



Microbiome of madila - a southern-african fermented milk product

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Abstract

This study was embarked upon to obtain strains of lactic acid bacteria from a traditional fermented milk product - Madila. The milk was obtained locally from three different locations. Samples of the Madila were taken at different intervals during the course of the preparation and analysed. Characterization of seven isolates through morphological, physiological, biochemical and carbohydrate fermentation test have been reported. Six species of lactic acid bacteria were identified and they include *Lactobacillus acidophilus*, *Lactobacillus delbrueckii*, *Lactobacillus plantarum*, *Lactococcus lactis*, *Lactobacillus fermentum* and *Lactobacillus brevis*; other non lactic acid bacteria identified were *Escherichia coli*, *Bacillus cereus* and *Staphylococcus aureus*. The counts ranged from 7.15×10^2 to 2.57×10^5 for non lactic acid bacteria and 2.25×10^7 to 8.63×10^9 for lactic acid bacteria. It was observed that the counts of non lactic acid bacteria reduced progressively as the number of lactic acid bacteria increased.

Keywords: Madila, Botswana, Milk, Traditional, Fermentation.

1 Introduction

Madila is a popular fermented food product of Botswana and other Southern African countries. Raising livestock (cattle and goats) has long been the most important agricultural activity in the country, which activity accounts for 2.4% of the country's GDP. The cattle reared in Botswana are used mainly for meat and milk production. The meat is one of the country's major exports while the milk is used for local consumption [9]. The milk is either sold fresh (pasteurised or unpasteurised) or sold as a sour milk product referred to as *Madila*.

Madila is traditionally brewed by cattle herders from cattle ranches, locally referred to as *meraka* or cattle posts. The making of *madila* is a slow process that requires patience. It can also be frustrating as traditional Tswana cattle do not produce a lot of milk. One has to milk about six cows to obtain a bucket full (20litres) of milk. The milk is then strained using a clean white cloth to remove exogenous particles. The clean milk is then kept in a clean covered enamel pail and allowed to ferment spontaneously for one day. The fermented milk is then poured into a cotton sack (*lekuka*) which is then hung on a tree. On fermentation, the milk separates into curd and whey. The *lekuka* allows the whey (*tloa*) to drain off from the fermenting milk, thereby resulting in the formation of a thick sour cream regarded as a delicacy in many Twana societies. Afterwards, variations in flavour may be made by the addition of various fruits including oranges, mangoes or *marula* – a Southern African fruit. The *madila* is dispensed into containers for sale.

Madila is consumed by people of all walks of life regardless of ethnicity and socio-economic class, making it the one of the commonest meals amongst the rich and the poor. It is consumed indigenously in various ways. It may be taken as dessert or enjoyed as a beverage; it could also be served on the rocks. It may be added to pap to make a dish called *ting* which is mostly consumed as breakfast.

Lactic acid bacteria (LAB) were first described as milk-souring organisms, due to the sour milk that arose from their production of lactic acid. They are a relatively diverse group of bacteria, but related by a number of typical metabolic and physiological features. Because they obtain energy only from metabolism of sugars, LAB are restricted to environments in which sugars are present. They have limited biosynthetic ability having evolved in environments that are rich in amino acids, vitamins, purines and pyrimidines; so they must be cultivated in complex media that fulfill all their nutritional requirements. LAB are naturally occurring in milk, milk products and decaying plant matter. In the human body they are present in the oral cavity, the intestinal tract and other mucosal linings where they play beneficial roles. Their health claims range from rather vague activities such as regulation of

bowel activity and increasing of wellbeing, to more specific activities such as exerting antagonistic effects on the gastro intestinal pathogens like *Clostridium defficille*, *Campylobacter jejunii*, *Helicobacter pylori* and rotaviruses, [7]; neutralizing food mutagens produced in the colon [6]; inhibition of the oxidation of human low density lipoprotein (LDL) [16]; removal of aflatoxin (Shahin, 2007); binding of dietary mutagen [17] and improving immunity [1]. LAB are among the most important groups of microbes used in the food industry. Since LAB occur naturally in many food systems and have been a part of the human diet for centuries, they can be regarded as safe organisms to consume. They have a great potential for extended use in bio-preservation of both food and feed products.

