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Microbiology of Keribo Fermentation: An Ethiopian Traditional Fermented Beverage

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Abstract: Keribo is an indigenous traditional fermented beverage and is being served on holidays, wedding ceremony and also used as sources of income of many households in Jimma zone. The aim of this study was to document the microbiology of the product and antibiotic susceptibility patterns of LAB. Samples of Keribo were collected from Jimma town and four of its districts. Keribo was fermented in the laboratory following the traditional techniques for microbial succession monitored at 6 h intervals. Finally, dominant LAB was evaluated for their antibiotic susceptibility patterns against eight antibiotics. Samples of Keribo from open markets and households in Jimma zone showed average Lactic Acid Bacteria (LAB), Aerobic Mesophilic Bacteria (AMB), Aerobic Spore-formers (ASF) and yeasts with mean counts of (log CFU mL^{-1}) 2.70±2.07, 2.34±2.37, 4.96±2.80 and 2.01±0.60, respectively. The mean counts of Enterobacteriaceae, staphylococci and moulds were below detectable levels. The early stage was dominated by AMB and ASF. However, the mean counts of LAB increased exponentially for the first 30 h and remain constant thereafter. *Leuconostoc mesenteroides,* identified as the most dominant LAB, were found to be susceptible to penicillin G, gentamicin, ampicilin, chloramphenicol, amikacin, bacitracin and norfloxacin but resistant to vancomycin.

Key words: Antibiotic, Jimma zone, keribo fermentation, LAB, susceptibility test

INTRODUCTION

Traditional fermented foods and beverages are those traditionally fermented products based on the skills of the household occupants by indigenous knowledge systems and is produced from a variety of locally available cereal ingredients using traditional techniques by the people of that area themselves (Abegaz *et al*., 2002). They became part of the cultural and traditional norm among the indigenous communities in rural areas. Ashenafi (2002) indicated that fermented foods and beverages constitute a major portion of peoples' diet in all parts of the world. In Ethiopia, like in many developing countries, fermented food products constitute a major portion of peoples' diet. Ethiopia is a country rich in cultural diversity and hence, varieties of foods and beverages are processed and consumed among the various ethnic groups. The fermented products are, however, produced on fairly small scale and usually for local consumption (Ashenafi, 2006).

The indigenous natural fermentation takes place by the microorganisms involved in the process but particular microbial community will succeeded the end product in any food fermentation. Isolation of such microbes should not only be confined to dominant organisms but also it should include other microbes found in lower numbers which might have an important function in the process. Microbiological, identification of the role of each organism, nutritional and technical investigation should be carried out on each of the fermentation processes. The various microorganisms involved in each fermentation process should be isolated, characterized, studied and preserved (Aidoo *et al*., 1992). Microbiologically, fermentation of traditional fermented products relies on the microorganisms (LAB) present in the substrates, fermenting vats and equipments. Lactic acid bacteria are the most frequently encountered groups in almost all fermented products. They are known to produce varieties of chemical compounds. Inhibition of the growth of pathogenic microorganisms by lactic acid bacteria in some fermented products is accounted to organic acids, low pH, hydrogen peroxide, diacetyl, competition and nutrient depletion, altered redox potential, CO_2, ethanol, crowding and production of antibiotics like substance such as bacteriocins (Adams and Nicolaides, 1997).

Keribo is an indigenous traditional fermented beverage produced and consumed in different parts of the country, including Jimma zone. It is produced mainly from barley and sugar. Fermented Keribo constitutes a major part of the beverages being served on holidays, wedding ceremony and also as sources of income of many households in Jimma zone. The popularity of this traditional fermented beverage is more reflected among the religious groups and those do not like alcoholic drinks. Being considered as a non- or low- alcoholic beverage, Keribo is popular among both adults and children. It has poor keeping quality with shelf-life of not

more than a day or two and it has a pronounced characteristic of the deteriorating beverage at the end of 48 h of fermentation.

