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# **African Journal of Microbiology Research**

# Review

# Technology and microbiology of traditionally fermented food and beverage products of Ethiopia: A review

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Fermented food and beverage products are made globally by using different practices, fresh materials and microbes. Fermented foods have ample sources of essential vitamins, minerals, enzymes, and antioxidants that are all enhanced through the process of fermentation. The advantageous effects related with fermented products have a special prominence during the production of these products in unindustrialized countries like Ethiopia. Therefore, coming to Ethiopia, fermented food and beverage products have practiced in a long history. During the production of traditional fermented food and beverage products, controlled natural fermentation process with the absence of starter cultures are used to initiate it. In Ethiopia, the local fermented food and beverage products are acid-alcohol type of fermentation. Moreover, the preparation of many traditionally fermented food and beverage products is still practiced in a household art thereby a wide variety of fermented foods and beverages are consumed in Ethiopia. Thus, this paper presents on the technology and microbiology of local fermented food and beverage products of Ethiopia.

Key words: Fermented food, kocho, keribo.

#### INTRODUCTION

In developed and developing countries, traditional fermented food and beverage products form an important part of the food. Therefore, these food products are prepared from plant and animal materials in which microbes play an important role by altering the material physically and nutritionally. African raw plant and animal materials are predominated by many lactic acid bacteria (LAB) and yeasts. Predominance of specific yeast species seems to be largely product specific (Christoph et al., 2017). Fermentation of row plant and animal material is one of the best and earliest methods of food

preparation and preservation. Thus, fermented foods have a role in social functions such as marriage, naming and rain making ceremonies, where they are served as weaning foods. In addition, fermentation delivers a natural way to reduce the volume of the material to be transported, to extinguish undesirable components, to improve the nutritive value and appearance of the food, to reduce the energy required for cooking and to make a safer product (Simango, 1997).

Fermented food and beverage products are made

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globally by using different practices, fresh materials and microbes. Therefore, there are only four main fermentation processes which are alcoholic, lactic acid, acetic acid and alkali fermentation (Soni and Sandhu, 1990). Alcoholic fermentation marks in the production of ethanol, and yeasts are the major organisms. Lactic acid fermentation is produced by the presence of LAB. A second group of bacteria of importance in food fermentations are the acetic acid producers from the *Acetobacter* species. *Acetobacter* spp. converts alcohol to acetic acid in the existence of surplus oxygen. Alkali fermentation frequently takes place through the fermentation of fish and seeds, popularly known as condiment (McKay and Baldwin, 1990).

The advantageous effects related with fermented products have a special prominence during the production of these products in unindustrialized countries like Ethiopia. These effects resulted in decreased miss of raw materials, minimized cooking time, enhancement of protein quality and carbohydrate digestibility, upgraded bioavailability of micronutrients and removal of toxic and anti-nutritional factors (Sanni, 1993). In addition, the probiotic effects (resistant to low pH and antibacterial activity) and the low rate of pathogenic bacteria seen in fermented food and beverage products are especially important when it comes to undeveloped countries where fermented foods have been stated to reduce the severity of diarrhea (Kimmons et al., 1999). Thus, a better understanding of the intestinal microbial populations will contribute to the development of new strategies for the anticipation and/or treatment of several diseases (De Almad et al., 2015).

Fermented food products have been renowned for their superior dietary value and digestibility compared to their row materials. Hence, fermentation of plant materials such as maize, millet, sorghum and rice, marks in enhanced protein quality, especially the level of available lysine (Hamad and Fields, 1979; Padhye and Salunkhe, 1979). And also fermentation process has the ability to improve the organoleptic properties by making different flavors in different foods (Khetarpaul and Chauhan, 1993). In most of these products the fermentation is natural and involves mixed cultures of microbes. Thus, some microbes may participate in parallel, while others act in a sequential manner with an altering dominant biota during the course of fermentation. The common fermenting bacteria are species of Leuconostoc, Lactobacillus, Streptococcus, Pediococcus, Micrococcus and Bacillus. The fungal genera are Aspergillus, Paecilomyces, Cladosporium, Fusarium, Penicillium and Trichothecium (Steinkraus, 1998). Yeasts have been reported to be involved in various types of local fermented foods and beverage products and the most dominant yeast species accompanying with African indigenous fermented foods and beverage products is Saccharomyces cerevisiae (Jespersen, 2003).

It is known that, as the people of the world, the race of Ethiopia has its own views and approaches relating to foods. Some of these are associated to foods and diseases, while others are to qualities, such as hot and cold or light and heavy foods (Gebrekidan and Gebrettiwat, 1982). However, the absence of a writing culture in most of the country marks their origin difficult to trace. Perhaps, the most accepted of the fermented foods is started at 1970s. The nature of fermentation in Ethiopia is not complex and does not required expensive equipment. During production of traditional fermented food products in Ethiopia, it is common to use and follow controlled natural fermentation process with no defined starter cultures used to initiate it. Ethiopian local fermented foods and beverages are products of acid-alcohol type of fermentation. The preparation of many local fermented foods and beverages is still practiced at household. It is known that a wide variety of fermented foods and beverages are consumed in Ethiopia.

These include injera, ergo, Ititu, ayib, qibe, arrera, kocho, tella, awaze, borde and tejj. Thus, at different time different researchers were conducted studies on the mentioned local traditional fermented food and beverage products. Bearing in mind the rich diversity in fermented food and beverage types in the country; few studies were carried out in widely different parts of Ethiopia, and included the major ethnic groups. Therefore, this paper presents the technology and microbiology of local fermented food and beverage products of Ethiopia.