Fermented foods are those foods, whose production is based on the activity of microorganisms. Fermentation has been said to serve five main purposes [15]

1. Enrichment of the diet through development of a diversity of flavors, aromas, and textures in food substrates;
2. Preservation of substantial amounts of food through lactic acid, alcohol, acetic acid and alkaline fermentations;
3. Biological enrichment of food substrates with protein, essential amino acids, essential fatty acids, and vitamins;
4. Elimination of antinutrients and
5. A decrease in cooking time and fuel requirement

Fermentation is generally regarded as one of the most economical methods of producing and preserving foods for human consumption. The fermented foods have been grouped in various ways including:

- Fermented starchy roots e.g. Garri, Fufu Lafun, Tapioca.
- Fermented cereals e.g. Ogiri, Uji, Masa, ogi
- Fermented legumes and oil seeds e.g. Iru (dawadawa) Ugba.[11]

A shortfall of this classification is that it did not include fermented foods from other substrates including dairy products and vegetables. A more comprehensive grouping is the United Nations' Foods and Agriculture Organisation's (FAO) grouping which has the following classifications:

- Bean based foods
- Grain based e.g. *Ogi, injera, maheu, uji, kvass, ikii* ;
- Vegetable based e.g. *Kimchi, sauerkraut, mixed pickle* .;
- Fruit based e.g. *Asinan, marula, visinata, perry* .;
- Honey based e.g. *Mead and metheglin*;
- Dairy based e.g. *Wara, Smetana, madila, mursik, nono* ;
- Fish based e.g. *Chorizo, salami, pepperoni* etc. and
- Tea based e.g. *Kombucha*

The arts of fermentation which have been developed for foods have achieved the following benefits: Larger keeping quality, Variety in flavour and making inedible foods edible. In addition, the fermented foods have enhanced nutritional values and decreased toxicity .

Lactic acid fermentation is used throughout the world to produce speciality foods, The following list outlines a number of these, their substrates with their countries or regions:

- Western world: yogurt, sourdough breads, sauerkraut, cucumber pickles and olives
- Middle East: pickled vegetables
- Korea: *kimchi* (fermented mixture of Chinese cabbage, radishes, red pepper, garlic and ginger)
- Russia: *kefir* (creamy drink with a low alcohol content made from fermented cow's milk)
- Egypt: *laban rayab* and *laban zeer* (fermented milk), *kishk* (fermented cereal and milk mixture)
- Nigeria: *gari* and *fufu* (fermented cassava), *Ogi* (fermented maize cereal), *Fura* (fermented milk), *Wara* (fermented milk)
- Uganda: *waragi* (alcoholic banana drink)
- Kenya: *Uji* (fermented maize porridge), *mursik* (fermented milk with ash)
- Zambia: *Maheu* (Fermented corn drink)
- South Africa : *magou* (fermented maize porridge)
- Nepal: *Gundruk* (fermented and dried vegetable product)
- Thailand : *nham* (fermented fresh pork)
- India: *Kanji* (traditional fermented carrot drink)
- Sudan: *Kawal* (Fermented drink from leaves of Sickle pod plant)

- Philippines : *balao balao* (fermented rice and shrimp mixture)
- Botswana: *Madila* (traditionally fermented milk product)[12, 5]

The presence of lactic acid, produced during the lactic acid fermentation is responsible for the sour taste and for the improved microbiological stability and safety of the food.

Nowadays, consumers demand large variations in flavor of dairy products besides consistency in overall quality. Therefore, the dairy industry is keen on exploring new possibilities for expanding the diversity of its product range. Accordingly, there is great interest in searching for potential starter organisms from the pool of wild LAB recoverable from raw milk or raw milk products [19]. The need for new products requires the use of new microbial strains with novel properties. This has led to a request for novel strains for the innovation and diversification of dairy products. These novel strains can be achieved either by exploring the biodiversity within nature from various ecological niches or by genetic modification of known production strains. The wild lactic acid bacterial flora represents a natural reservoir for cultures that are not exposed to any industrial selection. Some interesting characteristics of these microorganism are their ability to produce acid at a high and predictable rate, proteolytic activity, synthesize EPS and produce antimicrobial compounds which is essential in fermented milk starter strains. Therefore, the isolation and identification of new strains from *Madila* is necessary in order to bring novel strains to the industry.

