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Research Article

MICROBIAL DYNAMICS, ROLES AND PHYSICO-CHEMICAL PROPERTIES OF *KOREFE*, A TRADITIONAL FERMENTED ETHIOPIAN BEVERAGE

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ABSTRACT

There is a need to investigate the microbial dynamics, roles and physicochemical properties of *Korefe*. Therefore, the objective of this study was to investigate microbial dynamics, roles and physicochemical properties of *Korefe*. *Korefe* was prepared using traditional method. Physicochemical, dominant microorganisms of raw materials and risk assessment of *Korefe*were determined. Antagonistic activity of lactic acid bacteria (LAB) during fermentation of *Korefe* was also determined. Colony count of microorganisms during fermentation was varied between 3.6 log CFU/ml and 9.7 log CFU/ml. Colony count of microorganisms from vendors *Korefe* and final *Korefe* prepared in the laboratory were compared statistically and there was no significance ($p \geq 0.05$) difference for AMC and *Entrobacteriaceae*. Significant ($p \leq 0.05$) difference was observed in yeast and mould of the different samples. The numbers of *Lactococci* and *lactobacilli* were increased from 4 log CFU/ml to 9.7 log CFU/ml during fermentation. Yeast was increased from 3.6 log CFU/ml to 9.3 log CFU/ml. *Entrobacteriaceae* was eliminated within 72 h of fermentation. The pH of *Korefe* was dropped from 5.18 to 4.0 during fermentation processes. Titratable acidity was increased from 0.84% to 3.2% lactic acid. The ethanol content of *Korefe* was 2.7% v/v. Crude fat and ash content of *Korefe* was 7.01% and 4.73%, respectively. Lactic acid bacteria and yeast were played major role during *Korefe* fermentation through production of acid and alcohol, respectively. This study may serve as a basis for further investigations on the process of optimization of *Korefe* preparation.

Keywords: Alcohol, Fermentation, *Korefe*, Lactic acid, Yeast

INTRODUCTION

Fermentation is one of the oldest and most economical methods of producing and preserving food and is found to destroy undesirable components, enhance the nutritive value, flavor and taste of the food, and makes the product safe from pathogenic microorganisms (Mogessie, 2006). The preparation of many indigenous fermented foods and beverages remain today as a house art (Blandinob *et al.*, 2003). The method is inexpensive, easily acceptable and adaptable at household level in traditional communities (Nout, 1993). Fermented beverages are defined as products obtained through desirable biochemical changes caused by the action of microorganisms (Mogessie, 2006). They are typically unique and vary according to regions due to the variation in climate, social patterns, consumption practices and most importantly the availability of raw materials (Law, *et al*., 2011). Fermented foods and beverages are produced world-wide using various manufacturing techniques, raw materials and microorganisms. However, there are only four main fermentation processes: alcoholic, lactic acid, acetic acid and alkali fermentation. Alcohol fermentation results in the production of ethanol and yeasts are the predominant organisms (e.g. wines and beers).

Microorganisms have been playing a pivotal role in the fermentation of human beverages since the beginning of human civilization (Mogessie, 2006). A wide spectrum of microorganisms is involved during fermentation process. But a few types usually determine the quality of end products. Therefore, isolation, characterization and identification of the microorganisms involved in fermentation of cereal would be important to support the technical process and to obtain a predictable end product with a desired quality (Nwachukwu *et al*., 2010). The type of bacterial flora developed in each fermented beverage depends on intrinsic factors such as water activity, pH, salt concentration, availability of oxygen, composition of the food matrix, and extrinsic factors such as temperature, relative humidity and other parameters (Mogessie, 2006).

Lactic acid bacteria fermentation is a common way of preparing beverages traditionally in Africa. Most fermented products are dependent on LAB to mediate the fermentation process, although yeasts are also involved in cereal fermentations (Mogessie, 2006). Lactic acid bacteria are a large group of closely related bacteria that have similar properties such as lactic acid production, which is an end product of the fermentation (Chelule *et al.*, 2010). Basically, in addition to different species of lactic acid bacteria, various non-lactic acid bacterial species such as aerobic mesophilic bacteria (*Bacillus* spp.), coliforms, other members of *Entrobacteriaceae* and yeasts were shown to be present in fermenting beverages like *Borde* (Anteneh *et al.*, 2011).

Lactic acid fermentation is a traditional household-level technique, reported as effective in reducing or eliminating the growth of food borne pathogens (Anteneh *et al.*, 2011). It contributes towards the safety, nutritional value, shelf- life and acceptability of a wide range of cereal-based foods and drinks (Mogessie, 2006).

In Ethiopia, a wide range of cereal based traditional fermented beverages are prepared. Some of the known beverages are *Tella, Borde, Shamita, Cheka, Keribo, Bukire, Merissa, Korefe*and so forth. *Tella* is alcoholic, while the rest are considered to be low or non-alcoholic beverages (Kebede *et al*., 2002).

Korefe is the name of the local beer made in North West part of Ethiopia (North Gondar and North Wello). It is prepared from malted and non-malted barley (*Hordeumvuldare*), *Gesho* (*Rhamnusprenoids*) and water. However, there is no published scientific work on microbial dynamics, roles and physio-chemical properties of this traditional fermented product. Microorganisms may contribute a lot in the process of fermentation of *Korefe* and thereby help to produce quality product. A number of metabolites such as lactic acid and alcohol produced during the fermentation process may exhibit antimicrobial properties which may contribute to the safety of lactic fermented cereal drinks. The production of organic acids reduces the pH may making it difficult for some spoilage organisms to survive. Yeasts in this traditional semi-alcoholic beverage may contribute to produce alcohol. Therefore, there is a need to investigate the microbial dynamics, roles and physicochemical properties during the fermentation process of *Korefe*. To this effect, objective of the study was to investigate microbial dynamics, roles and physicochemical properties during fermentation of *Korefe.*

MATERIALS AND METHODS

Study area and collection of samples (*Korefe*)

The study was conducted in University of Gondar, North-west part of Ethiopia. Laboratory based experiment was conducted from February 2013 to October 2013 to study microbial dynamics, roles and physicochemical properties during fermentation of *Korefe.*

Nine samples (400 ml each) of *Korefe* were collected in sterile bottles randomly from *Korefe* vendors at three localities in Northwest part of Ethiopia, Gondar town, namely (Kebele- 18, Maraki and Lideta). After transportation of the samples (0.5 to 3 h), microbiological analysis, pH and ethanol content were immediately determined in the laboratory.