#### TRADITIONAL FERMENTED PLANT FOODS

#### *Injera* fermentation

Injera is thin, fermented Ethiopian dish made from grains particularly, teff flour by mixing water and starter (ersho), which is a fluid, saved from previously fermented dough. Teff (Eragrostis tef (Zucc) Trotter) is the most widespread grain for making injera, although other grains such as sorghum, maize, barley, wheat and finger millet are sometimes used. Teff has the largest part of area (23.42%, 2.6 million hectares) under cereal cultivation and third (after maize and wheat) in terms of grain production (18.57%, 29.9 million quintals) in Ethiopia (CSA, 2008). Due to its nutrition value, there is an increasing concern in teff grain utilization. For instance, the protein is essentially free of gluten. Gluten is a protein found in wheat, rye, barley and some lesser known grains. Generally, speaking the advantages of using gluten free diet translates to better health. However, people with celiac disease or allergies find the benefits of a gluten free diet to be life sustaining. Therefore, about 66% of Ethiopian nutrition covers of injera and it accounts for about two-third of the daily

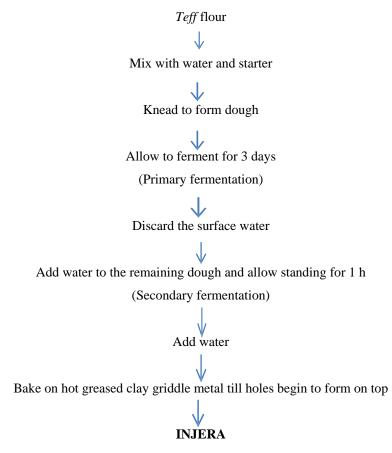


Figure 1. Flow diagram for the preparation of injera (Gebrekidan and Gebrettiwat, 1982).

protein consumption of the Ethiopian population (Arogundade, 2006).

The preparation of teff injera comprises of two stages of natural fermentation, which last for about 1 to 3 days depending on ambient temperatures. The method of making injera from its raw materials to the final product involves mixing the ingredients (teff flour and water) to dough, which is fermented and subsequently thinned to a batter. Then, the batter is poured and cooked onto a hot griddle to develop color, flavor and texture. The main quality attribute of a good injera is its somewhat sour taste due to low pH nature of injera. The storage period of injera does not exceed three days at ambient temperature (temperature in the highlands of Ethiopia is between 17 and 25°C). It is a common practice to discard moldy injera. However, in times of food scarcity, moldy injera is sun dried and prepared for consumption (Gashe, 1985).

The teff injera was prepared at household by mixing teff flour with clean water in the ratio 1:2 (w/w) and 16% of starter (ersho) and was kneaded by hand in a bowl in the traditional way. Then the resultant dough was allowed to ferment for 3 days at ambient temperature. And then, the external water formed on the upper of the

dough was discarded. The main dough was thinned by adding water equal to the original weight of the flour and stirred for 15 min. The batter was left covered for 2 h for secondary fermentation. The batter was left for about 30 min to rise (the second fermentation), before baking commenced. Then some more water was added to thin down and form the right batter consistency. Finally, about half a liter of batter was poured onto the hot clay griddle in a circular motion from the outside, working towards the centre. After 2 to 3 min of cooking using traditional baking equipment (metad), the injera was removed and stored in a traditional basket container messob (Figure 1).

According to Askal and Kebede (2013) report, a total of 34 samples from injera batter were collected during 96 h fermentation at 6 h intervals. The teff sample was bought from Hawassa open market. A total of 107 LAB and 68 yeast strains were isolated and identified. The LAB strains were identified as Pediococcus pentosaceus (49.53%),Lactobacillus fermentum (28.04%), Lactococcus piscium (5.61%), Lactococcus plantarum (4.67%), Pediococcus acidilactici (3.74%), Leuconostoc mesenteriodes subsp. mesenteriodes (2.80%),raffinolactis Lactococcus (2.80%),

mesenteriodes subsp. dextranicum (1.87%), Enterococcus casseliflavus (0.93%), and the yeast strains comprised Saccharomyces cerevisiae (48.53%), Candida humilis (22.06%), Candida tropicalis (17.65%), Saccharomyces exiguus (7.35%) and Pichia norvegensis (4.4%).

#### **Kocho fermentation**

Enset (*Ensete ventricosum*) is considered as large soft tissue plant, banana-like plant that grows up to 11 m high to the tip of the leaves (Mehtzun and Yewelsew, 1994; Admasu, 2002). Enset placed as cultivated main food crop found in south and southwestern Ethiopia (Berhanu, 1987; Almaz, 2001; Admasu, 2002). Records revealed that enset has been grown in Ethiopia for a long history (Jacob, 2004). Therefore, Enset is used for different purposes such as human food, livestock feed, industrial fiber, as rob material in fences and house-building, for mattresses and seats making, as local packaging material, and as substitute for table plates or umbrellas (Mehtzun and Yewelsew, 1994; Bizuayehu and Peter, 2003).

The plant is drought tolerant and accessible throughout the year thereby, it has the capability to serve for more number of people in the future as staple foods. Currently, the Ethiopian government has started a project on enset plant adaptation to non-enset growing regions (such as Tigray and Amhara) of the country. As a consequence, enset has increasingly growing in this non-enset region using another food security alleviating food source. In line with this, enset has a potential to use as alternative to alleviate the food security problem throughout the Nevertheless, the information microorganisms involved and the biochemical changes occurred during the fermentation processes are not well studied.

It is clear that different studies showed that, a number of people hang on this plant for their livelihood. Coming to Ethiopia, kocho is the fermented product of enset, which is the major food product, obtained by fermenting the mixture of the scraped pulp of the stem, pulverized corm and stalks inflorescence. And also the other food types obtained from enset are: bulla (extracted juice from edible part of enset) and amicho (non-fermented corm consumed after boiling). As scientific information reported that, the fermentation process of enset is carried out by different microbes. Thus, even though LAB are the dominant microorganisms involved during the fermentation of enset for kocho production, kocho contains a diverse group of microorganisms like aerobic and anaerobic spore-formers, Enterobacteriaceae, and yeast (Berhanu, 1987).