The traditional indigenous technology of cheese, bread, beer and wine is well documented. The microbiology and fermentation processes of some of the traditional Ethiopian fermented foods, condiments and beverages are also studied and documented. A review on the microbiology of Ethiopian foods and beverages (Ashenafi, 2006) has revealed the availability of scientifically documented information on the microbiology of a number of traditional Ethiopian fermented foods and beverages. Among the traditional Ethiopian fermented beverages, the fermentation processes and microbial dynamics during fermentation of 'Tella'(Samuel and Berhanu, 1991), 'Borde' (Bacha *et al*., 1998) and 'Shamita' (Bacha *et al*., 1999) are described. Moreover, the safety consideration of Ethiopian foods and beverages has shown the possibility of isolating some food-borne pathogens from some fermented products (Ashenafi, 2002). However, there is no scientifically documented information both on the microbiology and safety of 'Keribo' preparation. The aim of this study was, therefore, to document and analyze its microbiological flora with emphasis on the fermenting lactic acid bacteria, antibiotic susceptibility patterns of the isolates and safety of 'Keribo'. The present study reports on microbial succession occurred during traditional fermentation of Keribo.

MATERIALS AND METHODS

Enumeration of microbial groups: The counting and characterization of microbial groups was carried out following standard microbiological methods (Mugula *et al*., 2001). Samples of Keribo (25 mL each) were drawn aseptically at 6 h intervals during laboratory fermentation of Keribo and 25 mL each collected from local venders were mixed with 225 mL sterile peptone water (1.5%), homogenized and were spread-plated in duplicate on pre-dried agar plates of Plate Count(PCA) (CDH, India) for Aerobic Mesophilic Bacteria (AMB), Eosin Methylene Blue (EMB) (CDH, India) for Enterobacteriaceae (EB) , Mannitol Salt (MSA) (CDH, India) for Staphylococci, De man, Rogasa, Sharpe (MRS) (OXOID) for Lactic Acid Bacteria (LAB) and Chloramphenicol-bromophenol Blue (CBB) for yeasts/ moulds, CBB consisted of (g L^{-1} distilled water) yeast extracts 5.0 g, glucose 20 g, choramphenicol 0.1 g, Bromophenol-blue 0.01 g, agar 15 g, pH 6.0-6.4. The total Aerobic Mesophilic Bacteria (AMB) was enumerated on PCA plates after incubation at 30-32°C for 48 h. After incubation at 30-32°C for 20-24 h after which purplish red colonies surrounded by reddish zone of precipitated bile were counted as coliforms and large mucoid colonies that are pink to purple because of their lactose fermentation were enumerated as members of Enterobacteriaceae (EB). All snow white colonies of LAB were counted on MRS agar plates after anaerobic incubation using anaerobic jar (anaerobic Gas pack System, Oxoid) at 30-32°C for 48 h. Aerobic spore formers were counted on Plate Count (PC) agar after appropriate dilution was heat-treated at 80°C for 10 minutes in water bath and spread- plated. The numbers of AMB, EB, Staphylococci, LAB, yeasts/moulds or ASF from their respective duplicate countable plates are reported as log CFU mL^{-1} calculated from the arithmetic mean of total samples. After colony counting, 10 to 15 colonies were randomly picked from countable plates of MRS agar for further identification. Colonies of LAB were transferred into about 5 mL MRS broth (HIMEDIA, India) and purified by repeated streaking on MRS agar. The pure cultures of LAB were streaked on slants of MRS agar and were stored at 4°C for further characterization.

Characterization of the dominant LAB: Lactic acid bacteria isolated from Keribo samples were identified on the basis of key characteristics and tests (Facklam and Elliott, 1995; Ricciardi *et al*., 2005; Yousif *et al*., 2005; Bahiru *et al*., 2006). Morphological characterization of the pure culture was conducted microscopically using oil emulsion objectives.