Traditional preparation of *Korefe*

The ingredients utilized for this investigation were barley, *Gesho* and water. Barley was utilized as both malted and non-malted ingredients. The proportion of the malted and non-malted barley and *Gesho* was different among *Korefe* vendors, but in average *Korefe* vendor's use 17 kg of non-malted roasted barley (local name *Derekot*), 3 kg non- malted unroasted barley (*Kita*) and 1 kg malted non- roasted barley (*Bikel*, local name for malt) and 0.5 kg of *Gesho* powder. This method was used in the laboratory for *Korefe* preparation.

The major equipment used for the fermentation process were pots (plastic containers), grinding stones, wooden mortar and pestle, pan, baker, *Wontef* (a sieve with mesh of interwoven horse hair-fiber rethreads at the bottom) and *Wonnfit* (a sieve with mesh of interwoven grass-fiber

rethreads at the bottom).Generally, fermentation of *Korefe* has four or three major phases marked by the introduction of ingredients into the fermentation pot at different times but four phase fermentation is a frequently used method.

Each four phases were designated as stage I, II, III and VI. In stage I**,** 4.5 g *Gesho* was mixed with 22.5 ml water and left at ambient temperature in a clean Erlenmeyer flask for 72 h. This was an initiation stage for the extraction of the flavor, aroma, bitterness and antibiotic from *Gesho* before the real microbial fermentation was begun. In stage II, 4.5 g *Bikel* powder was added with 45 ml water and fermented for 12 h. This was the first step for the real fermentation process. Stage I and stage II are commonly known as *Tigit.* In stage III, 13.5 g non-malted barley bread (*Kitta*) and 30 ml water were added and fermented for 48 h. The semisolid mixture formed at this stage is known as *Tinsis*. In stage IV, 76.5 g roasted non-malted barley powder (*Derekot*) and 30 ml water was added and fermented for 72 h. The semisolid mixture formed at this step is known as *Liwes*. Then (1:3 ratio) of *Korefe* and water was mixed after the end of fermentation and foam was formed. Formation of foam indicates that the beverage is ready for consumption.

Three phase fermentation of *Korefe*

In stage I**,** 4.5 g *Gesho* and 4.5 g *Bikel* powder was mixed with 67.5 ml water and left at ambient temperature in a clean Erlenmeyer flask for 72 h. This was the first fermentation process of *Korefe* and an initiation stage for the extraction of flavor, aroma, bitterness and antibiotic substances of *Gesho*. During the process a brown semisolid mixture called *Tinsis* was formed. In stage II, 13.5g non-malted barley bread (*Kitta*) and 30 ml water was added and fermented for 72 h. The semisolid mixture formed at this stage is known as *Tinsis.* In phase III, 76.5g roasted non-malted barley powder (*Derekot*) and 30 ml water was added and fermented for 48 h. The semisolid mixture formed during this stage is known as *Liwes.* Then a small quantity of the mixture was taken, water was added (1:3 ratio) after the end of fermentation, foam was formed.

Physicochemical analysis of *Korefe* sample

During the fermentation process, *Korefe* sample was taken aseptically at 24 h intervals and analyzed for physicochemical characteristics and microbiological load in each phase.

The pH was measured using a digital pH meter after calibration at 25°C using buffers of pH 4 and 7. The pH of thick sample was measured after blending with distilled water at a 1:1 ratio (w/v) into thick slurry at 24 h interval in each phase of fermentation (Kebede *et al*., 2002).

With regard to titratable acidity, 5 ml of the *Korefe* sample was mixed with 20 ml of distilled water. From the mixture, 3-5 drops of phenolphthalein indicator was added. The solution was titrated against 0.5 N NaOH. The amount of lactic acid produced in 100 ml of medium was calculated as percent lactic acid (Fite *et al.*, 1991).

Alcohol content was determined using distillation through direct heating and calculated using distillate specific gravity (Rosendal and Schmidt, 1987). The

temperature of the fermentation process was measured by digital thermometer (ORION 420A, Boston, USA) at each phase within 24 h interval. The moisture content of the *Korefe* was determined by drying 3 g of *Liwes* sample in a forced draft oven at 102 ± 2°C for 3 h. The moisture and ash content of the *Korefe* was calculated according to (Bradley *et al*., 1993).For fat determination, 2.0 g sample was solubilised in alcohol (2 ml) and hydrolyzed with 10 ml concentrated hydrochloric acid at 70-80°C for 40 min in water bath. The hydrolyzed fat was extracted with petroleum ether for 24 h. The ether was evaporated from the extract and the fat was dried to constant weight at 100°C for 90 min (Ahmed and Kanwal, 2004).The ash content was determined by igniting the pre-dried *Korefe Liwes* (2.0 g) in a Muffle Furnace at 550^0C. The sample was ignited until constant mass achieved (4 h).

Microbiological analysis of *Korefe*

Two and half g of sample was transferred aseptically to 22.5 ml normal saline and homogenized for 1 min by a homogenizer. The homogenate was then serially diluted (10^{-1}-10^{-10}) and aliquots of 0.1 ml from appropriate dilutions was spread-plated in triplicate on pre-dried plates of Violet Red Bile agar (VRBA), Plate Count Agar (PCA), MRS, M-17 and Potato Dextrose Agar (PDA); All the media used were Oxoid(England, registration number 3291857). The plated samples were allowed to solidify and then incubated at 30^0C for 48 -72 h. Colony counts were made using colony counter (R000102371, UK).

Purple-red colonies on VRBA plates were counted as *Entrobacteriaceae* after incubation at 30°C for 24 h. Aerobic mesophilic count (AMC) was enumerated on PCA plates after incubation at 30°C for 48 h. Colonies of LAB were counted on MRS and M-17 agar plates after anaerobic incubation in Gas Pak jars at 30°C for 48 to 72 h. Yeast and mould colonies were counted on PDA at 25°C for 72 to 120 h and each was preserved for further study (Kebede *et al*., 2002). The number of *Entrobacteriaceae*, AMC, LAB and yeast were reported as log $CFUg^{-1}$ calculated from the mean of three replicates.

In this study, identification of LAB was carried out using the following procedures. The LAB isolates were characterized using a microscope, biochemical and physiological tests. All isolates under examination were separately cultured twice in de Man-Rogosa-Sharpe (MRS) broth and an overnight culture was used for all tests and incubated. Each sample was initially examined for colony and cell morphologies, cell grouping and motility as well as gram reaction using microscope (Gregersen, 1978) and catalase test was undertaken. Growth at different temperatures was observed in MRS broth after incubation at 4, 10, 15, 37 and 45^oC for 120 h – 240 h. Growth in the presence of 4%, 8% and 9.6% NaCl was performed in MRS broth and incubated at 30^0C for 120 h. Gas (CO_2) production from glucose was determined in modified MRS broth containing ammonium sulphate using inverted Durham tubes (Samelis *et al.*, 1994). The growth of LAB on acidic and basic media was checked by MRS broth media using HCl and NaOH (Kebede, 2007).