The corms of mature enset plants were used as main

raw material for the preparation of starter culture. The unwanted parts of the corm were removed with knife and then make it ready for fermentation. All the prepared corms were wrapped with fresh enset leaves near the farm site and left at ambient temperature for about 8 days. At the 5th day, it was exposed to the sun for 5 to 12 h and again wrapped with fresh enset leaves and allowed to further ferment for 3 to 5 days. Enset has two traditional processing phases such as phase I (surface fermentation), beginning of fermentation and continued for about 15 days and phase II (pit fermentation), completion of fermentation for about 15 additional days. After comprehensive fermentation of starter culture (gamancho), and ready to use, the selected enset plants were cut and processed in to fresh dough (Tiruha et al., 2014). Hence, the fermented starter was sliced and carefully mixed with fresh enset dough and distributed into treatments. The treatments were: (A) Traditional kocho fermentation in pit. (B) Fermenting mass in bucket with starter culture (not buried), (C) Fermenting mass in bucket without starter culture (not buried), (D) Fermenting mass with starter culture in bucket buried in pit, and (E) Fermenting mass without starter culture in bucket buried in pit (Figure 2).

Different studies indicated that the length of time necessary to complete kocho fermentation varied depending on the locality, environmental temperature and mass of the fermenting dough. Therefore, the majority agreed that 15 to 30 days are required for complete kocho fermentation. Thus, the fermented dough can be kept in a pit up to one year. Nevertheless, during fermentation of kocho, the value of pH was gradually decreased. In line with this, the number of microorganisms during fermentation of kocho was seen gradual increment. The decrease in pH and increase in titrable acidity during the entire kocho fermentation could be attributed to the activities of acid producing microorganisms mainly LAB and yeast. At the initial fermentation Enterobacteriaceae increased and thereafter counts of Enterobacteriaceae reached below detectable level (Tiruha et al., 2014). And also according to Negasi et al. (2017) study on in vitro characteristics of LAB isolated from Ethiopian traditional fermented shamita and kocho for their desirable properities as probiotics, the LAB like Lactobacillus, Leuconostoc, Pediococcus and Lactococcus were found in kocho. Moreover, that Lactobacillus isolates were the most frequently isolated groups from kocho samples followed by Leuconostoc.

## TRADITIONAL FERMENTED DAIRY PRODUCTS

In a long history, fermented dairy products are made from domesticated milk animals. Milk is known as the most nutritious food due to its rich nutrient content. It is an excellent source of proteins, minerals (especially calcium and phosphorus) and vitamins. It is known that,

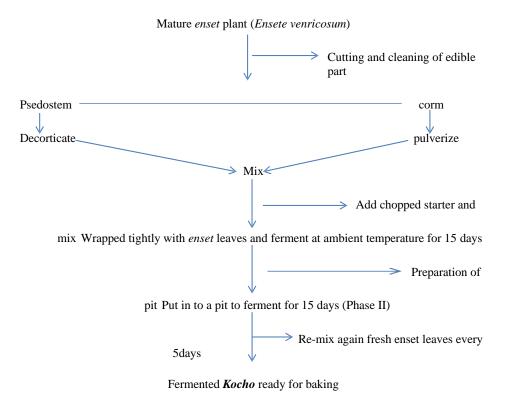


Figure 2. Flow chart of traditional enset fermentation (Tiruha et al., 2014).

fermented milk products are widely spread throughout the world. Fermentation of milk is carried out by the activities of natural flora present in the food or added from the surroundings. Hence, the microbes mainly encountered on the dairy industry are LAB, AMB, coliforms, Enterobacteriaceae, yeasts, molds and viruses. Some bacteria such as LAB are useful on milk processing, causing milk to sour naturally, and leading to fermented products. These products were an important component of nutritional food in age. Alongside, natural milk can also contain pathogenic bacteria, such as species, Mycobacterium tuberculosis. Salmonella Listeria species, and Brucella species, and can thus transmit disease and produce poor yields of products (O'Connor, 1995).

Traditional dairy fermentation process is conducted naturally without controlling the fermentation processes. Due to this, fresh milk is left at room or ambient temperature to assist fermentation process. In rural areas, especially among the pastoralists, raw milk is mostly kept in properly smoked container which is used for killing any organism remaining after washing, improves smell of equipment, improved aroma of butter when used as hair dressing and improved taste. And fermented milk from a previous fermentation uses it as source of inoculums. In addition to this, LAB from the internal surface of the container can also serve as starter culture. Regarding the quality and taste of the

fermented product, the incubation temperature does have significant role in the lowlands. In Ethiopia, the main fermented milk products include ergo, ititu, ayib, kibe, arerra, etc.

#### Ergo (Sour Milk)

Ergo is a naturally processed native Ethiopian fermented milk product, which is usually prepared at house level. It is made by natural fermentation of milk under ambient temperature. As a result, the microbial load of fermented milk samples, including Ergo, could vary from sample to sample based on the microbial number and types of microorganisms in the original raw milk (Abdulkadir et al., 2011).

During ergo fermentation, LAB were the dominant all other microorganism, followed by yeasts and then molds. Almaz (2001) showed that ergo fermentation is carried out by the genera, *Lactobacillus*, *Lactococcus*, *Leuconostoc*, *Enterococcus* and *Streptococcus* (LAB). And also the same authors also revealed that *Micrococcus* species, coliforms and spore formers were also present in fairly high numbers during the first 12 to 14 h of fermentation. Their population decreased substantial thereafter, which implies an antimicrobial activity besides low pH in the fermented milk (Savadog et al., 2004). Misganaw and Teketay (2016)

demonstrated that there is a diversity of LAB (homofermentative and hetrofermentative) in raw cow's milk. The presence of LAB in milk and milk products enhances bioavailability of nutrients, act as a preservative and serve as source for beneficial lactic acid.

