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MICROBIOLOGICAL AND ACIDITY CHANGES DURING THE TRADITIONAL PRODUCTION OF *KIRARIO*: AN INDIGENOUS KENYAN FERMENTED PORRIDGE PRODUCED FROM GREEN MAIZE AND MILLET

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ABSTRACT

Using a previously pre-tested structured questionnaire, the traditional processing method for *kirario* (a traditional fermented porridge of the Merus in Kenya) was studied and documented. The biochemical and microbial profile changes during fermentation of *kirario*, both by the traditional method and in the laboratory were monitored for 48 hours. Samples of *kirario* from ten localities in the study region were analyzed. Samples of the final products from the traditional method were analyzed for total viable counts (TVC), lactic acid bacteria (LAB), lactococci, yeasts and moulds and coliforms, while the laboratory samples were taken at six (6)-hour intervals and analyzed for TVC, LAB, lactococci, and yeasts and moulds for 48 hours. The traditional product showed average TVC, LAB, lactococci, yeasts and moulds of 9.30, 9.63, 8.62, and 4.83 $\log_{10}$ cfu/ml, respectively. Coliform counts were detected in only two of the samples at <1 $\log_{10}$ cfu/ml. Analysis of the laboratory samples showed similar results. This showed that the production of *kirario* was reproducible and could be simulated in an industrial set-up for commercialization. In both the laboratory and traditional samples, the microbial counts were monitored at 6 hourly intervals for 48 hours. The initial pH of 6.4 dropped to 3.3 at the end of the fermentation, while the total titratable acidity increased to 3.15% from an initial value of 1.04%. The TVC, LAB, lactococci, yeasts and molds increased from initial counts of 8.20, 8.18, 7.20 and 5.86 $\log_{10}$ cfu/ml to 9.64, 9.55, 5.38 and 0.70 $\log_{10}$ cfu/ml, respectively at the end of the 48-hour fermentation. The coliform counts were low or not detected at all in majority of the samples. These results indicated high degree of hygiene in traditional processing of *kirario* as indicated by the very low or undetectable coliforms. This was also attributed to the effect of inhibition of growth of coliforms during fermentation. The results were also substantiated by unusually high levels of acid in both the traditional and laboratory products, corresponding to pH 3.0 to 3.5, which indicated high activity of the lactic acid bacteria in *kirario*.

Key words: Fermentation, *Kirario*, Microbial counts, Acidity

INTRODUCTION

Kirario is an indigenous non-alcoholic lactic-fermented porridge that is widely prepared and consumed in Kenya. Traditionally, it is prepared by spontaneous lactic fermentation, at ambient temperature. The porridge is produced from mixtures of green maize and millet/sorghum in different combinations and proportions. Kenyan *kirario* is one of the most popular cereal-based traditional fermented beverages that is increasingly being consumed by many communities in the country. It is consumed as a low cost meal substitute and therefore, provides a cheap food alternative for the impoverished rural communities. Currently, it has become a refreshing drink for all segments of the population and is also a choice drink in celebrations in most cultural festivals such as dowry payment festivities, customary weddings, circumcision and initiation ceremonies among other festivals.

In many African countries, cereal-based traditional fermentation products are consumed both as beverages and as foods [1, 2]. Scientific studies of fermentation products in Kenya, standardization and promoting utilization of their processes, and developing local food processing capabilities can help to alleviate food insecurity if accorded research priorities [3].

Traditional food fermentation processes of alcoholic and non-alcoholic beverages in Africa have been extensively investigated and documented [1, 2, 4]. However, little information is available on traditional fermentation and microbial profile of *kirario*. *Uji*, is a Kenyan lactic acid fermented thin porridge that has been studied and documented extensively [4, 5]. Approximately, 10-12% of maize in Kenya is consumed in the form of *uji* and other fermented porridges [5].

Quin, after a life-long study of Africans, concluded that recognition and encouragement of the traditional foods and feeding habits of African tribes, could contribute to alleviation of and perhaps even solving the problem of malnutrition and disease among the people [6]. Steinkraus, a renowned scientist, reported that if the world wanted to improve the nutritional status of its poor and hungry, as well as modernize the traditional fermented foods, it ought to look at the processes involved in these indigenous foods in order to achieve its aims [7]. Many of the recent studies on indigenous processes of food fermentation have led to or are aimed at upgrading or modernizing the methods involved in their preparations [8].