In the case of identification of yeast, morphologically distinguished colonies were selected under a dissection microscope by using lacto phenol as a stain. Yeasts were purified by subsequent streaking on yeast extract peptone dextrose agar (YEPDA) medium. Chloramphenicol (0.1 gm/l) was added to each media to inhibit bacterial growth. The samples were incubated at a temperature of 25^{o}C for 48 h. Colonies were characterized and recorded. Pure culture of each was kept on YEPDA slants and stored at 4^{o}C until further investigation (Warren and Shadomy, 1991).

Yeast fermentation broth media were used for identification of yeasts based on patterns fermentation of specific carbohydrate. The carbohydrates used were: glucose, galactose, maltose, lactose, fructose, sorbitol, dextrose, cellulose, starch and raphinose. Yeast fermentation broth was prepared using the modification of media developed by Warren and Shadomy (1991) for the determination of carbohydrate fermentation by yeasts. The ability of fermentation by each isolate was evaluated by looking for the formation of gas (CO_2) in Durham's tube (Warren and Shadomy, 1991; Barnett *et al.,* 2000).

Assessment of raw materials as source of the dominant fermenting and non-fermenting microorganisms in *Korefe* preparation

One g of each sample was mixed with 9 ml sterile distilled water in a sterile flask for serial dilutions. The mixture of each sample of *Bikil*, *Gesho*, *Kita*, and *Derekot* was placed on shaker for 3 h, filtered and centrifuged for 10 min in 3000 rpm. One ml of the supernatant was taken and serially diluted in test tubes containing sterile distilled water. This was followed by spread plating aliquots of 0.1ml from dilutions (10^{-1}-10^{-4}) on pre-dried plates of VRBA for *Entrobacteriaceae*, PCA for AMB, MRS for bacilli spp, M-17 for *lactococci* spp and PDA containing Chloramphenicol (0.1 gm/l) for yeast and moulds (Pons *et al.,* 1986). The plated samples then incubated from 25ºC -30ºC for 48 -72 h.

Risk assessment of *Korefe*

Hundred ml *Korefe liwes* was mixed with 200 ml H_2O and filtered with filter paper. Five ml of the mixture was taken to the test tubes and autoclaved. On the autoclaved sample, refreshed loop full of standard pathogenic bacteria such as *Shigella flexneri*(ATCC12022),*Salmonella typhi*(clinical isolate, *Escherichia coli* (ATCC25922), *Streptococcus pneumoniae*(ATCC14619) and *Staphylococcus aureu*s (ATCC 25923) were deliberately inoculated and then incubated at 37^{0}C for 48 h. Autoclaved sample without inoculation of pathogenic bacteria was used as control. After incubation, a loop full from the sample was taken and streaked on Muller Hinton agar and incubated at 37^{0}C for 24 h to check whether the pathogen can grow or not.

Determination of antagonistic activity of LAB during fermentation of *Korefe*

Korefe associated LAB isolates were separately grown in test tubes without agitation in 10 ml MRS broth (Oxoid). The antimicrobial activity of cell free filtrate against standard pathogenic bacteria such as *E. coli* (ATCC25922), *S. aureas* (ATCC 25923), *S. flexneri*, (ATCC 12022) and *S.*

typhi (clinical isolate) was performed using the well diffusion assay (Saranya and Hemashenpagam, 2011). The pathogenic test bacteria were incubated in nutrient broth at 37ºC for 24 h. Petri dishes containing 20 of cell-free filtrate and incubated at 37ºC for 24 h. Then the diameter of the inhibition zone was measured in mm to determine antimicrobial activity.

Statistical analysis

Data were analyzed using SPSS version 16.0. Means and standard deviations of the triplicates analysis were calculated using analysis of variance (ANOVA) to determine the significant differences between the means followed by Duncan's Multiple range test ($p \leq 0.05$) when the F-test demonstrated significance. The statistically significant difference was defined as $p \leq 0.05$.

RESULTS

The physicochemical changes along with the fermentation process of *Korefe* were shown in Table1. The temperature, moisture content, pH and TA changes were monitored during the spontaneous fermentation of *Korefe* at every 12 h interval ml of Muller Hinton agar were prepared and inoculated with 0.1 ml of 24 h broth culture of pathogenic bacteria. Once solidified, the dishes were stored for 2 h in a refrigerator. Then wells were made and filled with 100 µl for 9 consecutive days. Preparation of *Korefe* for social occasions is normally prepared by the more experienced women to ensure and maintain the right quality. In this study, *Korefe* was prepared in the laboratory based on the protocol obtained from *Korefe* vendors in Gondar town. In 3- phase fermentation, the first stage was used to extract the different chemical components of *Gesho*. At the beginning of fermentation time, the liquid portion of both 3- and 4-phase fermentation were shown greenish color but the control was whitish in color. The next two and three stages were the time of fermentation and production of alcohol take place. The final product of all 3- and 4-phase fermentation have *Tella*-like flavor, smooth texture and white brown color. The fermenting *Korefe* shelf life can be extended up to 6 weeks or longer depending on the environmental factors, sanitation and storage conditions.

Table1.Changes in TA, pH, moisture content and temperature occurring along with fermentation process of *Korefe;* Chemical and nutritional properties of *Korefe,* collected from four *Korefe* vendors and prepared in the laboratory.