The preparation was observed under Digital Olympus spectro-microscopy connected to Screen display. Cell grouping, motility, presence or absence of endospores and cell shape were the basic features to be evaluated during morphological observation. Gram-reaction was tested based on the KOH test of Gregersen (1978). Production of the enzyme oxidase was tested according to Kovacs (1956) and formation of catalase was determined by flooding young colonies with 3% solution of H_2O_2 and Oxidative or fermentative utilization of glucose by each isolate was assessed by the O/F test (Hugh and Leifson, 1953). Gas production from glucose was assigned in MRS broth containing inverted Durham tubes. The broth was inoculated with two colonies from fresh grown MRS agar plate. The broth was then sealed with melted petroleum jelly and the tube is incubated at 30-32°C for 48 h. Gas production was indicated when the inverted Durham tubes pushed upward. Salt tolerance was done using MRS broth containing 4, 6.5, 8, 15 and 20% (w/v) NaCl. Heat tolerance at 15, 40 and 45°C in MRS broth (Oyewole and Odunfa, 1990), deamination of arginine (Pilone *et al*., 1991), acid production from carbohydrates (Nair and Surendran, 2005) and production of yellow

pigment (Farrow *et al*., 1989) in MRS broth was conducted. Coagulase test was done by placing isolates from pure culture on a clean microscopic slide and mixed with blood plasma. Agglutination or clumping of cocci within 5 to 10 seconds was taken as positive result. Nitrate reduction in nitrate broth and indole production in tryptone broth was evaluated (Facklam *et al*., 1989).

Source of dominant LAB involved in Keribo fermentation: To evaluate the dominant LAB involved in Keribo fermentation, sample of table sugar (25 g), which is commonly used for Keribo preparation, was mixed with 225 mL sterile peptone water (1.5%) and homogenized using vortex mixer. The homogenate was serially diluted (10^{-1} to 10^{-2}) and 0.1 mL aliquot of appropriate dilution was spread-plated in duplicate on pre-dried plates of De-Mann Rogossa and Sharpe (MRS) agar for counts of LAB. The inoculated plates were incubated anaerobically using anaerobic jar (anaerobic Gas pack System, Oxoid) at 30-32°C for 48 h. Repeatedly purified colonies of LAB were subjected to morphological and biochemical analysis.

Physico-chemical analysis: The pH of samples was determined by dipping an electrode of a digital pH meter (HANNA-211 meter, Portugal) into 10 mL aliquot sample drawn during laboratory fermentation. The pH meter was calibrated against standard buffer solutions at pH 4.0 and 7.0 (Merck). The total amount of lactic acid present in each of the sample drawn was determined by titration against a 0.1 N NaOH (Byaruhanga, 1998). The percent of lactic acid present in the sample was calculated using the formula:

$$\text{Lactic acid (\%)} = \frac{\text{Amount of NaOH x Normality of NaOH x 9}}{\text{Volume of sample (mL)}}$$

Determination of antibiotic susceptibility patterns of Isolates: Susceptibility of the LAB to 8 types of antibiotics was performed by the disc diffusion method as described by Bauer *et al*. (1966) and Liasi *et al*. (2009) using commercially available antibiotic disc (Oxoid). The commercial antibiotics used were penicillin G (Pen, 10 unit), ampicillin (Amp, 10 μg), amikacin (Amk, 30 μg), norfloxacin (Nx, 10 μg), chloramphenicol (Chl, 30 μg), Vancomycin, (Van, 10 μg), gentamycin (Gen, 30 μg) and bacitracin (B, 10 μg). After incubation of the plates, inhibition zone diameters were measured inclusive of the diameter of the discs. The isolates were classified as sensitive S (≥21 mm); intermediate, I (16-20 mm) or resistant, R (≤15 mm), respectively according to Vlkova *et al*. (2006). For purpose of data analysis, the intermediates were considered as sensitive (Ferraro, 2000; Rojo-bezares *et al*., 2006).

Statistical analysis: To see if there was significant difference in microbial counts among study areas was analyzed by analysis of variance (ANOVA) and means were separated by Duncan's test at $\alpha = 0.05$.