According to Anteneh et al. (2011a) report during fermentation and storage of ergo, at the end of fermentation at 72 h, the pure LAB cultures decreased the mean count of the target enteropathogens by 3 log units. In addition to this, the count of all target pathogens was also decreased almost by 2, 3 and 5 log units at 24, 48 and 72 h, respectively during fermentation by mixed LAB cultures. During storage of ready-toconsume ergo at ambient condition, the count of test organisms decreased by 3 to 4 log units at 24 h; and the test strains were totally excluded within 30 to 48 h. In contrast, during storage of ergo at refrigeration condition, the average count of the test pathogens was reduced by 3 to 4 log units at 72 h. The LAB strains survived at counts of log 8.0 cfu/ml or higher up to 72 h during ambient and refrigeration conditions. Moreover, the study results showed that the strains are possible candidates for the formulation of bioprotective starter cultures that can be employed for production of safe and potentially probiotic ergo.

In line with this, Abebe et al. (2013) revealed that two LAB isolate from ergo showed antibacterial activity against clinical and standard human pathogens. The antibacterial activities of these isolates were effectively compared to Ampicillin. Excellent amount of lactic acid and H<sub>2</sub>O<sub>2</sub> was documented at 72 h incubation time. Thus, fermented ergo was the potential source of LAB that can produce antibacterial agents for the treatment of human pathogens. This can give direction for finding antibacterial agent producing microorganisms from fermented drinks use for preservation of foods as well as prevention of health risk problems associated with food borne diseases like Listeria monocytogenes, Shigella species, Staphylococcus aureus, Staphylococcal Streptococcus, Vibrio cholerae, enteritis. enterocolitica, Yersinia pseudotuberculosis, molds and noro virus (Abebe et al., 2013).

Kassahun (2013) revealed that washing of milk and milk products equipment using different kinds of plant materials are a normal practice. *Eucalyptus globules* and *Cymbopogan martini* are the major plant materials used by the majority of households in all locations. The major reason is to improve the taste and/or flavor of the milk products and/or to increase the shelf life. Traditional milk preparing techniques involve smoking of processing utensils using residues of *Olea africana*. This smoking practice is useful to keep better quality of ergo through its inhibitory effect on spoilage and pathogenic organisms. To control the harmful microbes, the effect of lower pH of ergo is more effective after 24 h of incubation. At this time, the ergo is considered

to be too sour for direct consumption since ergo coagulates within 24 h and preferably consumed at this time for its good flavor (Mogossie, 2006).

### Kibe (traditional butter)

Traditionally, Ethiopian butter is the most shelf stable of all traditionally processed of fermented dairy foodstuffs and has always been made from sour milk. It is semisolid at room temperature with white and sometimes yellowish color, depending on age. It has a typical diacetyl taste and flavor when fresh, but extended storing at room temperatures results in putridity and rancidity. Butter is important component of Ethiopian traditional fermented milk products (Gemechu et al., 2017). This traditional milk product processed and sold by women in every society. It has been used by women for hair dressing and also used as the source of diet both in rural and urban areas, and is also utilized by children of weaning age and the elderly.

The moisture content of the traditional dairy product, batter ranges from 20 to 43% as compared to the international commercial standard for butter of 16%. Putrefactive microorganisms cause spoilage of kibe when stored at room temperature in age. However, it is highly stable against microbial spoilage after 2% salt addition, low moisture and nitrogen ratio. But microbes having lipolytic activity are highly liable for disorders such as rancidity or loss of flavor. In general, the microbiological information on this product is not common in Ethiopia. However, there are some studies published on the microbiological quality of traditional butters from the country (Almaz, 2001).

According to Zelalem (2010) report the average total bacterial counts (TBC) ranged from 6.18 log cfu/g in butter samples collected from Selale area to 7.25 log cfu/g samples from Sululta. On other studies, the average TBC of 7.49 log cfu/g and the presence of high variability among samples depending on the sources were reported. Samples collected from open markets and rural producers, for instance, had higher counts as compared to that obtained from dairy farms and urban producers of southern Ethiopia. In addition to this, the TBC of fresh butter sampled from rural and public butter markets in Addis Ababa ranged from 8.27 to 4.7 log cfu/g of butter (O'Mahoney, 1998).

# Arera (defatted sour milk)

Arrera is another byproduct of ergo obtained after removal of kibe after churning. It has a similar color to ergo, but its appearance slightly smoother, although thicker than fresh milk and basically contains the casein portion of milk. In contrast to other traditional dairy products, arrera has fewer calories. It contains

91.5% moisture, 3.1% protein, 1.4% fat, 3.4% carbohydrate, and 0.6% ash (EHNRI, 1997). And also arrera has a shorter shelf life compared to all other fermented milk products (only 24 to 48 h). It is consumed in all parts of the country where fermented milk is made and it serves as a beverage either plain or spiced. It is chosen by women for consumption as a side dish or as drink. Extra of the products are given to calves, lactating cows and dogs. However, it may indirectly serve as additional income for the women by its use as raw material for cottage cheese (ayib) manufacture, which may be sold in the market. Due to its relatively short shelf life and some traditional beliefs, arrera is not sold in the market for direct consumption (Almaz, 2001).