The traditional fermentation systems of products like *kirario* can lead to microbial evolution of strains with unique technological and other beneficial properties. Fermented foods are consumed in every country of the world and, there is growing scientific evidence that many fermented foods are good for health. Fermented foods that improve or change the intestinal microflora are of particular interest because of increased knowledge of the beneficial role of intestinal microflora in health and disease prevention.

The information in this paper consists of the results of a study on the traditional processing of *kirario*. Microbiological and acidity changes that occur during fermentation of *kirario* were investigated. The microbial count, pH and titratable acidity of the traditionally processed *kirario* were measured, and the same monitored during the laboratory processing of *kirario* following the traditional methods.

MATERIALS AND METHODS

Collection of samples

The traditional fermented porridge samples were obtained from four villages in Meru North, two in Tigania West (Uringu and Mbeu locations) and two in Tigania East (Athwana and Kimirii locations). The raw materials [green maize and millet] used in preparation of the porridge were bought at a local market in the study region and divided into two batches. One of the batches was used for traditional preparation, while the other batch was used for the laboratory study. For the traditional processing, the materials were given to ten randomly selected local women experienced in preparation of *kirario*. They were requested to prepare the product using the traditional method. Households with women experienced in the art of *kirario* preparation were selected and the numbers randomized by the calculator random method. The samples of the fermented porridge from the women were collected in sterile sample containers, cooled to 4-6^{0}C and transported to the laboratory in a cool box containing freeze packs to maintain them at the temperature between collection and analysis. The samples were analyzed within 8 hours. The raw materials used for the laboratory-simulated production were also maintained at 4-6^{0}C prior to the microbiological analysis.

Traditional production of *kirario*

A survey was carried out among women in the study region [Meru North] to obtain the specific and relevant information on the traditional preparation processes of *kirario*. Specific information with regard to the type of raw materials used, fermentation equipment, fermentation time and conditions were acquired with the help of questionnaires and personal observation. The raw materials were supplied to a hundred randomly selected local women experienced in preparing *kirario* in the study regions. The experienced women were selected in randomized block design with the different regions representing the blocks. They were requested to prepare the product in the traditional way. The proportions of the ingredients used by the women were calculated using standard measures and used for replication of the process in the laboratory.

These ingredients were then processed and a standardized traditional process by the women, for *kirario* developed and presented in a flow diagram. This process was simulated in the laboratory and data on microbial counts pH and total titratable acidity taken within the course of fermentation.

Analytical methods

During fermentation of *kirario* in the laboratory, samples were taken at 4-hour intervals and analyzed for pH, titratable acidity (TA), Total Viable Counts (TVC), Lactic acid bacteria (LAB – *lactococci* and *lactobacillus*), yeasts and moulds counts and total coliforms.

Determination of pH and titratable acidity

The pH was measured using a pH meter (PYE Unicam Model 290 Mk 2). The pH meter was calibrated using buffers of pH 4 and 7. Titratable acidity was determined according to AOAC methods [9]. A sample of 10 ml was titrated with 0.1 N NaOH using phenolphthalein as an indicator. The titratable acidity was calculated as percent lactic acid.

Microbiological analysis

The methods and procedures used were as described by Harrigan and McCance [10], FDA, bacteriological analytical manuals [11], and ALPHA, compendium of methods for microbiological examination of foods [12]. Total viable counts (TVC) were determined using the pour plate method of Harrigan and McCance [10]. Decimal dilutions were made with 0.1 percent bacteriological peptone. One millilitre of the 10^{-8} to 10^{-14} dilutions was used to prepare pour plates using plate count agar (PCA). The plates were incubated at 30 ^{o}C for 48 hrs. The colonies on plates with between 30-300 colonies were counted. LAB counts were determined using MRS (deMan Rogosa Sharpe) and M17 agar incubated anaerobically at 30 ^{0}C for 3 days. The counts of yeasts and moulds were determined using potato dextrose agar (PDA), acidified with 10% tartaric acid to pH 3.5 by incubating at 30^{0}C for 3-5 days. Coliform numbers were determined using violet red bile glucose agar incubated at 37^{0}C for 48 hrs.