	Control					3-phase fermentation				4-Phase fermentation				
P	T(day)	Temp	Moist	TA	pH	T(^{0}C)	moist	TA	pH	**P**	T(^{0}C)	Moist	TA	pH
I	0	25	96.3	0.72	5.98	25	97.3	0.92	5.18	**I**	25	97.3	0.84	5.15
	1	25	96.3	NM	3.18	25	97.3	NM	4.71		25	97.3	NM	4.83
	2	26	96.1	0.75	2.90	25	97.2	1.25	4.05		25	97.2	1.O8	4.08
II	3	26	95.3	1.60	6.12	26	96.0	1.82	6.70	**II**	25	93.0	1.25	5.73
	4	27	95.2	ND	3.07	25	96.6	ND	3.78	**III**	25	92.6	ND	3.67

	5	26	95.0	1.72	5.13	26	96.6	2.6	5.95		26	92.30	1.44	5.86
III	6	25	84.5	1.72	5.09	25	85.2	2.8	5.57	**IV**	25	85.20	1.43	5.53
	8	25	83.3	2.55	3.84	25	85.8	3.2	4.1		25	85.83	3.00	4.0

Chemical and nutritional properties of *Korefe*

***Korefe* sample**	**Crude fat (%)**	**Ash (%)**	**pH**	**TA(% LA)**	**Ethanol content (%)**
Arada	6.98± 0.4[a]	4.6± 0.30[a]	4.65± 0.65[a]	3.24± 0.24[a]	2.71±.0.3[a]
Kebele-18	7.33± 0.3[a]	4.62±0.24[a]	4.12± 0.12[a]	3.40± 0.5[a]	2.77 ± 0.7[a]
Lideta	6.82± 0.42[a]	4.75±0.55[a]	3.90+ 0.1[a]	3.25± 0.5[a]	2.64± 0.4[a]
4-Phase (LB)	7.00± 0[a]	4.80± 0.4[a]	4.2 ± 0[a]	3.15± 0.15[a]	2.705+0.6[a]
3-Phase (LB)	6.98± 0.8[a]	4.80± 0.7[a]	4.1 ± 0[a]	3.26± 0.28[a]	2.706+0.7[a]
Control	7.00± 0.2[a]	4.80± 0.2[a]	4.1± 0[a]	3.84± 0.4[a]	2.707+0.8[a]
Total mean	7.01 ± 0.28	4.73± 0.37	4.15± 0.34	3.19± 0.3	2.706±0.7

Key: P = phase; T = temperature; moist = moisture; TA = Titratable acidity (%lactic acid), LB = *Korefe* prepared in the laboratory. Values are means of triplicate determinations; Values within the same column followed by different superscripts are significantly different at ($P \leq 0.05$).

Main physicochemical changes in 3-phase fermenting *Korefe*

There was no temperature change in the first stage for 3- phase fermenting *Korefe* and the temperature (i.e., 25ºC) was in line with room temperature. In the second stage of fermentation, temperature was gradually increased up to 26ºC at the third day. Titratable acidity was increased from a value of 0.92% lactic acid to 3.2% lactic acid to the end of fermentation process. Increment of TA was continued to the end and finally, TA was reached to 3.2% lactic acid. The pH value was dropped from a value of 5.18 to 4.05 at the end of first stage of fermenting *Korefe*. Finally at the end of the last fermentation day, pH was reduced to 4.1. Moisture content was decreased from a value of 97.3% to 85.8% for 3-phase of *Korefe* at the end of the fermentation day. Moisture content was greatly changed in the first stage. From the begging of second stage to the first day of the third stage, there was a steady decline of moisture content (from 96% to 85.2%) but after the beginning of the third stage to the end, moisture content variation was not clearly observed and finally it was reached at 85.8%.

Main physicochemical changes in 4-phase fermenting *Korefe*

There was no temperature change (25ºC) in the first stage for the 4-phase *Korefe* fermentation process. Temperature change was shown only at the third stage of

this fermentation time (26ºC). Titratable acidity was increased from a value of 0.84% lactic acid to 3.00% lactic acid to the end of fermentation process. There was declination of pH during the first stage (from a value of 5.15 to 4.08). In the third stage, there was no significant variation of pH value and finally the pH was reduced to the value of 4.0. Moisture content was decreased from 97.3% to 85.83%. Like 3-phase and the control, there was no great change in moisture content in the first stage. However, from the last day of the second stage to the second day of the third stage, there was a steady decline (93.0% to 92.3%). After the beginning of the third stage to the end, there was a rapid decline of moisture content and finally reduced to 85.83%.

Some chemical and nutritional properties of *Korefe* collected from vendors and compared with *Korefe* prepared in the laboratory

Mean value of crude fat, ash, TA, pH and ethanol content of *Korefe* samples collected from both vendors and prepared *Korefe* in the laboratory were determined (Table 1). Their chemical and nutritional properties of *Korefe* products were compared statistically and there was no significance ($P \geq 0.05$) difference for crude fat, ash, pH and TA of all samples. The earn alcoholic content of *Korefe* was 2.71% (v/v).

Microorganisms found in association with ingredients used for *Korefe* preparation.

The microorganisms found along with the fermentation process of *Korefe* were also found in the ingredients (raw materials) of *Korefe* preparation such as *Bikil, Kita, Derekot and Gesho*. LAB and yeasts were the dominant microorganisms in the raw materials used for *Korefe* production. *Entrobacteriaceae*, *lactobacillu*s spp, yeasts, *Streptococcus* spp and total aerobic group were found in *Bikil*. Except *Lactobacillus*spp, all the above four groups were also found in *Gesho*. From the five main groups of microorganisms, only *Lactobacillus*spp and aerobic microorganisms were found in *Kita*. *Derekot* was not a good source of fermenting microorganisms such as yeast and LAB, only total aerobic group were isolated from *Derekot* (Table 2). *Bikil* was the only ingredient that contains the five types of microorganism which were also found in the course of fermenting *Korefe.*

Risk assessment of *Korefe*

From the five standard pathogenic bacteria used for the risk assessment, *S. pneumoniae (*ATCC14619*)* and *S. aureu*s(ATCC 25923) were not grown on the *Korefe* sample; while the rest three pathogenic bacteria such as *E. coli (*ATCC25922),*S. flexneri (*ATCC 12022) and *S. typhi* (clinical isolate) were grown on *Korefe* sample. In the contrary, all standard photogenic bacteria and the clinical isolate were grown on the control group as shown in (Table 2).

Characterization and identification of lactic acid bacteria during the fermentation process of *Korefe*

Lactic acid bacteria from each phase of *Korefe* fermentation was isolated, characterized and identified using morphological, physiological and biochemical test methods as shown in Table4. All the isolates were Gram-positive and catalase negative rods and cocci. Most of the isolates were grown at the

temperature of: 10ºC, 15ºC and 37ºC but only few isolates were grown at the temperature of 4^0C and 45ºC.

All the isolates were also grown on the media containing 4% and 6% NaCl. Only few isolate of LAB were grown on the media containing 8% NaCl (Table 2). Finally, a total of 32 colonies were grouped into 5 main different genera of the lactic acid bacteria based on biochemical, morphological and physiological characteristics. These genera of the lactic acid bacteria were; *Lactobacillus, Lactococcus, Pediococcus, Enterococcus* and *Weissella*. *Weissella* spp was the dominant LAB in fermenting *Korefe*, while genus *Pediococcus*spp was the dominant one for the cocci group (75%).

