssp.* paracasei *(Nt-emas2-6); 8:* Lb. plantarum *(Nt-emas-5); 9:* Lb. brevis *(Nt-emas-6); 10:* L. mesenteroides *ssp.* mesenteroides/dextranicum *(L-emah-1); 11:* Lb. plantarum *(L-emah-3); 12:* Lb. brevis *(L-emah-5); 13:* Lb. brevis *(L-emah-6); 14:* Lb. collinoides/Lb. fermentum *(L-emah-7); 15:* L. mesenteroides *ssp.* mesenteroides/dextranicum *(L-emah-8); 16:* Lb. plantarum *(L-emah-9); 17:* L. mesenteroides *ssp.* mesenteroides/dextranicum *(L-emah-13); 18:* Lb. plantarum *(L-emah-16); 19:* Lb. plantarum *(L-emah-18); 20:* Lb. fermentum *(Mot-emah-4); 21:* Lb. collinoides *(Mot-emah-6); 22:* Lb. fermentum *(Nt-emah-2); 23:* Lb. paracasei *ssp.* paracasei *(Nt-emah-6); 24:* Lb. plantarum *(S-emah); 25:* Lb. plantarum *(S-emah-5)*

the predominant strains in *hussuwa*, a Sudanese fermented sorghum food. Also, in *gari*, a cassava-based fermented food from Benin, *Lb. plantarum* was the most commonly isolated species followed by *Leuconostoc fallax* and *Lactobacillus fermentum*.[29] Muyanja et al.[21] also identified the LAB isolated from the spontaneously fermented Ugandan *bushera* as *Lb. plantarum*, *L. paracasei* subsp. *paracasei*, *Lb. fermentum*, *Lb*. *brevis* and *Lb. delbrueckii* subsp. *delbrueckii*. Similarly, *Lactobacillus* and *Weissella* were the common genera isolated from Munkoyo and Chibwantu, traditional non-alcoholic fermented beverages popularly consumed in Zambia.[8] Therefore, the common LAB strains in Swazi *emahewu* belong to *Lb. plantarum, Lactobacillus* spp., *Leuconostoc* spp., *Lactococcus* spp. and *Weissella* spp. This finding is consistent with LAB strains reported in other products similar to Swazi *emahewu*. Notably, there were very few differences in identification of LAB for some isolates between the two methods (API 50 CH test and sequencing 16S rDNA). The accuracy of the API 50 CH test is limited to species available on the databases on the Internet-based APIWEB™ service, and the accuracy of 16S rDNA analyses strongly depends on the choice of primers.

Notably, the common LAB strains in Swazi *emahewu* belong to *Lactobacillus,* which suggests that *Lb. plantarum*, in particular, is a typical biota of spontaneously fermented maize and sorghum non-alcoholic beverages and plays a key role in defining the attributes of these products. Some strains of *Lb. plantarum* have been found to be amylolytic, that is, they break down starch in pearl millet slurries[30]; further studies on these *emahewu* strains is needed.

Carbohydrate profile of LAB

Almost all *Lactobacillus* spp. were able to utilise mainly ribose, galactose, D-glucose, D-fructose, D-mannose, N-acetylglucosamide, amygdaline, arbutine, esculine, salicine, cellobiose, maltose, lactose, melibiose, saccharose and trehalose (Table 5). *Lactococcus* ssp. metabolised carbon source ribose, D-xylose, galactose, D-glucose, D-fructose, D-mannose, N-acetylglucosamide, amygdaline, arbutine, esculine, salicine, cellobiose, maltose, lactose and trehalose. Most *Leuconostoc mesenteroides* ssp. utilised substrate ribose, galactose, D-glucose, D-fructose, D-mannose, cellobiose, maltose, lactose, melibiose, saccharose and trehalose. In general, LAB in the current study fermented other carbohydrates such as L-arabinose, rhamnose, mannitol, sorbitol, α-methyl-D-mannoside, α-methyl-D-glucoside, melizitose, D-raffinose, starch, B gentiobiose, D-turanose, D-tagatose, D-arabitol, gluconate, 2-ketogluconate and 5-ketogluconate (Table 5).

The metabolism of carbohydrates by LAB is a similar observation to that made by Negussie et al.[31] who observed that LAB isolated from Ethiopian naturally fermented buttermilk were able to utilise carbohydrates such as galactose, maltose, glucose, fructose, mannose and lactose. The results of the current study are supported by those of Ashmaig et al.[32] who observed that LAB isolated from traditional Sudanese fermented camel's milk were able to ferment some carbohydrates. The common substrates that were fermented include carbohydrates such as lactose, fructose, galactose, trehalose, melibiose, mannose, xylitol and sorbose.

Conclusions

Emasi and *emahewu* are fermented foods of Swaziland. *Leuconostoc mesenteroides, Lb. plantarum* and *Lb. lactis* subsp. *lactis* were typical strains in *emasi*, while the *Lactobacillus* genus, especially *Lb. plantarum,* was typical in *emahewu*. Other LAB strains commonly found in *emahewu* were *Lb. acidophilus, Leuconostoc lactis, Lactococcus lactis* and *Weissella confusa*. Nevertheless, there is still a need to broaden the LAB isolates to be identified by sequencing 16S rDNA, carefully considering the choice of primers. *Emasi* and *emahewu* enhance dietary diversity and are popular foods for both children and adults in Eswatini. Studies are therefore needed to develop starter cultures for easier production of these foods.

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Authors' contributions

P.S. performed all the methodology, including data collection, sample analysis and data analysis as part of PhD studies; worked on the original concept of the manuscript write-up and revisions of the manuscript. M.S. and T.H.G. provided supervision and contributed significantly to the final version of the manuscript.

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