RESULTS

Microbial load of Keribo samples and identification of LAB: The minimum counts of Aerobic Spore Forming bacteria (ASF) was over 5 log CFU mL^{-1} with the mean count of 4.96 log CFU mL^{-1} and maximum counts of 7.97 log CFU mL^{-1} in Keribosamples. Similarly, the mean count of LAB in collected samples was 2.70 log CFU mL^{-1} with maximum count of 6.89 log CFU mL^{-1} (Table 1). The counts of staphylococci, Enterobacteriaceae and Molds were below detectable level (<log 2 CFU mL^{-1}).

LAB, AMB and yeasts were among the commonly isolated microbial groups in Keribosamples next to ASF. The Keribo samples from the five study areas did not have significant differences in counts of LAB and yeasts ($p>0.05$) (Table 2). However, samples from areas 1, 2 and 5 had significantly higher counts of AMB than samples from areas 3 and 4 ($p<0.05$). Similarly, samples from areas 1, 2, 3 and 5 had significantly higher counts of ASF than samples from area 4 ($p<0.05$) (Table 2).

About 52% (n = 26) of Keribo samples had counts of LAB ranging from Log 2 to Log 6 CFU mL^{-1}. However, about 48% (n = 24) of the sample had counts of LAB below Log 2 CFU mL^{-1}. Even though about 28%

Table 1: Microbial count (log CFU mL^{-1}) of different microbial groups detected in Keribo samples

Microbial group	Mean±SD	%CV	Minimum	Maximum
LAB	2.70±2.07	76.66	0.0	6.89
AMB	2.34±2.37	101.28	0.08	8.31
ASF	4.96±2.80	56.45	0.0	7.97
Yeasts	2.01±0.60	29.85	0.81	3.10
EB	<2	-	-	-
Staph	<2	-	-	-
Molds	<2	-	-	-

LAB: Lactic acid bacteria, AMB: Aerobic mesophilic bacteria, ASF: Aerobic spore former, Staph, staphylococci, EB: Enterobacteriaceae

Table 2: Microbial counts (CFU mL^{-1}) of Keribo samples from five areas (n = 50)

Microbial Groups	Log CFU mL^{-1} (Mean±S.D) Area 1	Area 2	Area 3	Area 4	Area 5
LAB	2.47±2.02^a	2.69±1.80^a	3.07±2.12^a	2.56±2.65^a	2.69±2.06^a
AMB	2.27±1.82^a	3.04±0.56^c	1.26±1.99^b	1.46±0.94^b	3.69±3.34^a
ASF	5.69±2.17^a	5.37±1.89^a	6.73±2.24^a	2.44±2.58^b	4.54±3.35^a
Yeasts	2.48±0.44^a	2.08±0.49^a	2.10±0.26^a	1.60±0.67^a	1.87±0.77^a

*Averages in rows followed by the same letters are not significantly different ($p>0.05$)

(n = 14) of the samples had counts of mesophilic spore formers below detectable level, majorities of the samples (72%, n = 36) had these counts ranging from Log 2 to Log 7 CFU mL^{-1}. Contrary to this, majorities of keribo samples (64%, n = 32) had counts of Aerobic mesophilic bacteria below Log 2 CFU mL^{-1} although 36% (n = 18) of the samples had counts ranging from Log 2 to Log 8 CFU mL^{-1}. All samples had mould counts below detectable level. About 56% (n = 28) of the samples had yeast counts = Log 2 CFU mL^{+}. Only 2% (n = 1) of samples had counts of Enterobacteriaceae at levels >log 2 CFU mL^{-1} (Fig. 1).

Physico-chemical change during laboratory Keribo fermentation: Changes in pH and TA during Laboratory keribo fermentation are as shown in Fig. 2. The initial pH of unfermented Keribo at 0 h was around 5.75. The pH dropped from 5.75 to around 4.5 within the first 6 h of fermentation. The pH further dropped gradually to as low as 4.0 after 30 h of fermentation with maximum drop down to 3.7 in 48 h fermentation (Fig. 2). Generally, titratable acidity increased from 0.07 to 0.13% during the first 6 h of fermentation. Thereafter, the amount of lactic acid increased gradually up to about 20% during 36 h of fermentation. The amount of lactic acid produced reached a value of 25% at 48 h of fermentation (Fig. 2).