Table 2.**Chemical and nutritional properties of *Korefe,* collected from four *Korefe* vendors and prepared in the laboratory.**

Organism	*Bikil*	*Kita*	*Derekot*	*Gesho*
Enterobacteriaceae	+	_	_	+
L*actobacillus*spp	+	+	_	_
Yeast	+	_	_	+
Streptococcus spp	+	_	_	+
Aerobic Mesophilic Bacteria	+	+	+	+
Total aerobic group	+	_	_	_

Risk assessment of pathogenic bacteria in *Korefe*

Standard micro-organism	control	*Korefe*
*S. pneumonia (*ATCC14619*)*	+	_
*E. coli (*ATCC25922)	+	+
*S. flexneri (*ATCC 12022)	+	+
*S. aureu*s (ATCC 25923)	+	_
S. typhi (clinical isolate)	+	+

Key=Values are means of triplicate determinations; Values within the same column fol different superscripts are significantly different at ($P \leq 0.05$). +present; - absent;+ grow growth.

Morphological characteristics of yeast isolates

Identification of yeast isolates were carried out using physiological and biochemical properties of morphologically selected yeast isolates from *Korefe* (Table 3). Based on colony morphological characteristics, isolate A had been shown rough surface, cream in color and oval in shape, while isolate B had been shown smooth surface, white in color and spherical in shape. The yeast obtained from the market (Baker's yeast), which was used as positive control, had been shown similar morphological characteristics of isolate B. In addition to morphological test, isolate A and B were identified using ten different carbohydrates as carbon source during fermentation process (Table 3). The result was compared with the reference isolate of the *S. cerevisiae* obtained from the market. According to the data obtained, isolate A was highly fermentative (produce large amount of gas), but isolate B was shown similar fermentation pattern with that of *S. cerevisiae*.

Table 3. Physiological and biochemical properties of morphologically selected yeast isolates from *Korefe* and from the market, using Durham tube fermentation

Carbone source provided during fermentation

Isolate	CE	GL	FR	GA	MA	LA	RA	ST	DX	SO
A	-	+++	++	++	-	++	+	+	+	+
B	-	+++	++	+	-	+	++	-	-	+
S. c	-	++	+++	++	-	+	++	-	-	+

Diameter of zones of inhibition (mm) produced by cell free filtrates of LAB isolates against test pathogenic bacteria

LAB	*S, typhi* (clinical)	*S. flexneri* (ATCC1202)	*S. aureus* (ATCC25923)	*E. coli* (ATCC2592)
LB	17 ± 1^{b}	16 ± 1^{a}	16 ± 1^{a}	15 ± 1^{a}
LC	14 ± 1^{a}	15 ± 0^{a}	15 ± 0^{a}	14 ± 0^{a}
PC	16 ± 1^{b}	15 ± 1^{a}	15.3 ± 0.6^{a}	14 ± 1^{a}
SC	15 ± 1^{a}	15 ± 2^{a}	15.3 ± 0.6^{a}	14 ± 0^{a}

Key: += fermentative,++= high fermentative,+++=very high fermentative (Durham tube empty)CE=cellulose, GL=glucose, FR=fructose, GA= galactose, MA=maltose, LA=lactose, RA= raphinose, ST=starch, DX= dextrose,

SO= sorbitol, *S. c=S. Cerevisiae*, LB= *Lactobacillus* spp; LC =*Lactococcus* spp; PD=*Pediococcus*spp; SC=streptococcus spp. Values are means of triplicate determinations; Values within the same column followed by different superscripts are significantly different at ($P \leq 0.05$)

Antagonistic activity of LAB isolated from *Korefe*

Antagonistic activity of LAB isolated from *Korefe* was presented on Table 3. Cell free filtrates of lactic acid bacteria from *Korefe* were shown some inhibitory activities against the test standard and clinical isolate. The cell free filtrates of *Lactobacillus* spp and *Pediococcus* spp had significantly ($P \leq 0.05$) greater diameter of inhibition zone than all the rest cell free filtrates of LAB. Among the test organisms, *S. typhi* (clinical isolate) was the most sensitive one, while *E. coli* (ATCC25922) was the least sensitive in all cases. Except *S. typhi*(clinical isolate), the inhibition zones produced by all cell free filtrates of lactic acid bacteria against each test standards and the clinical isolate were compared and there were no significant ($p \geq 0.05$) difference.

Microbial colony counts of *Korefe*

Microbial colony counts (log CFU/ml) of *Korefe* sample collected from three *Korefe* vendors and prepared in the laboratory were shown in the Table 4. All the microorganisms isolated from the fermenting *Korefe* in the laboratory were also found in the *Korefe* samples collected from the vendors, except that of *Entrobacteriaceae*. *Entrobacteriaceae* was isolated only from Kebele-18 and Lideta *Korefe* samples.

Table 4. Morphological and physiological properties of dominant LAB isolated during *Korfe* fermentation.

	Number of isolated colonies, morphological, physiological and biochemical properties			
Group	A	B	C	D
No. of isolated colonies	3	8	3C/1R	16
Cell morphology	Rod	Cocci	Rod &cocci	Rod
Gram	+	+	+	+
Catalase	_	_	_	_
CO_2 from glucose	75	0	0	0
Growth at 4°C	100	75	75	50
10°C & 15^0C	100	100	100	100
37°C	100	75	100	100
45°C	0	100	50	50
Growth at 4% & 6 % NaCl	100	100	100	100
8% NaCl	75	100	100	87.5
Growth at pH 3.9	100	100	50	100
Growth at pH 9.6	0	25	25	25

A = *Lactobacillus* spp*;* B =*Lactococcus* spp*;* C= *Pediococcus*spp and *Enterococcus* spp; D= *Weissella* spp

The mean microbial colony count of each sample was compared statistically. There was no significant (P≥0.05) difference in AMC of each sample, but significant (P≤0.05) difference was observed in yeast and mould of each sample. The number of yeasts and moulds of laboratory fermented *Korefe* products (3-phase, 4-phase and control) are significance (P≤0.05) greater in number than all the collected *Korefe* samples. Also significance (P≤0.05) difference (range from 6. 1 log CFU/ml to 9.4 logs CFU/ml) was found under each sample for *Lactococci* spp. For *Lactobacillus,* there was significant (P ≤ 0.05) difference (range from 5.3 log CFU/ml to 9.2 log CFU/ml) of each sample, but there was no significant (P ≤ 0.05) difference in *Entrobacteriaceae* of Kebele-18 and Lideta *Korefe* samples.