Statistical analysis of the data

The data collected was entered in MS Access and MS Excel solver as data base for optimization. The data was analyzed using Genstat 5 Release 3.2 statistical package and Statistical Package for the Social Sciences (SPSS 12.0 for Windows). . The data validity and interpretation was monitored to ensure quality control of the results.

RESULTS

Traditional production of kirario in Meru North District

Results of the survey showed the procedure of the traditional preparation of kirario to be according to the flow diagram presented in Figure 1. The raw materials, mainly green maize in admixture with dry millet or sorghum, is suspended in a measured quantity of water and extracted by wet milling on a stone quern. The quern or grinding stone is normally made from a hard stone like granite. The operator rests on her knees or bends over at a slightly elevated end of the base-plate on which she places a handful of grains. She puts both hands on the roller stone on top of the grain. Milling is achieved by moving the roller repeatedly forwards and backwards. The milled grains, now a slurry, fall into a receiver at the distal end of the quern. Figure 2

shows a picture of a traditional grinding stone or stone quern. The slurry is then poured into a container, which is used for fermentation. Traditionally, the slurry was fermented in either earthen or clay pots, but today, plastic buckets and jerricans are used. The slurry is covered with a lid or green banana leaves and left to ferment for about two days at ambient temperatures. After fermentation the slurry, now sweet and sour, has the water adjusted as desired if necessary, and is then placed in a pan and cooked into porridge. The porridge is stirred continuously during cooking, which takes about 10 -15 minutes. The porridge is normally taken warm using either a calabash directly, or using a spoon from a plate, calabash or cup. The mixing of *kirario* during cooking is achieved by employing a special wooden hand blend or traditionally called *kibiro*. *Kibiro* has a T-shaped blade at the end of a long handle. The *kirario* cooking process requires a great deal of effort and skill in order to produce a product with no defects. The final product usually has high viscosity, smooth texture and consistency, and a milk-like flavor. The name *kirario* denotes the sour nature of the product resulting from overnight holding. The product can be stored for one week or longer depending on the hygiene and storage conditions. Traditionally, kirario preparation was the domain of the older experienced women, but nowadays even young unmarried girls do it, assisted by the older and more experienced women. Preparation of kirario for social occasions and gatherings is done by the more experienced women to ensure that it comes out right.

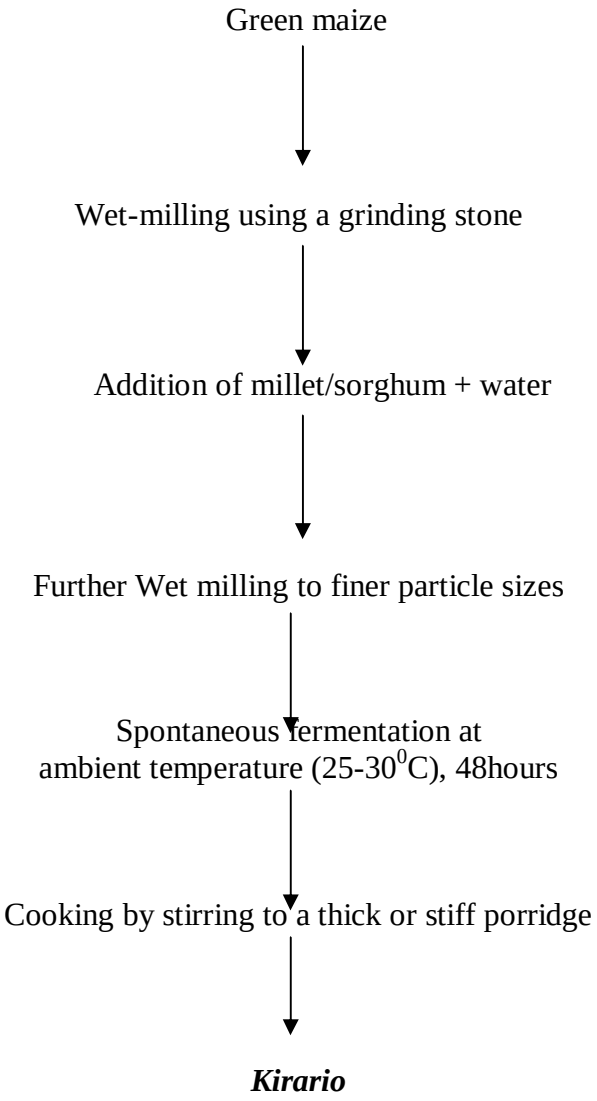


Figure 1: Flow diagram of traditional preparation of *kirario*

Figure 2: Picture of a traditional stone quern or grinding stone, showing the base stone on which sits the handle