The average counts of total bacteria, Enterobacteriaceae and coliforms were greater than 9, 4.7 and 4.2 log cfu/ml, respectively of arera sampled from Addis Ababa (Zelalem, 2010). Similar study showed that arera sampled from Wollayta area had total bacterial count of about 9 log cfu/ml (Rahel, 2008). The same author also reported coliform count of 4.86 log cfu/ml. Different species of bacteria were identified in arera samples collected during both dry and wet which include Klebsiella pneumoniae. seasons. Klebsiella oxytoca, Escherichia cloacae, Escherichia sakazakii, Escherichia coli and some species of Salmonella (Zelalem et al., 2007).

#### Ayib (Ethiopian cottage cheese)

Ayib, a traditional Ethiopian cottage cheese, is a popular milk product consumed by the several local groups of the country. It is prepared from sour milk after the butter is removed by churning. Churning of the sour milk is carried out by slowly shaking the contents of the pot until the butter is separated. The defatted milk is heated to about 50°C until a distinct curd forms. It is then allowed to cool slowly, and the curd is filtered through a muslin cloth. Ayib comprises 79% water, 14.7% protein, 1.8% fat, 0.9% ash and 3.1% soluble milk constituents (O'mahoney, 1988).

In a study on the microbiological quality of ayib (Mogessie, 1990), samples collected from an open market in Awassa had counts of mesophilic aerobic bacteria (AMB), yeasts and enterococci of 10<sup>8</sup>, 10<sup>7</sup> and 10<sup>7</sup> cfu/g, respectively. Above 60% of the samples had psychotropic count of 10 log cfu/g and about 55% of the samples were positive for coliforms and fecal coliforms. The pH values of the samples varied between 3.3 and 4.6 with about 40% having pH lower than 3.7. During preparation of ayib, the high initial count of microbes in milk, which raises the fermentation process, is shown to fall by the combined action of cooking and low pH. The presence of high microbial load of ready-to-consume ayib is assumed to be introduced from plant

parts used for packaging and imparting flavor, and from handlers, too. Its low pH value should also assist in maintaining the low count for a certain period of time (Anteneh et al., 2011b).

Further analysis of ayib micro flora revealed that bacterial and yeast counts did not relate with pH value of ayib samples (Mogessie, 1994). It is clear that ayib samples with pH greater 4.0 contained more bacterial groups than those with pH less than 4.0 (Anteneh et al., 2011b). The Gram-positive rods dominated the aerobic mesophilic bacterial flora, being the most abundant. Enterobacteriaceae and Pseudomonas species constituted the bulk of the Gram-negative rods. The count of LAB was around  $10^6$  cfu/g and L. fermentum and L. plantarum dominated the flora. Though the low pH of ayib inhibits the growth of many food-borne pathogens, higher numbers of LAB and yeasts are not desirable in ayib. A considerably lower pH due to the activity of LAB may result in a too sour product with a low sensory quality.

Study done by Anteneh et al. (2011b) showing the incompatible effect of mixed lactic cultures against foodborne pathogens (Escherichia coli, Salmonella Typhimurium DT104 and S. aureus) were evaluated during preparation and storage of ayib. Ayib was prepared by cooking pasteurized milk product with the numerous mixed starter cultures. The test pathogens were separately inoculated in duplicates into 200 g of cooled ayib in sterile stomacher bags to give initial inoculum level of 6 log cfu/g mixed thoroughly and incubated at ambient condition. Separately, ayib samples was similarly processed and stored at refrigeration condition. Enumeration of pathogens was done at 24 h intervals for 9 days. When counts were clug1 cfu/g, enrichment followed by streaking on the nutrient agar plates were done to determine complete inhibition (Mekonnen and Mogosie, 2005).

In milks soured in the presence of mixed starter LAB cultures, the test organisms (TOS) were reduced by 2 and 4 log factors at 24 and 48 h, respectively. The pH of souring milk was about 4.0 at 48 h. In the control milks (milks inoculated with test pathogens in the absence of LAB), the test pathogens grew to 8.4 log cfu/g at 48 h. The mean pH value of control milks dropped from initial 6.44 to 5.43 (Table 1).

#### Ititu fermentation

Ititu is popular fermented camel milk consumed by pastoral communities of the Kereyu area of the Oromia Region in the eastern part of Ethiopia (Eyassu et al., 2012). The people prepare ititu during the rainy season (Almaz, 2001). Ititu has good nutritional quality and wait for about two months at ambient temperature (25 to 30°C). It is served as side dish with traditional thin-baked cereal chips or consumed as food or drink alone. And also it is considered as one of the special foods and

**Table 1.** The inhibitory effect of mixed LAB cultures on the test organisms during souring of milk (Anteneh et al., 2011a).

| Tast summisms         | Average values |                |       |                |       |                |
|-----------------------|----------------|----------------|-------|----------------|-------|----------------|
| Test organisms        | 0 h            |                | 24 h  |                | 48 h  |                |
| (TOS)-MLCs            | рН             | Count (log/ml) | рН    | Count (log/ml) | рН    | Count (log/ml) |
| E. coli - MLCs        | 6.42           | 3.57           | 5.24  | 5.56           | 4.21  | 4.78           |
| S. Typhimurium - MLCs | 6.47           | 3.61           | 5.23  | 5.32           | 4.24  | 4.74           |
| S. aureus - MLCs      | 6.47           | 3.53           | 5.47  | 5.65           | 4.20  | 5.30           |
| SD1                   | ±0.01          | ±0.17          | ±0.31 | ±0.19          | ±0.14 | ±0.36          |
| TOs alone (control)   | 6.44           | 3.30           | 5.97  | 7.73           | 5.43  | 8.48           |
| SD2                   | ±0.04          | ±0.18          | ±0.06 | ±0.22          | ±0.13 | ±0.11          |

SD: Standard deviation.