Microbial change during Laboratory Keribo fermentation: At early stage of fermentation, the mean counts of LAB, yeasts and moulds were below detectable level. Both yeasts and moulds remained below detectable level throughout fermentation with no significant rise in the counts of yeasts and a total elimination of molds at the end of 48 h fermentation. The counts of LAB, however, increased exponentially for the first 24 h of fermentation followed by gradual increment thereafter (Fig. 3). Likewise, counts of ASF increased exponentially for the first 30 h of fermentation with gradual decline up to 48 h of fermentation. On the other hand, the counts of AMB decreased rapidly in the course of fermentation for the first 24 h and began to decrease gradually for the rest of the fermentation period. In the later part of fermentation both AMB and ASF had related count.

Grouping of the dominant LAB: Lactic acid bacteria isolated from Keribo samples were classified into the genus *Leuconostoc* and were further classified into the species level on the basis of their physiological and other biochemical characteristics (Table 3). The identified isolates of *Leuconostoc* species were non-spore-forming, single cocci, gram-positive, catalase positive and non-motile. When they are cultivated in MRS broth, cells precipitated rapidly and growth did not occur on the surface. The determination of fermentation products showed that the isolates could convert glucose to lactic acid and CO_2 through a typical hetero-fermentative pathway. It produced ammonia from arginine and hydrolyzed bile esculine, but indole production was negative in tryptone broth. These bacteria also produced a characteristic yellow pigment in MRS broth. Moreover, tests for temperature tolerance showed the organism could tolerate temperature of up to 40°C. Prolonged incubation in MRS broth at 4, 6.5, 8, 15 and 20% NaCl resulted in turbidity growth that shows increased in the number of bacteria. Based on these characteristics the isolates were identified as *L. mesenteroids*.

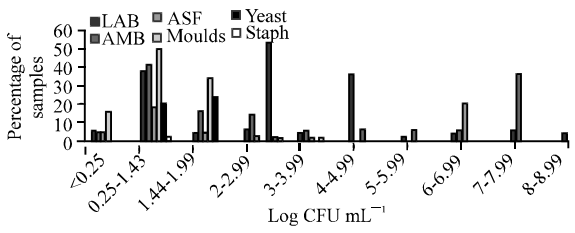

Fig. 1: Distribution of counts of major microbial groups in Keribo samples. AB, lactic acid bacteria; AMB, Aerobic mesophilic bacteria; ASF, Aerobic spore former; EB, Enterobacteriaceae; Staph, staphylococci

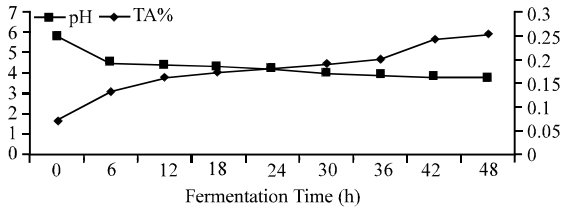

Fig. 2: pH and TA Changes in traditionally fermenting Keribo

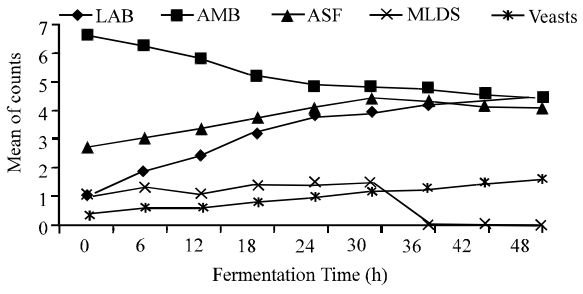

Fig. 3: Dynamic of LAB, AMB, ASF and Mould/ Yeast during traditional Laboratory fermentation of Keribo, Where, LAB: Lactic acid bacteria, AMB: Aerobic mesophilic bacteria, ASF: Aerobic spore formers, MLDS: Molds