Microbial content of kirario

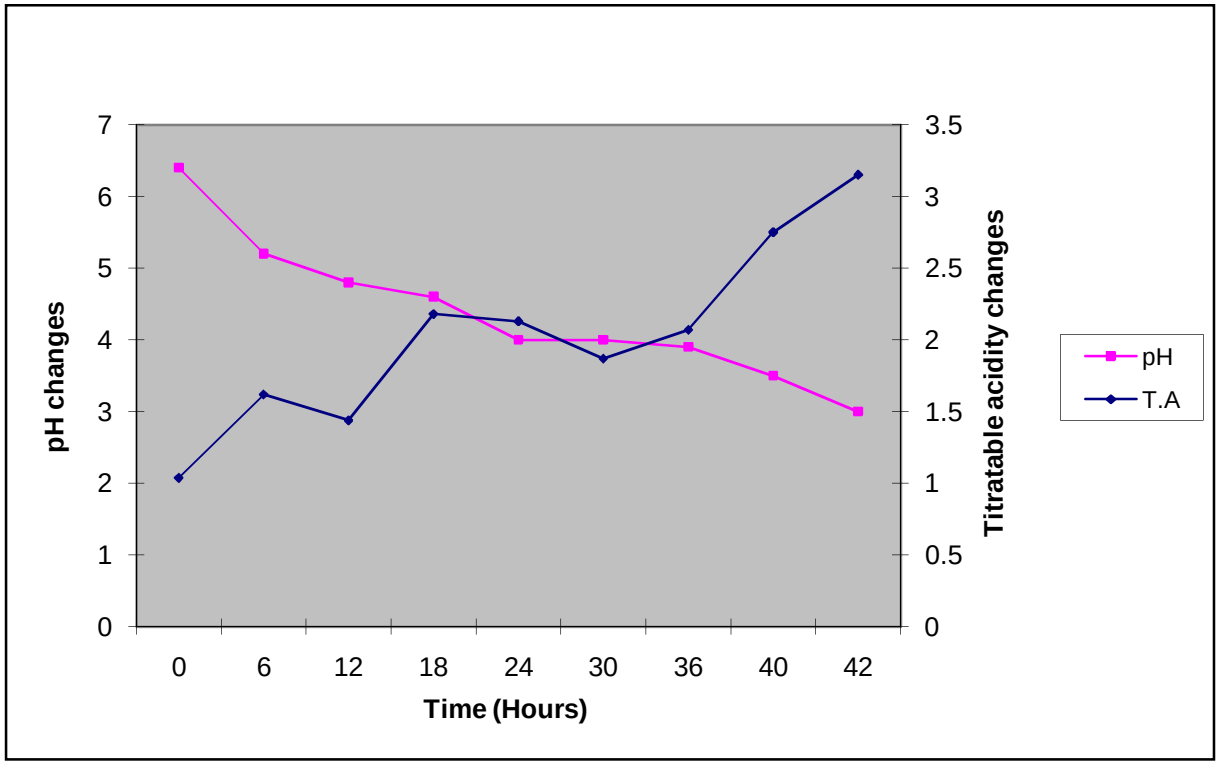
The total viable microorganisms, lactic acid bacteria, yeasts and moulds, and total coliforms were enumerated in the traditional samples and laboratory –produced *kirario*. Table 1 shows the microbial counts in *kirario*. High total viable counts [>9.51 log cfu/ml] were observed, with the lactic acid bacteria being the predominant microbes. There was no significant difference [$p<0.05$] between total viable counts, LAB, lactic-acid cocci, and yeasts and moulds counts between the traditional and laboratory produced products. The results showed that the microbial contents in *kirario* produced in the laboratory, were comparable to those of the traditional products. No coliforms were detected in both the traditional and laboratory-simulated *kirario*.