2 Materials and methods

Sample Collection -: Fresh cow milk was collected early from three different locations. The milk was collected in sterile 1,000ml wide mouth bottles (Nalgene, Germany) with caps and transported at 5 – 10°C in an ice box to the Laboratory. The samples were labelled A, B and C respectively.

Fermentation -: The milk was subjected to spontaneous fermentation in a capped container at 30°C. 48 hours after the initial fermentation, the milk was aseptically poured into a clean cotton drain bag and hung, so as to separate the curd from the whey. Fresh cow milk was added in the ratio of 4:1 (Fresh milk to fermented milk). The mixture was then allowed to ferment for 24 hours before addition of fresh milk again. Further fermentation time of 24 hours was allowed after which the *Madila* was ready.

Microbiological Analysis -: 10ml of each *Madila* sample was aseptically added to 90 ml of sterile peptone water (Fisher BioReagents, UK) and mixed thoroughly. A seven fold serial dilution of the samples was prepared by transferring 1ml of the sample into 9ml of peptone water. 1 ml aliquots of appropriate dilution were inoculated in triplicate on the MRS agar (Oxoid, UK) and Nutrient agar (Oxoid, UK) and incubated anaerobically in an Anaerobic Gas-Pack system (Biomerieux, France) for 48 hours at 37°C for isolation of lactobacilli.

RESULTS AND DISCUSSION

In this study, serial dilutions of the samples were done for three consecutive days of fermentation. 1ml of appropriate dilutions were inoculated on Nutrient and MRS Agars for the enumeration of heterotrophic organisms and Lactic acid bacteria present in the *madila* respectively. The mean plate counts of these organisms are represented in Table 1 in cfu/ml. The hydrogen ion concentration and Titratable acidity of the samples was estimated, Figs. 1 and 2 shows the results of these parameters, highlighting the comparison. Tests to identify the isolates were carried out as well and the organisms found are represented in Table 2, while Table 3 shows the distribution of the organisms isolated in the samples.

Table 1: Total Heterotrophic and Lactic acid bacteria count(cfu/ml)

Days	A		B		C	
	Non LAB	LAB	Non LAB	LAB	Non LAB	LAB
1	4.37 x 10 ⁷	2.25 x 10 ⁷	4.53 x 10 ⁷	3.37 x 10 ⁷	6.34 x 10 ⁷	5.21 x 10 ⁷
2	7.82 x 10 ⁸	3.46 x 10 ⁸	8.32 x 10 ⁸	3.92 x 10 ⁸	7.21 x 10 ⁸	6.37 x 10 ⁸
3	6.75 x 10 ⁹	5.64 x 10 ⁹	7.79 x 10 ⁹	4.41 x 10 ⁹	8.35 x 10 ⁹	7.24 x 10 ⁹

Table 1 shows the total viable counts of Lactic acid bacteria and non Lactic acid bacteria from the *madila* samples. From the results, it can be deduced that as the counts of the LAB increase, the non LAB counts reduce.