Microbial dynamics along with fermentation process of *Korefe*

Along with fermentation process of *Korefe,* different microorganisms were isolated in each stage of fermentation from the two main *Korefe* products (such as 3-phase and 4-phase fermented *Korefe)* and the control group (*Korefe* without *Gesho*). As shown in Table 5, the main microorganisms found during the fermentation process of *Korefe* were grouped under: total aerobic groups, *Lactococci, Lactobacili, Entrobacteriaceae*, Yeasts and moulds. Most of these microorganisms were found in each stage of fermentation. The number of *Entrobacteriaceae* was gradually reduced and finally disappeared at third day of the fermentation time. *Entrobacteriaceae* was isolated only in the first day from the control group, but there was isolation of *Entrobacteriaceae* from 3-phase and 4-phase fermenting *Korefe* up to 72 h. The number of moulds was decreased gradually along with the increment of fermentation times and yeasts became dominant over moulds. After the third day fermentation time, moulds were totally disappeared from *Korefe* fermentation.

The main microbial groups, such as *Lactococci, Lactobacili,* yeast and the total aerobic groups were increased to 2 log CFU/g up to fifth day and reached at highest level (9 log CFU/g to 9.5 log CFU/g) during the end of the second stage (fifth day) for control and 3- phase *Korefe*. Increment trend of these microorganisms was also the same for 4-phase *Korefe*. Most of the microorganisms were reached at their peak on the end of the fermentation time.

The mean microbial colony count of the three samples within a day was compared statistically. At the beginning of the fermentation, for *Entrobacteriaceae,* there was significance (P≤0.05) difference among the 3-phase and 4-phase fermenting *Korefe*and the control group. In the first and second fermentation day, *Entrobacteriaceae* was disappeared from the control group and only 3- phase and 4-phase fermenting *Korefe* were compared statistically. During the first day, there was significance (P≤0.05) difference. There was also significance (P≤0.05) difference in the second day of the fermentation time for *Entrobacteriaceae* between the 3- phase and 4- phase fermenting *Korefe.* For *Lactococci,* there was significance (P≤0.05) difference in the

first day of the fermentation time. For all the rest microorganisms, there was no significance ($P \leq 0.05$) difference among the different samples in each day.

Table 5. Changes in mean counts (log cfu/ml) of major microorganisms along the fermentation process of *Korefe*

Control		Three phase									Four phase				
AMC	EB	LB	LC	Y+M	AMC	EB	LB	LC	Y+M	P	AMC	EB	LB	LC	Y+M
3.7 ± 0.2^{a}	9.3+ 0.3^{c}	4 ± 0.2^{a}	4.2 ± 0.2	3.7 ± 1.4^{a}	3.9 ± 0.4^{a}	6.6 ± 0.8^{b}	4.7 ± 0.5^{a}	4 ± 0.2^{a}	3.9 ± 0.8^{a}	**I**	3.6 ± 0.6^{a}	4.2 ± 0.2^{a}	4.6 ± 0.6^{a}	4.5± 0.4^{a}	3.6± 0.4^{a}
3.6 ± 0.4^{a}	-	5.2 ± 0.2^{a}	5.3± 0.3^{a}	5.0 ± 0.4^{a}	5.3 ± 0.3^{a}	5.2 ± 0.8^{b}	5.3 ± 0.3^{a}	5.2 ± 0.4^{a}	5.5 ± 0.3^{a}		5.0 ± 0.2^{a}	2.4 ± 0.2^{a}	5.3 ± 0.6^{a}	6.3± 0.8^{b}	5.1± 0.1^{a}
7.3+ 1^{a}	-	6.3 ± 0.7^{a}	6.3 ± 0.3^{a}	7.4 ± 0.6^{a}	6.3 ± 0.3^{a}	4.6 ± 0.6^{b}	6.2 ± 0.2^{a}	6.2 ± 0.3^{a}	6.3 ± 0.5^{a}		6.0 ± 0.6^{a}	2.4 ± 0.2^{a}	6.0 ± 0.8^{a}	6.3± 0.4^{a}	6.0± 0.8^{a}
8.3 ± 2^{a}	-	8.3± 1^{a}	7.7 ± 0.7^{a}	9.2 ± 0.6^{a}	8.1 ± 1^{a}	-	9.3± 0.5^{a}	8.1± 0.3^{a}	7.1± 1^{a}	II	8.2 ± 0.2^{a}	-	8.6 ± 0.4^{a}	8.3± 0.28^{a}	8.4± 0.6^{a}
9.0 ± 0.6^{a}	-	8.9 ± 0.7^{a}	8.9 ± 0.7^{a}	8.5 ± 0.28^{a}	9.1 ± 0.6^{a}	-	8.0 ± 1^{a}	9.1 ± 0.3^{a}	8.2 ± 0.6^{a}	III	9.0 ± 0.2^{a}	-	8.4 ± 0.8^{a}	8.8± 0.5^{a}	8.8± 0.8^{a}
9.3 ± 0.7^{a}	-	9.0 ± 1^{a}	9.7 ± 0.7^{a}	8.6 ± 0.8^{a}	9.3 ± 0.4^{a}	-	9.4± 0.6^{a}	9.5 ± 0.4^{a}	9.0 ± 0.8^{a}		9.0 ± 0.8^{a}	-	9.1 ± 0.14^{a}	9.3± 0.4^{a}	9.3± 0.7^{a}
9.1 ± 9.1^{a}	-	8.7 ± 0.5^{a}	9.1± 0.4^{a}	9.3 ± 0.3^{a}	9.2 ± 0.3^{a}	-	9.0 ± 1^{a}	9.3 ± 0.3^{a}	8.5 ± 0.4^{a}	IV	9.3 ± 1.3^{a}	-	9.4 ± 0.8^{a}	9.3± 0.7^{a}	8.6± 0.6^{a}
8.2 ± 0.2^{a}	-	8.4 ± 0.4^{a}	8.6 ± 1.2^{a}	8.5 ± 1^{a}	8.4 ± 1.4^{a}	-	8.2 ± 0.8^{a}	8.5 ± 0.4^{a}	8.3 ± 1^{a}		8.6 ± 0.7^{a}	-	8.5 ± 0.8^{a}	8.2± 0.8^{a}	8.4± 0.8^{a}

Key: P=phase; T=time; AMC= aerobic mesophilic count; EB=*Entrobacteriaceae*; LC=*Lactococci*; LB= *Lactobacilli*

DISCUSSION

The production of *Korefe* is carried out by women and fermentation process accomplished with nine days. It is prepared from malted and non-malted barley (*Hordeumvuldare*), *Gesho* and water. Some of the processing techniques and characteristics of *Korefe* show similarities to other Ethiopian fermented cereal products. For example baking of *Kita* and addition of malt is the same as *Tella* brewing (Sahle and Gashe, 1991) and *Borde* fermentation (Mogessie, 2006). Small amount of *Gesho* was also added in *Korefe* as common to *Tella* but the amount of *Gesho* added in *Tella* fermentation was relatively high (Sahle and Gashe, 1991). The nature and type of equipments used for cooking and fermentation process and the adjuncts added to each phase are more or less common to those Ethiopian traditional alcoholic products (Sahle and Gashe, 1991; Mogessie, 2006).