Biochemical and microbiological changes during kirario fermentation

The microbial profile, pH and titratable acidity changes were monitored during the spontaneous fermentation of *kirario* at a 6-hour interval for 48 hours. The changes in pH and titratable acidity during fermentation of *kirario* are shown in Figure 3. The changes in the microbial counts are shown in Figure 4. The titratable acidity increased from a value of 1.04 to 3.15 during fermentation of *kirario*. The pH showed a rapid decline from an initial value of 6.4 to 4.0 within 24-hour fermentation and then a steady decline from a pH of 4.0 to 3.0 in the remaining 24 hours of fermentation.

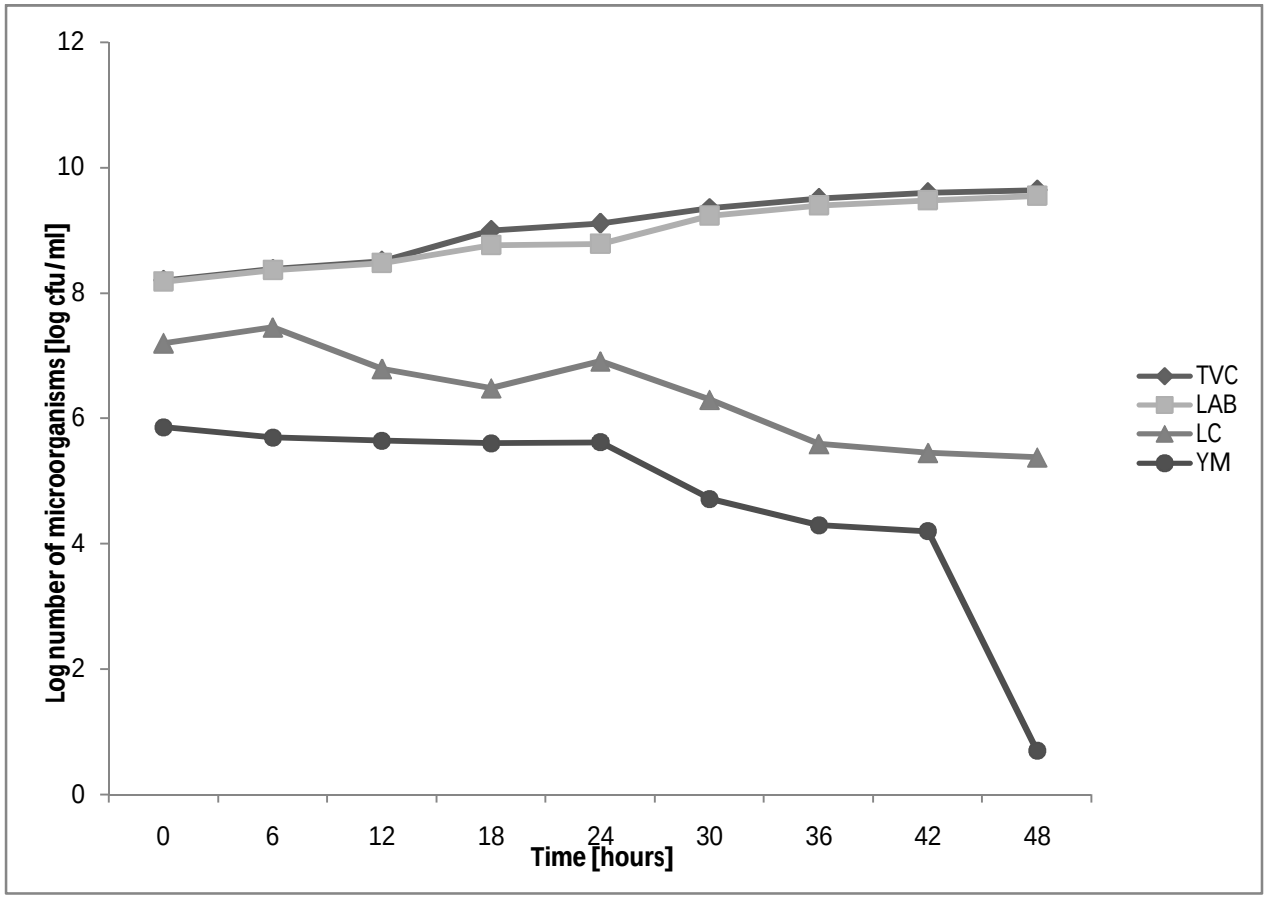
The total viable counts were high and continued to increase steadily throughout the fermentation of *kirario* with the LAB being the predominant microorganism. The lactococci (Lactic *Streptococci*) showed a fairly constant trend during fermentation with values ranging from 5 - 8 $\log_{10}$ CFU/ml. The yeasts and moulds counts were