Table 3: Morphological, physiological and biochemical characteristics of LAB

	Isolates						
Characteristics	S_1	S_2	S_3	S_4	S_5	S_6	S_7
Cell morphology	Cocci	Cocci	Cocci	Cocci	Cocci	cocci	Cocci
Cellular arrangement	Single	Single	Single	Single	Single	single	Singe
Motility test	-	-	-	-	-	-	-
Catalase activity	+	+	+	+	+	+	+
Coagulase test	-	-	-	-	-	-	-
Gas from glucose	+	+	+	+	+	+	+
Yellow color from MRS broth	+	+	+	+	+	+	+
Growth at temperature (°C)							
15	+	+	+	+	+	+	+
40	+	+	+	+	+	+	+
45	-	-	-	-	-	-	-
Tolerance to NaCl (%)							
4	+	+	+	+	+	+	+
6.5	+	+	+	+	+	+	+
8	+	+	+	+	+	+	+
15	+	+	+	+	+	+	+
20	+	+	+	+	+	+	+
Indole production in Tryptone broth	-	-	-	-	-	-	-
Arginine hydrolysis	+	+	+	+	+	+	+
Bile esculine hydrolysis	+	+	+	+	+	+	+
Acid from							
Lactose	+	+	+	+	+	+	+
Mannitol	+	+	+	+	+	+	+
Nitrate broth	-	-	-	-	-	-	-

+: Positive result and -: Negative result

Source of dominant lab involved in keribo fermentation: The data gathered in this study showed, during traditional keribo preparation, barley was deeply roasted and boiled in boiling water at 65 to 70°C for 10 to 20 min. Thereafter, it was filtered and table sugar and yeast was added to the filtrate. The microbiological analysis indicated that the dominating LAB isolated from both collected Keribo sample and Keribo prepared by experienced woman showed catalase positive and all were hetero-fermentative. The result showed that only a single genus and/or a single species of LAB involves in Keribo fermentation. Result obtained from microbiological analysis of table sugar revealed that the isolate was gram-positive, catalase-positive, fermentative, non-oxidative, non spore- forming, hetero-fermentative and single cocci that considered as *Leuconostoc* species, mainly *Leuc. mesenteroides.*

Determination of antibiotic susceptibility patterns of Isolates: Results of the sensitivity studies of the LAB isolates tested against 8 different types of antimicrobial agents are shown in Table 4. All isolates were susceptible to penicillin G, Norfloxacin, gentamycin, ampicillin chloramphenicol, amikacin and norfloxacin. However, the isolates were resistant to Vancomycin.

Besides sharing similarity in morphological and physiological characteristics, the isolates shared similarity in antibiotics resistance/sensitivity. The isolates could be species of the same strains.

Table 4: Antibiotic sensitivity of *Leu. Mesenteroids*

	Groups of LAB isolated from different localities				
Antimicrobial agents	$Leum_1$	$Leum_2$	$Leum_3$	$Leum_4$	$Leum_5$
Penicilin G	+	+	+	+	+
Vancomycin	-	-	-	-	-
Amikacin	+	+	+	+	+
Bacitracin	+	+	+	+	+
Ampicilin	+	+	+	+	+
Norfloxacin	+	+	+	+	+
Chloramphenicol	+	+	+	+	+
Gentamicin	+	+	+	+	+

-: Resistant, +: Susceptible, Leum: *Leuconostoc mesenteroides*

DISCUSSION

Keribo is a traditional, non-alcoholic, dark brown colored fermented beverage commonly consumed in rural and urban areas of Jimma zone, southwestern of Ethiopia, with some similarity to Boza of Bulgaria, Albania, Turkey and Romania (Blandino *et al*., 2003). It is produced by an over-night fermentation of cereal (barley) predominantly by activities of LAB like the fermentation of shamita (Bacha *et al*., 1999).

High count of LAB could account for acidification of the product with extension of fermentation periods. LAB have been involved in the natural fermentation of many traditional Ethiopian fermented foods and beverages (Bahiru *et al*., 2006).