Several researchers have indicated that the microorganisms involved in the natural fermentation of cereals are

essentially the micro flora of the raw materials and equipments (Kunyanga *et al.*, 2009). This was in agreement with the present study on *Korefe*. All of the microorganisms isolated in the course of fermentation of *Korefe* were also isolated from the raw and roasted ingredients of *Korefe*. Increasing microbial load, TA, temperature of the *Liwes* and decreasing pH were common features in each phase of *Korefe* fermentation. The high microbial load observed in *Korefe* is similar to Ethiopian *Borde* (Kebede *et al.,* 2002) and *Tella* (Sahle and Gashe, 1991).

The *Liwes* temperature increased from the value of 25^0C to 27^0C during the course of *Korefe* fermentation. The increment of temperature in the course of fermentation of *Korefe* might be the dramatic increment of LAB and yeasts almost in all phases with high rate of primary metabolic activity. At the beginning of each stage, pH was higher than that found at the end of its immediately preceding stage. This could be the effect of the newly added ingredients (adjuncts) on the fermenting microorganisms and production of lactic acid by LAB. The final pH of *Korefe* was 4.1 and 4.2 for 3-phase and 4-phase *Korefe* products, respectively. The low pH in *Korefe* was also in agreement with other cereal based beverages like Ethiopian *tella* (Sahle and Gashe, 1991), Kenyan *Kirario* (Kunyanga *et al.*, 2009, Ugandan *Kwete* (Namugumya and Muyanja, 2009)**,** Zimbabwean *Mukumbi* (Kock *et al.*, 2008), Ethiopian *Bord*e (Kebede *et al.*, 2002) and *Shamita* (Mogessie and Tetemke, 1995).

Lactic acid bacteria and yeasts were observed in higher numbers and increased with progression of fermentation. A symbiotic relation could explain the simultaneous presence of yeast and LAB (Munyaja *et al.,* 2003).*Korefe* provides a good environment where both yeast and LAB for their growth, multiplication and metabolism. Some LAB were acid tolerant. They have the ability to grow in a wide range of pH in the presence of organic acids. Their mechanism of acid-tolerance is not completely known (McDonald *et al*., 1990).

Lactic acid-fermented products inhibited the proliferation of some pathogenic bacteria provided that the pH is below 4.2 (Mogessie, 2006; Chelule *et al.,* 2010; Anteneh *et al.,* 2011). Thus, acidic fermentation would be expected to contribute for the safety of *Korefe*. According to the risk assessment experiment done on *Korefe,* Gram positive bacteria was unable to grow in comparison with Gram negative bacteria. This is due to the presence of an extra outer membrane in Gram negative bacteria, which consist of lipopolysaccharide and makes them impermeable to lipophilic extracts; whereas Gram positive bacteria where more susceptible because of having only an outer peptidoglycan layer which is not have effective permeability barrier (Tortora and Funke, 2001).

Along the progression of *Korefe* fermentation, *Entrobacteriaceae* disappearance corresponded to increase in

acidity (low pH). The value of pH 3.5–4.2 and antibiotic substances produced by LAB like bacteriocin and lactic acid have been reported to inhibit *Entrobacteriaceae* and other Gram-positive bacteria (Steinkraus, 1996). The final pH of *Korefe* was within the range of 3.5 to 4.2 and played great role on the elimination of *Entrobacteriaceae* from fermenting *Korefe*. In previous study, elimination of *Entrobacteriaceae* was observed along the production of *Kenkey*, Ghanaian fermented maize dough (Namugumya and Muyanja, 2009). In the contrary, *Entrobacteriaceae* was isolated from the two different collected *Korefe* samples and this may be due to the contamination of utensils used for preparation and filtering process of *Korefe* for consumption but not at fermentation process.

The cell-free filtrate from LAB was tested by disc diffusion method to know whether the antimicrobial metabolites produced by LAB. All the LAB isolates belonging to the four genera (*Lactobacilus*, *Lactococcus*, *Pedeococcus* and *Streptococcus*) were inhibited the growth of the test standard pathogenic bacteria to different degrees. Similar to this finding, Girum *et al.* (2005) observed varying degrees of inhibition of various food borne pathogens by cell-free filtrates of LAB. The study of Paella *et al.* (1992) has also shown the inhibitory activity of *Lactobacillus casei* and *Lactobacillus acidophilus* on the growth of *Shigella sonnei*. Traditional *Korefe* vendors should follow safety procedure in the production of *Korefe*, especially during the filtering process in order to avoid food borne contamination.

The major yeasts involved in *Korefe* fermentation were dominated by *S. cerevisiae*. It is known for its role in alcoholic fermentation. Similar findings were reported by Lyumugabe *et al*. (2010) for Rwandese traditional beer *Ikigage*, Ethiopian *tella* (Sahle and Gashe, 1991) and *tej* (Ethiopian honey wine) (Mogessie, 2006).

CONCLUSION

The traditional brewing technology of *Korefe* is exposed for contamination. Therefore, there should be development of culture starters for the improvement of the quality, flavor and aroma for *Korefe* production, but it needs screening and characterization of qualified strains of yeasts and LAB for the desired products. This technique is important to avoid contamination. Fermentation of traditional fermented drinks in such a way is significant in terms of food quality, preservation and decontamination of pathogens and other undesirable chemicals associated with the ingredients.

The result of this study may serve as a basis for further investigations on the process of optimization and qualification of *Korefe* preparation. The results of this study indicated that the Ethiopian traditional alcoholic beverage, *Korefe,* had a specific community of yeasts and complex LAB. Yeast flora in *Korefe* was not diverse in terms of spices as other Ethiopian alcoholic drinks like *Tella* and *Borde* but the type of LAB was diverse as *Borde* and *Shamita*, common Ethiopian traditional drinks. The low ethanol content of *Korefe* is significant for consumer and most consumers consider

as food rather than alcoholic beverage. Since *Korefe* had low ethanol content, it could be an alternative drink for individuals who do not take high alcoholic drinks. Another, further studies are necessary to determine the involvement and role of different types of microorganisms in determining the overall product quality of *Korefe.* Fermentation technique should be further developed to enhance safety production. In other words, production should be carried out in aseptic condition in order to avoid environmental contaminants.