Table 2. LAB species isolated from the traditional fermented camel milk ititu (Eyassu et al., 2012).

| Species                                     | Number of isolates | % of total isolates |
|---|--------------------|---------------------|
| Lactobacillus salivarius                    | 47                 | 32.3                |
| Lactobacillus plantarum                     | 13                 | 8.9                 |
| Lactobacillus delbrueckii subsp. Bulgaricus | 25                 | 17                  |
| Lactococcus lactis subspecies cremoris      | 10                 | 6.8                 |
| Lactococcus lactis subspecies lactis        | 26                 | 17.8                |
| Enterococcus faecalis                       | 25                 | 17                  |
| Total                                       | 146                | 100                 |

served to much respected guests as well as to weaningage children and elderly.

During the traditional fermentation of ititu, fresh milk is collected in a well smoked fermenting vessel called gorfa. Gorfa is woven from fibers of selected plants into a lidded container with a capacity up to three litters. The lid of the gorfa is treated with leaves of *Ocimum basilicum* for cleaning and imparting desirable flavor to the product (Kassaye et al., 1991). When the milk coagulates, whey is removed by wooden pipette and an additional volume of fresh milk is added. Eyassu et al. (2012) reported that genus *Lactobacillus*, genus *Lactococcus* and genus *Entrococcus* carried out the souring process of ititu. Thus, *Lactobacillus* species was the dominant genus and comprised of 58% of the total LAB isolates (Table 2).

Lactobacillus salivarius, L. plantarum, Lactobacillus delbrueckii subsp. bulgaricus, Lactococcus lactis subsp. lactis, and Enterococcus faecalis are the isolated LAB species from ititu. Hence, L. salivarius was a comparatively fast acid producer bringing the initial pH of the skim milk to the final value of 4.6 before 48 h of incubation followed by L. plantarum and L. delbrueckii subsp. bulgaricus.

According to Kassaye et al. (1991) report, ititu had an average pH of 3.65, titratable acidity (as lactic acid) of 1.92%, fat and protein content of 9.05 and 7.17%, respectively. Moreover, these values varied significantly

among samples, though. Ititu had improved contents of free and total amino acids when compared with fresh whole milk and was rich in amino acids such as glutamic acid, alanine, proline, leucine and serine. In a study on farm-made fermented milk in southern Ethiopia, Fekadu and Abrahamsen (1997) reported that ititu had 3.3 to 3.7% fat, 3.3 to 3.6% protein and 3.3 to 3.5% lactose. Kassaye et al. (1991) further reported that the total bacterial count was 10<sup>12</sup> cfu/g, mainly dominated by LAB.

#### **Dhanaan fermentation**

Dhanaan is naturally fermented sour milk produced by pastoralists in the areas of Shinile and Jigjiga zones of eastern part of Ethiopia (Seifu, 2007). It has well nutritional quality and stay up to five months. Dhanaan is made by placing fresh camel milk in a smoked container, packaging the container with a piece of cloth and keeping it in ambient temperature place for about 12 to 24 h to allow spontaneous fermentation. Similar products from camel milk were reported from Kenya, Somalia and Sudan. Naturally fermented camel milk products, namely susac and shubat are produced in Kenya, Somalia and Sudan. Similarly, fermented sour milk called gariss is prepared from camel milk in Sudan by placing raw camel milk in a skin bag hitched to the

saddle of a camel that is allowed to go about its business (Abdulkadir et al., 2011).

In Ethiopia, no study was conducted about the bacterial characteristics, manufacturing protocols and potentials of the fermented camel milk product, dhanaan. Pastoralists prepare dhanaan from camel milk because they consider that it has high nutritional value and long shelf life, it enables collection of milk over a few days and thus facilitates delivery of milk to the market, it eliminates seasonal surpluses of milk, its taste is liked by the consumers, it has high demand in the market especially by urban dwellers, and it reduces thirst.

Dhanaan is made by natural fermentation without adding a starter culture. However, some of the producers mentioned that when a small amount of previously fermented milk is added as a starter into fresh camel milk it takes only 6 h to obtain dhanaan (Seifu, 2007). Kenyan researchers reported that the quality of susac improved using selected mesophilic lactic starter cultures rather than spontaneous fermentation; the resulting fermented milk had a uniform taste and a longer shelf life (Farah et al., 1990; Lore et al., 2005). Screening of microorganisms that is responsible for the fermentation and production of the dhanaan would help to develop a commercial starter culture and to standardize the manufacturing method for this product in the future. The producers also mentioned that during making dhanaan, the milk in the container should be kept closed. This suggests that the microorganisms responsible for souring or fermentation of camel milk are probably thermophilic anaerobic types.

#### TRADITIONAL FERMENTED BEVERAGES

#### Tella fermentation

Tella is popular Ethiopian traditional beverages, which is made from diverse ingredients. It is, by far, the most commonly consumed alcoholic beverage in Ethiopia. It is assumed that over two million hectoliters of tella to be brewed annually in households and drinking houses in Addis Ababa alone (Shale and Gashe, 1991). Some of them consider as local beer. It is traditionally drunk on major religious festivals, saint's days and weddings.

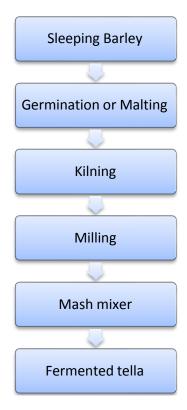
Depending on the type of cereal ingredients used to make, tella has different names: Amhara tella, Oromo tella, and Gurage tella (Fite et al., 1991). Amhara tella has gesho (*Rhamnus prinoides*) and concentrated. Gurage tella is delicately aromatized with a variety of spices. Oromo tella has no gesho (*R. prinoides*), and it is thick and sweet (Vogel and Gobezie, 1983). Generally, tella is brewing from substrates such as barley, wheat, maize, millet, sorghum, teff or other cereals. The quality of tella is variable from local to local, from individual to individual. Even within the same individual, the quality is variable from time to time.