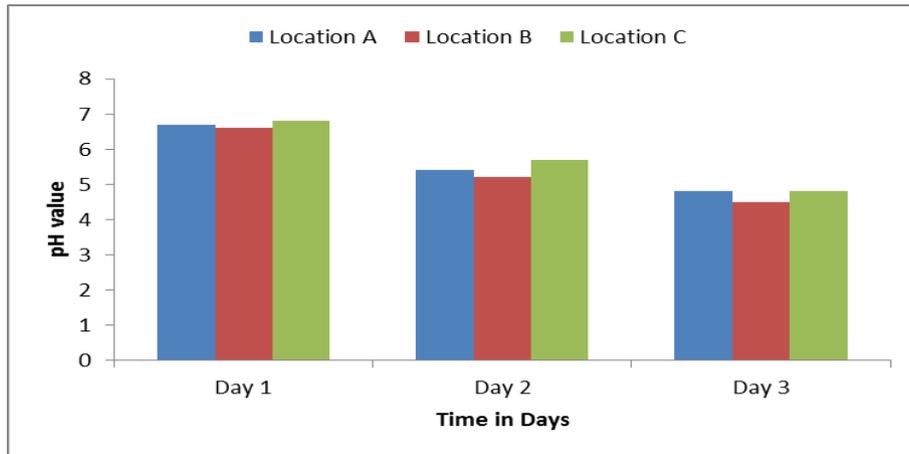


Figure 1: pH values of the madila samples

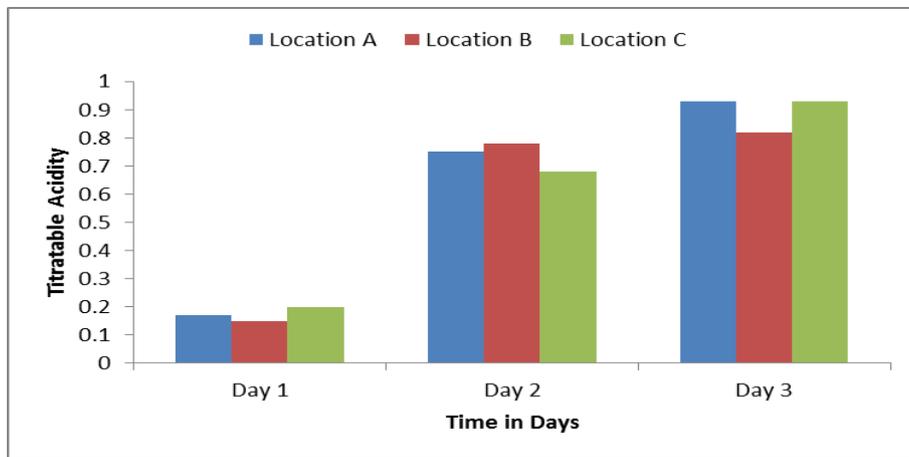


Figure 2: Titratable acidity values of the madila samples

From Figures 1 and 2 as the pH decreased , the TA increased.

Table 2: Organisms isolated from the madila samples.

Days	Isolated Organisms
1	<i>L. plantarum</i> , <i>L. acidophilus</i> , <i>S. aureus</i> , <i>B. cereus</i> , <i>L. delbrueckii</i> , <i>L. brevis</i> , <i>E. coli</i>
2	<i>L. plantarum</i> , <i>L. acidophilus</i> , <i>L. delbrueckii</i> , <i>S. aureus</i> , <i>B. cereus</i> , <i>L. fermentum</i> , <i>L. brevis</i> , <i>E. coli</i>
3	<i>L. plantarum</i> , <i>L. acidophilus</i> , <i>L. delbrueckii</i> , <i>L. lactis</i> , <i>L. brevis</i> , <i>S. aureus</i> , <i>B. cereus</i> , <i>E. coli</i>

Six Lactic acid bacteria species were isolated and identified from the samples and three species of non-lactic acid bacteria

Table 3: Occurrence of LAB in the different samples

Isolates	A			B			C		
	Day 1	Day 2	Day 3	Day 1	Day 2	Day 3	Day 1	Day 2	Day 3
<i>L. plantarum</i>	+	+	+	+	+	+	+	+	+
<i>L. acidophilus</i>	-	+	+	+	+	+	+	+	+
<i>L. delbrueckii</i>	+	+	+	+	+	+	+	+	+
<i>L. lactis</i>	-	-	+	-	+	+	-	+	+
<i>L. fermentum</i>	-	+	+	-	-	-	-	+	+
<i>L. brevis</i>	+	+	+	-	+	+	-	-	-
<i>S. aureus</i>	+	+	+	+	+	+	+	+	+
<i>B. cereus</i>	-	-	-	+	+	+	+	+	+
<i>E. coli</i>	+	+	+	-	-	-	+	+	+

Key: + = Present - = Absent

MRS medium selects for Lactic acid bacteria, so organisms which grew on MRS were taken as Lactic acid bacteria. This was confirmed by gram staining and catalase tests, isolates determined to be Lactic acid bacteria were subjected to further biochemical tests.