CONFLICT OF INTERESTS

The authors declare that they have no competing interests.

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REFERENCES

Ahmed T and Kanwal R (2004). Biochemical characteristics of lactic acid producing bacteria and preparation of camel milk cheese by using starter culture. Pakistan Veterinary Journal, 24: 87-91.

Anteneh T, Tetemke M and Mogessie A (2011). Antagonism of lactic acid bacteria against food borne pathogens during fermentation and storage of *Borde* and *Shamita*, traditional Ethiopian fermented beverages. International Food Research Journal, 18: 1189-1194.

Apella G, Nader D, Romero M and Oliver B (1992). *In vitro* studies on the inhibition of the growth of *Shigella sonnei* by *Lactobacillus casei* and *Lactobacillus acidophilus*. Journal of Applied Bacteriology, 73: 480-483.

Barnett A, Payne W and Yarrow D (2000).Yeasts Characteristics and identification, 3rd Ed. UK: Cambridge University Press.

Blandinob A, Aseeria E, Pandiellaa S, Canterob D and Webba C (2003). Cereal-based fermented foods and beverages**.** Food Research International, 36: 527–543.

BradleyL, Arnold E, Barbano D N andSemerad G (1993).Chemical and physical methods.In Marshal, T. (ed.), Standard methods for the examination of dairy products, Washington: American Public Health Association, 16thed:433-531.

Chelule P, Mokoena M and Gqaleni N (2010). Advantages of traditional lactic acid bacteria fermentation of food in Africa. Current Research, Technology and Education Topics in Applied Microbiology and Microbial Biotechnology, 2.

Fite A, Tadesse A, Urga K and Seyoum E(1991). Methanol fusel oil and ethanol contents of some Ethiopian traditional alcoholic beverages. Sinet: Ethiopian Journal of Sciences14: 19-27.

Girum T, Eden E and Mogessie A (2005). Assessment of the antimicrobial activity of lactic acid

bacteria isolated from borde and Shamita, traditional Ethiopian fermented beverages, on some food-borne pathogens and effect of growth medium on the inhibitory activity. International Journal of Food Safety, 5: 13-20.

Gregersen T (1978). Rapid method for distinction of gram-negative from gram-positive bacteria. European Journal of Applied Microbiology, 5: 123-127.

Kebede A (2007). Isolation, characterization and identification of lactic acid bacteria involved in traditional fermentation of *borde*, an Ethiopian cereal beverage. African Journal of Biotechnology, 6: 69-78.

Kebede A, Fekadu B, Thor L and Judith A (2002). Parameters of processing and microbial changes during fermentation of borde, a traditional Ethiopian beverage. Journal of Food Technology in Africa, 7: 85-92.

Kock J, Pretorious E and Pohl C(2008). Identification of yeasts isolated from *mukumbi*, Zimbabwean traditional wine. Journal of Sustainable Development in Africa, 10: 70-78.

Kunyanga C, Mbugua S, Kangethe E and Imungi J (2009). Microbiological and acidity changes during the traditional production of *kirario*: an indigenous Kenyan fermented porridge produced from green maize and millet**.** African Journal of Food, Agriculture, Nutrition and Development, 9: 107-116.

Law S, Abu B, Mat H and Abdul H (2011). Popular fermented foods and beverages in South East Asia. International Food Research Journal, 18: 475-484.

Lyumugabe F, Kamaliza G, Bajyana E and Thonart P (2010). Microbiological and physio-chemical characteristic of Rwandese traditional beer, *Ikigage.* African Journal of Biotechnology, 27: 4241-4246.

McDonald C, Fleming P and Hassan M (1990). Acid tolerance of *Leuconostoc mesenteriodes* and *Lactobacillus plantarum*. Applied Environmental Microbiology, 56: 2120-2124.

Mogessie A (2006). A review on the microbiology of indigenous fermented foods and beverages of Ethiopia. Ethiop Journal of Biological Sciences, 5: 189-245.

Mogessie A and Tetemke M (1995). Some microbiological and nutritional properties of borde and Shamita, traditional Ethiopian fermented beverages. Ethiopian Journal of Health Development, 9: 105-110.

Munyaja M, Narvhus J, Treimos J and Langsrud T (2003). Isolation, characterization and identification of lactic acid bacteria from bushera: Ugandan traditional fermented beverage. International Journal of Food Microbiology, 8: 201-210.

Namugumya B and Muyanja C (2009). Traditional processing, microbiological, physiochemical and sensory characteristics of *kwete*, Ugandan fermented maize based beverage. African Journal of Food,

Agriculture, Nutrition and Development, 9: 63-77.

Nout M (1993).Processed wearing foods for tropical climate. International Journal of Food Microbiology, 4: 213-221.

Nwachukwu E, Achi K and Ijeoma O(2010). Lactic acid bacteria in fermentation of cereals for the production of indigenous Nigerian foods**.** African Journal of Food Science Technology, 1: 021-026.

Pons M, Rajab A and Engasser J (1986). Influence of acetate on growth kinetics and production control of *Saccharomyces cerevisiae* on glucose and ethanol. Journal of Applied Micro Biotechnology, 24: 193-198.

Rosendal and Schmidt (1987). The Alcohol table for beer analysis and polynomials for alcohol and extract. Journal of International Brewing, 6: 93-373.

Sahle A and Gashe B (1991).The microbiology of *tella* fermentation. Sinet; Ethiopian Journal of Sciences, 14: 81 - 92.

Samelis J, Maurogenakis F and Metaxopoulos J (1994). Characterization of lactic acid bacteria isolated from naturally fermented Greek dry salami. International Journal of Food Microbiology, 23: 179-196.

Saranya S and Hemashenpagam N (2011). Antagonistic activity and antibiotic sensitivity of lactic acid bacteria from fermented dairy products. Advances in Applied Science Research, 2: 528-534.

Steinkraus H (1996). Keith H. Steinkraus (Eds).Handbook of indigenous fermented foods, New York, NY: Marcel Dekker: 776.

Tortora G and Funke B (2001). Case clinical microbiology: an introduction. Benjamin Cummings, San Francisco:88.

Warren P and Shadomy L (1991). Yeast fermentation broth base with carbohydrate and Durham tube. Manual of clinical microbiology. Washington D.C:5thed.