Therefore, the way of making tella varies among the ethnic groups and depends on traditional and the economic situation. The clay container (insera) is washed with grawa (Vernonia amygdalina) and water numerous times and then smoked with wood from weyra (Olea europaea subsp. cuspidate) for about 10 min, in order to get it as clean as possible. Germinated grain of barley, or corn, or wheat (bikil), bought in the local market or prepared at home, are dried and milled. For making bikil, the grains are moistened in water and the moist grains are placed between fresh leaves, left to germinate for 3 days and after that dried. Gesho (R. prinoides), local hops, is available dried in the local market. The leaves of gesho are separated from the stem and dried again in the sun for about 1/2 h and then pounded. The ground gesho leaves are placed in a clay container with water and left to ferment for 2 to 3 days. Gesho is responsible for the bitter taste of tella. It is also thought to be the source of various chemicals (Sahle and Gashe, 1991; Kebede, 1994). It is assumed that gesho maintains acidic pH during tella fermentation so as to modify the nature of the mash and impedes the growth of unwanted microorganism (Kebede, 1994).

Some of the grains intended for tella preparation are toasted and milled, and then mixed with water and baked on the mitad to prepare what is known as kita (a thin, 5 to 10 mm thick, pancake- like bread). This kita, broken into small pieces, part of the milled bikil and the pounded gesho stems are added to the water and allowed to ferment for 1 to 2 days. The rest of the flour is toasted on mitad, sprinkled with water and toasted until dark brown to form what is known as enkuro. This mixture of enkuro, the rest of the germinated grains (bikil), some gesho, and water are added to the container. The mixture is kept covered overnight, after which more water is added and the container is kept sealed for 5 to 7 days, until when the beverage is ready. Tella can be kept for 10 to 12 days (Figure 3).

According to Shale and Gashe (1991), who made a detailed study of tella fermentation, there are numerous recipes for preparing tella and it appears as if every housewife has her own version of the recipe. The fermenting organisms of tella are composed of *S. cerevisiae* and *Lactobacillus* spp. Increase in ethanol content (2.2 to 5% (v/v)) is directly associated with growth in the population of yeasts and decrease in reducing sugar and total carbohydrate. The pH of tella is in the range of 4.5 to 4.8 (Debebe, 2006).

For tella considered to be a good quality, the final ethanol content is in the range of 2 to 8% (v/v) and pH is 4 to 5. The biochemical changes, the microorganisms involved in the fermentation and those which bring about necessary and unwanted changes in the process of tella making are described (Shale and Gashe, 1991). According to the report, the fermentation process of tella is divided into four phases. The first occurs in the original



**Figure 3**. Flow chart of traditional preparation of tella.

mixtures of ingredients, and the second and third phases occur after successive additions of more carbohydrate materials. The three main carbohydrate materials are mentioned to be bikil, kitta and enkuro. The latter phase is where acidification takes place, which is actually not desirable. Maximum ethanol production occurs during the third phase and at the beginning of the fourth phase.

Shale and Gashe (1991) reported that the extent of heat treatment the asharo (roasted barley) receives and the degree of steaming the enkuro (roasted barley steamed after grinding) is subjected to have the direct bearing on the color of tella, which is determined by the housewife preparing the tella. Tella is actually a beverage of variable viscosity and having a variety of colors (grayish-white to dark brown). Several samples of tella and other traditional alcoholic beverages collected from three regions of Ethiopia (Gojam, North Shoa, and Addis Ababa) were analyzed for their ethanol, methanol, and fuel oil contents by Fite et al. (1991). The mean values for methanol, fuel oil, and ethanol were found to be 35 ppm, 66 ppm, and 3.6%, respectively.

According Abegaz and Kebede (1995) report there were no microbes at the end of tella fermentation phase, especially in tella made with gesho. This is because of the synergic effect of both *R. prinoides* antibacterial substance, high alcohol concentration,

reduction of pH as fermentation time increases and reduction of nutrient content of tella. Moreover, yeasts were dominated at the middle of tella fermentation phases but at the last phase, they were dramatically reduced. Gesho and bikil (malt) were main sources of yeasts and bikil was the major source of LAB. Acetic acid bacteria were not detected from any ingredient; similarly Enterobacteriaceae and yeasts were not detected in ashero and kita. Therefore, the counts of Lactobacillus, Lactococcus, yeasts and AMB showed increment during the first two phases in both fermenters but gradually reduced at phase IV in both fermenters. The counts of Enterobacteriaceae were high at day zero and not detected at phase II in both fermenters. Acetic acid bacteria were detected at the beginning of phase II in traditional fermenter but at phase III in modified fermenter. In line with this, Belay and Wolde (2014) indicated that based on the nature of the ingredients of tella, the distribution of the microbial community is variable (Table 3).

# Tej fermentation

Tej is a honey wine with alcohol content varying from 8 to 14% ABV, which is made from honey, water and leaves of gesho. Previously, upper class were used, but now it is widespread among all social groups, consumed on holidays and at weddings as well as served in hotels and bars across the country. It is a home-based as well as commercially available honey wine. So tej is mainly used for great feasts, such as weddings and the breaking of fasting. Sometimes, widely for commercial purposes, mixture of honey and sugar could be used for its preparation. In cases where sugar is used as part of the substrate, natural food coloring is added so that the beverage attains a yellow color similar to that made from honey (Fite et al., 1991).