Deep-roasting o f the cereal and boiling at about 65-70°C for 15 to 20 min during Keribo preparation must have eliminated most of the contaminant associated with the raw materials. As most of the isolates failed to tolerate

temperature above 40°C, the single species of LAB that dominated in the final product must have joined the system from sugar used for fermentation. Efiuvwevwere and Akoma (1997) reported similar treatment of ingredients at 70°C for 30 min during preparation of pasteurized Nigerian beverage, Kunun-zaki, in which most of the microorganisms were destroyed except the *Bacillus* species and the thermo-tolerant lactic acid bacteria.

Since the cooking process (deep roasting and boiling at 65 to 70°C) and low pH inactivates the contaminants, contamination of Keribo with *Staphylococcus* and Enterobacteriaceae could be due to post production contamination. The occurrence of *Staphylococcus* (0.83%) and Enterobacteriaceae (0.75%) are evidence of poor hygienic conditions of some of the Keribo samples. These organisms may be contaminants from unsafe water used either to dilute the ready-to-consume Keribo or wash utensils. The utensils used for preparation of Keribo and serving are made of low quality plastic and necked-bottles that are difficult to be cleaned.

Although, there are no microbiological standards set for the traditional fermented foods/beverages of Ethiopia, the mean counts of staphylococci, Enterobacteriaceae, yeasts and molds observed among the samples of Keribo were on the lowest margin of the standards set for fruit juices served in the Gulf region, indicating the maximum count permitted for total colony count of coliforms, yeast and molds are 1×10^4, 100 and 1×10^3 CFU mL^{-1}, respectively (Gulf Standards, 2000). However, the means counts of aerobic spore-formers and aerobic mesophilic bacteria of the samples were 4.96 log CFU mL^{-1} (with the maximum count of 7.97 log CFU mL^{-1}) and 2.34 log CFU mL^{-1} (with maximum of 8.31 log CFU mL^{-1}), respectively. On the basis of the Gulf Standards, it is clear that the colony counts of LAB, AMB and ASF in our Keribo samples exceeded the standard by considerable margin. From long history of its safety, the high counts of LAB may not pose hazard to the health of consumers. The low mean counts of staphylococci also avoid the risk of enterotoxin production as toxin production among these groups is possible after the counts exceed or equals 10^6 CFU mL^{-1} (James, 2000). High counts of aerobic mesophilic bacteria may trigger health problems provided that there are potential pathogenic strains among the strains including *E. coli* and *Salmonella* species.

The microbiology of Keribo samples drawn an intervals during controlled laboratory fermentation were observed to have mean counts of Coliforms, *Enterobacteriaceae*, *Enterococci* and *Staphylococci* below detection level. The two steps heat treatment during Keribo preparation (deep roasting of barley and boiling of roasted barley in water to dissolve it) has contributed to eliminate these bacterial groups. Moreover, the drop in pH level in the course of fermentation due to rise in the level of percent lactic acid could account to the betterment and microbiological safety of the fermented product. Laboratory prepared Keribo had comparable microbial counts with samples obtained from local Keribo brewers in Jimma Zone. Although with steady increase and below detectable level at the end of fermentation, the mean counts of yeasts increased throughout fermentation (over a period of 48 h) of the laboratory prepared Keribo. Likewise, there was an increase in the number of LAB and aerobic spore formers. The growth of yeasts appeared not to be inhibited by the acidity developed by the activities of lactic acid bacteria and proliferation with ease (Etchells *et al*., 1943).

The positive reaction to catalase test of our LAB isolates is in contradiction to the common characteristics of LAB (Aguirre and Collins, 1993). The result of the present study is in agreement with the rare observations reported in the distant past (Johnson and Mccleskey, 1957; Whittenbury, 1964; Yousten *et al*., 1975; Lucey and Condon, 1986) and even some recent reports (Bayane *et al*., 2006; Azizpour *et al*., 2009).

In the present study utilization of mannitol in MRS broth which was not reported among other *Leuconostoc* spp, accumulation of H_2O_2 that did not affect better growth of the *leuconostocs* and showed catalase activity (Lucey and Condon, 1986), high salt concentration (20%NaCl), high temperature (40°C) tolerance (Oyewole and Odunfa, 1990) and vancomycin-resistant were the specific criteria to decide on isolated LAB to be *Leu. mesenteroids*.