relatively constant during the fermentation with a rapid decline of 3-log cycles towards the end of the fermentation (42nd hour). This decrease was up to about a value of <1 $\log_{10}$ cfu/ml. There was a steady increase in total viable counts, LAB, and lactococci within the first 24 hours by a value of 1 log cycle.



Key: T.A – Titratable acidity

Figure 3: Changes in pH and titratable acidity during fermentation of *kirario*



Key:
TVC – Total viable counts
LAB – Lactic acid bacteria
LC – Lactic acid cocci
YM – Yeasts and mould

Figure 4: Changes in the microbial profile during fermentation of *kirario*

DISCUSSION

Traditional production of *kirario*

The fermentation of *kirario* is a spontaneous process, which has its own unique features. The raw materials used in the preparation are unique since green maize is used as opposed to other documented lactic fermented beverages where dry cereals are used. This unique feature is likely to influence the type of lactic acid bacteria involved in the fermentation of *kirario*. Several researchers have indicated that the microorganisms involved in the natural fermentation of cereals are essentially the microflora of the raw materials and equipment [13, 14, 15]. The resulting starchy material from wet-grinding have characteristic sour taste and aroma which has become an essential and desired element in the product that is subsequently cooked before consumption [16]. Many authors have reviewed several African fermented

products for example porridges and dumplings, which are prepared by soaking and wet-milling processes [16, 17]. Examples of local cereal-based products that have been scientifically studied are *ogi* and *agidi* of Nigeria, *koko, akasa*, and *kenkey* of Ghana, *uji* of East Africa, and *mahewu* of southern Africa. Many of these products are also made commercially for local markets [18]. The study of these fermented products is of significance in the fight against hunger and malnutrition in the developing countries. However, commercialization and utilization of these products cannot be attained until the analysis of the traditional production and process is documented.

Microbial content of *kirario*

A high microbial load was observed in *kirario* with very high initial counts of between 6-8 log $_{10}$ CFU/ml. This initial count could be attributed to the raw material (green maize) and unintentional back slopping. Studies have shown that maturing green maize kernel contains carbohydrates other than starch such as simple sugars. The total sugars found in the green maize range between 1 and 3 percent, with sucrose as the major component [19]. Higher levels of monosaccharides, disaccharides and trisaccharides are present in the mature green maize [20]. These relatively high levels of reducing sugars and sucrose are possibly the reason why there were high counts of LAB during the fermentation of *Kirario* and the low final pH achieved. The carbon sugars are readily fermentable substrates by the lactic acid bacteria during catabolic processes in fermentation of cereals. The initial counts increased to high levels of 9.3 - 9.5 log CFU/ml during fermentation. The high microbial load of 9.3 - 9.5 log CFU/ml achieved in *kirario* is similar to microbial counts reported in the Zimbabwean *mangisi* [21]. It has also been reported that in fully fermented slurry of *ogi* (a Nigerian lactic fermented cereal-based porridge), a microbial load of more than 10^9 CFU/g, very similar to the results showed for *kirario,* were achieved [13]. The low pH of 3.0 - 4.5 in *kirario* is also in agreement with other cereal based fermented beverages with pH values in the range of 3.0 - 4.8 like. Tanzanian *togwa* [1], Ethiopian *tella* [22], Sudanese *merissa* [8], Zimbabwean m*angisi* [21] and Egyptian *bouza* [23]. The low pH in *Kirario* can be linked to the high lactic acid bacteria (LAB) counts observed in the product with an average count of 9.50 $\log_{10}$ CFU/ml. The count of lactococci was high initially with an average count of 7.96 log CFU/ml and increased slowly as the fermentation progressed. In previous studies, it has been found that the lactococci group of LAB decreases during fermentation as a result of the decrease in pH [24]. It also reported that the lactococci group, especially the *Leuconostoc,* is less resistant to low pH than the *Lactobacillus.*