The counts of the microbes (Table 1), ranged from 7.15×10^2 to 2.57×10^5 for non-LAB and 2.25×10^7 to 8.63×10^9 for LAB. It was observed that the counts of non-LAB reduced progressively as the number of LAB increased. This presupposes that the LAB inhibit the growth of spoilage organisms in the milk. This finding is in consonance with the findings of so many authors, who in their studies stated that despite the large number of different bacterial species, the lactic acid bacteria (LAB) group dominated the microbiota.

The results of the various biochemical tests and cultural and morphological characteristics of the isolates when compared with Bergey's Manual of Systematic Bacteriology identified the isolates to be as follows:

- Lactic acid bacteria: *Lactobacillus plantarum*, *Lactobacillus acidophilus*, *Lactococcus lactis*, *Lactobacillus fermentum*, *Lactobacillus brevis* and *Lactobacillus delbrueckii*.
- Non-Lactic acid bacteria: *Bacillus cereus*, *Escherichia coli* and *Staphylococcus aureus*.

It was also observed that in all the instances, the LAB were dominating the microbial population of the milk. This is important because of the key role they play in the fermentation process and their production of lactic acid and other antimicrobial substances. They may therefore be important as starter organisms in *madila* making in standardised production.

The occurrence of LAB in this fermented milk product helps in its keeping quality with the aid of the acid produced and other products of the metabolism of these bacteria [11]. Moreover, cow milk which is used for the preparation of *madila* favours the growth of these species.

The results obtained from microbial analysis of the *madila* show that the food product was contaminated with microorganisms of public health concern. The likelihood of culture contamination was to a large extent ruled out due to the high aseptic measures taken in the course of the laboratory work. The occurrence of these organisms may therefore be a consequence of poor levels of hygiene during milking of the cows. A poorly washed udder and teats of a cow; and unwashed hands readily introduce this sort of organisms to milk at the point of milking. The presence of *E. coli* indicates possible faecal contamination [18]. The faecal oral route transmission plays an important role in food-borne diseases therefore high hygiene measures should be taken in the handling of milk. The detection of *Bacillus cereus* is also of public health significance due to its pathogenicity and resistance to desiccation by means of sporulation. *Staphylococcus aureus* is also of public health importance due to its ability to cause many infections especially food intoxication [4]. These organisms were also isolated from fermented milk products by [18] and [10].

Study of technological properties of LAB strains isolated from *madila* is an important criterion for selection of starter cultures to be used in the standardized production of *madila* and yoghurt. According to Figure 2, it was observed that pH was lowest on the third day and that as the pH lowered, the titratable acidity increased. These results concurred with those reported by other authors [2,3 and 8].

LAB are also able to survive in highly acidic environments of pH 4 to 5 or even lower; due to this, they are responsible for the latter fermentation stages in *madila* production when the pH is very low. This further shows that the low pH favours the growth of LAB as observed by [14]

The abundance / predominance of LAB in *madila* through the stages of production shows their importance in the fermentation process. Consequently, there is a high potential for starter organisms for use in standardised *madila* production, and yoghurt production. It has also been observed by [9] that if one acquires a desired taste / blend of *madila*, an amount of this may be used as a starter for the production of other batches with the same qualities if the same conditions are maintained.

Standardised *madila* production can also improve economic conditions of cattle herders as the duration of the process is greatly reduced when ready made *madila* is used as a starter.

On another note, microbiological standards have not been defined for locally produced foods in Nigeria and most African countries. Botswana is an exception due to the regulations like the Botswana Bureau of Standards. Unfortunately this is not entirely seamless as this regulatory body does not take the trouble to visit cattle posts (*meraka*) where *madila* is produced for sale to the general population. Due to the fact that *madila* is consumed by people of all walks of life, and is a ready to eat food consumed without further processing, it is recommended that regulatory bodies should be set up and be strictly involved in maintaining the microbiological quality of *madila*. This is also applicable to other locally produced foods in Nigeria and elsewhere in Africa where such bodies do not exist or are passive in their duties.

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