Although, the first report from traditional Ethiopian fermented beverages, the dominance of *Leuconostoc* species was reported earlier during the cassava fermentation for gari production (Okafor, 1977). Besides dominating microflora of the final product, several studies have shown that *Leu. mesenteroides* could also initiate fermentation processes such as the fermentation of idli (Mukherjee *et al*., 1965), sauerkraut (Pederson and Albury, 1969; Steinkraus, 1992), teff (Gashe,1985), Cassava (Oyewole and Odunfa, 1990) and Burukutu (Kolawole *et al*., 2007).

Aerobic Mesophilic Bacteria (AMB) initiated Keribo fermentation at 0 h to 6 h as shown by their early leading rate of growth followed by the succession of LAB. The initial high pH 5.75 of the Keribo fermentation at 0 h would explain the reason for growth of Aerobic Mesophilic Bacteria (AMB) while the lower pH (pH = 4.47) at 6 h fermentation began to inhibit their growth. The high

numbers of LAB attained after 6 h fermentation was responsible for a marked reduction of pH and increment in TA resulting in inhibition of most Aerobic Mesophilic Bacteria (AMB). Thus, fermentation for 24 h appeared to be a turning point for an accelerated reduction in number of aerobic mesophilic bacteria and stabilization of the maximum numbers of acid producing bacteria involved in Keribo fermentation. Thereafter, the LAB entered steady growth and showed relative decline during 36 h fermentation that corresponds with lactic acid production. This was because the cocci which would normally initiate fermentation were suppressed by rapid decrease in pH with accelerated increase in acidity followed by high growth rate of LAB responsible for end fermentation. This result was in agreement with the disappearance of *Leu. mesenteroides* beyond the first 48 h of the cassava fermentation during *fufu* production due to its inability to tolerate the increasing acidity of the fermenting mash (Oyewole and Odunfa, 1990).

The microbiological analysis of common sugar used in Keribo preparation was showed LAB with the same morphological and physiological characteristic which was similar to LAB obtained from dynamics and sample collected from venders. Overall, the results obtained from analysis of Keribo samples, laboratory fermented Keribo (dynamics) and samples of sugar used for the making of Keribo were similar in morphological and physiological characteristics. Therefore, the addition of sugar and yeast to un-malted, deeply roasted and boiled barley to initiate fermentation of Keribo is the possible source of LAB responsible for Keribo fermentation.

Antimicrobial resistance has been increasing in many parts of the world; it becomes increasingly important to monitor the antimicrobial susceptibility of lactic acid bacteria isolated from food and drinks including Keribo. All *Leu. mesenteroids* isolates were resistant to vancomycin and susceptible to penicillin G, gentamicin, ampicilin as also reported by Bacha *et al.* (2010). The low resistance to the commonly used antibiotics of these strains could show low contribution of these strains in the dissemination of resistance genes to potential pathogens in the environment, including fermented foods. Thus, the observed Intrinsic resistance of LAB to vancomycin could be the result of natural resistance of the isolates (Salminen *et al*., 1998).

To sum-up, during production and sales, venders and local processors must always keep their personal hygiene to discourage contamination. Sellers should also ensure that they do not expose the fermented products during display because this may predispose them to contamination. Improving the processing condition and upgrading traditionally fermented food production could improve the food in-security problems of the community. In order to produce the desired amount of traditional fermented beverages, it calls for optimization of the production processes and/or techniques. Hence, future studies should include the selection of most suitable strains for starter culture development that may be used to scale up the production of Keribo from households-level to large scale production.

CONCLUSION

This study indicated that LAB inoculated from yeast and sugar added and the yeasts are involved in the fermentation process. The prevalence of isolation of potential pathogen in traditional Keribo was low as mean counts of coliforms, Staphylococci, Enterococci and Enterobacteriaceae were below detectable level. It requires further study on the effect of heat treatment on nutritional value of the final product.

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