Biochemical and microbiological changes during *kirario* fermentation

Lactic acid fermented cereal gruels inhibit the proliferation of pathogenic bacteria provided that the pH is below 4.0 [1, 25]. Thus the low pH achieved during the spontaneous fermentation of *kirario* can be expected to contribute to its safety. This explained the decline of coliforms, which were undetectable due to the dramatic decline of pH through rapid acidification. The results obtained for total viable and LAB counts in *kirario,* and the predominance of the LAB are similar to those reported by authors who have studied the microbial profiles of traditional fermented cereal-based gruels similar to *kirario*. Results from the microbiological analysis of the

Ethiopian fermented beverage, *borde*, reported an average aerobic mesophilic count [AMC], LAB and yeasts counts of 9.9, 10.1 and 8.1 log cfu g^{-1}, respectively [26]. Studies carried out on the microbial groups involved in the spontaneous fermentation of a Sudanese cereal-based product *medida*, showed that *Lactobacillus spp*., and *acetobacter spp*., were the predominant microbes in the product [27]. The decrease observed in yeasts and moulds to levels of <2 log cfu/ml indicate that they were destroyed by the more dominant LAB as the fermentation progressed.

The biochemical and microbiological changes that occur during production of m*asvusvu* and *mangis*i, both traditional Zimbabwean cereal-based beverages are similar to those of *kirario*. Microbiological studies carried out during fermentation and production of *mangisi*, showed a total aerobic mesophilic and lactic acid bacteria increased with fermentation time [21]. However, the total titratable acidity increased from 0.51 to 4.10 percent lactic acid, and pH decreased from 6.10 to 3.98. These results are slightly different to those obtained in *kirario* whereby the titratable acidity increased from 1.04 to 3.15 percent lactic acid, and pH decreased from 6.4 to 3.0. This is a vital characteristic in *kirario* fermentation in terms of microbial and lactic acid development. The high levels of fermentable simple sugars and lack of nutrient inhibitors in the green maize were attributed to the high initial count of LAB and rapid acid development.

The study has shown that *kirario* has unique characteristics as reflected in its unique raw material (green maize), high counts of lactic acid bacteria and a high pH. The traditional processing was therefore, documented during the microbial and biochemical analysis. This is essential in analyzing *kirario*'s unique properties. The results obtained from the laboratory-produced *kirario* showed similar microbial counts and profile, indicating the ability to replicate *kirario* production under laboratory conditions. This was vital for further research work on *kirario*.

CONCLUSION

The traditional art of *Kirario* preparation as documented in this study involves spontaneous fermentation of green maize and millet gruel at ambient temperature for 24-48 hours. Traditional fermenting vessels like earthen ware pots or clay pots [nyung'o in Meru language] are rarely used currently and have been replaced by the more popular plastic jars and buckets. Fermenting vessels that are frequently used for fermentation are most preferred for use during the preparation of *kirario* based on the belief by the local women that they accelerate the fermentation of the gruel. The fermented porridge is cooked by boiling to a thick consistency and served into calabashes and plates; the consumer uses a spoon [usually wooden] to drink. It was noted that the consumption of *kirario* is mostly for thirst quenching purposes, a special drink during festivities/rituals and is a special drink for the invalids, lactating mothers, infants and circumcised youth who are undergoing recovery. Relatively high acid contents were observed in both the traditional and laboratory-produced *kirario*, corresponding to pH between 3.0 and 3.5, which indicated relatively high activity of the lactic acid bacteria in *kirario*. There is still a considerable lack of information with

regard to lactic acid fermented porridges (soured porridges and dumplings), in spite of the significance of these foods for millions of people in Africa and Asia.

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Table 1: Microbiological counts in traditional and laboratory-simulated *kirario*

	Log counts [log_{10}cfu/ml]	
	Traditional	Laboratory-simulated
Total viable counts	9.30 (0.14)	9.51 (0.68)
Lactic acid bacteria	9.63 (0.11)	9.40 (0.60)
Lactic-acid cocci	8.62 (0.98)	5.59 (0.98)
Yeasts and molds	4.83 (2.50)	4.20 (2.17)
Coliforms	<1	<1

Standard deviation in parentheses 10 samples each of traditional and laboratory-produced *kirario*

Number of replicates of laboratory-produced *kirario* samples = 3

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