Tej fermentation, like other traditional beverages of Ethiopia, is a natural fermentation and no starter culture or other modern techniques are used. So, the fermentation depends upon microorganisms the present in the environment. Thus, to determine the major source of the yeast cells in tej attention was given to honey and gesho. The dominant yeast, S. cerevisiae, counts ranged from 10<sup>2</sup> to 10<sup>3</sup> cfu/g in gesho samples while 0 to 10<sup>2</sup> cfu/g in honey samples. And gesho was considered the major source of the dominant yeast in tej because it contained greater number of the yeast than honey.

According to Vogel and Gobezie (1983), during the preparation of *tej*, the fermentation pot is seasoned by smoking over smoldering *R. prinoides* stems and olive wood. One part of honey mixed with 2 to 5% (v/v) parts of water is placed in the pot, covered with a cloth for 2 to 3 days to ferment after which wax and top scum is removed. Some portion of the must is boiled with washed and peeled *R. prinoides* and put back to the

| Ingredient | Enterobacteriaceae | Yeasts          | Lactobacillus   | Lactococcus     | Acetic acid bacteria | Aerobic mesophilic counts |
|------------|--------------------|-----------------|-----------------|-----------------|----------------------|---------------------------|
| Gesho      | $5.86 \pm 0.01$    | 5.55± 0.21      | $0.00 \pm 0.00$ | $0.00 \pm 0.00$ | $0.00 \pm 0.00$      | 5.85 ± 0.91               |
| Bikil      | $5.88 \pm 0.11$    | $5.77 \pm 0.10$ | $5.52 \pm 0.32$ | $5.79 \pm 0.00$ | $0.00 \pm 0.00$      | $5.97 \pm 0.63$           |
| Kita       | $0.00 \pm 0.00$    | $0.00 \pm 0.00$ | $0.00 \pm 0.00$ | $0.00 \pm 0.00$ | $0.00 \pm 0.00$      | $4.8 \pm 0.12$            |
| Ashero     | $0.00 \pm 0.00$    | $0.00 \pm 0.00$ | $0.00 \pm 0.00$ | $0.00 \pm 0.00$ | $0.00 \pm 0.00$      | $4.83 \pm 0.34$           |

Table 3. The distribution of Microflora of the major ingredients of tella (Belay and Wolde, 2014).

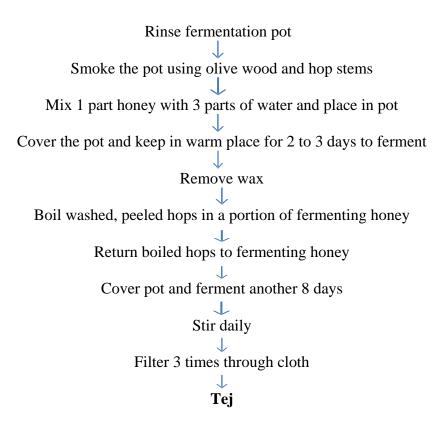


Figure 4. Flow chart of traditional preparation of tej (Vogel and Gobezie, 1983).

fermenting must. The pot is covered and fermented continuously for another 5 days, in warmer weathers, or for 15 to 20 days, in colder cases. The mixture is stirred daily and finally filtered through cloth to remove sediment and *R. prinoides*. Good quality tej is yellow, sweet, effervescent and cloudy due to the content of yeasts. The flavor of *tej* depends upon the part of the country where the bees have collected the nectar and the climate (Figure 4).

According to Bekele et al. (2006) a total of 200 samples of tej, an indigenous Ethiopian honey wine, was collected from ten production units at different production times. The pH values of samples varied between 3.07 and 4.90 and 77% of the samples had pH values <4.0. Therefore, change in pH value among all samples was significant (p<0.01). The variety for titratable acidity

was 0.1 to 1.03 g/l00 ml and mean values for the different production units were 0.34 to 0.6 g/l00 ml. About 65% of the samples had titratable acidity values of 4 g/l00ml and variations within samples of production units (CV>10%) or among all samples (p<0.01) were significant. Mean total alcohol content for the various production units was 6.98-10.9%. About 58% of the samples had alcohol content of 5 to 10%.

In line with this, the antagonistic activities of LAB studied by Abebe et al. (2013), a total of 18 ergo and tej samples were collected from Gondar town. During fermentation of ergo and tej, lactic acid and  $\rm H_2O_2$  were able to hinder the growth of all clinical and standard human pathogens. The antibacterial activity of these was effective compared to ampicillin. Therefore, according to their report, much amount of lactic acid

| 11-1     | la sub etter berne | Measurements in mg |                   |  |  |
|----------|--------------------|--------------------|-------------------|--|--|
| Isolates | Incubation hours   | Lactic acid        | Hydrogen peroxide |  |  |
|          | 24                 | 810.72             | 1.93              |  |  |
| 2        | 48                 | 873.78             | 2.14              |  |  |
|          | 72                 | 1396.24            | 1396.24           |  |  |
|          | 24                 | 720.64             | 3.10              |  |  |
| 3        | 48                 | 747.66             | 3.32              |  |  |
|          | 72                 | 810.72             | 5.35              |  |  |

**Table 4.** Determination of lactic acid and  $H_2O_2$  at 37°C for different incubation hours (Abebe et al., 2013).

and  $\rm H_2O_2$  were recorded at 72 h incubation time. Thus, isolation and screening of LAB from potential fermented drinks are the sources of antibacterial agents for the treatment of human pathogens (Table 4).

#### **Borde fermentation**

Borde is a local beer mostly consumed by people in southwestern parts of the country. It is considered as a drink for people in the lower socio-economic status. Borde is prepared by women from fermented maize, sorghum, barley, or a mixture of the three. Borde can be very thick and serve as a substitute for meals during long trips. According to the villagers attitude borde is also used for medical and ritual purposes. The users consider that it enhances lactation and mothers are encouraged to drink substantial amounts of it after giving birth (Kebede et al., 2002).

Borde is produced by natural fermentation of a diversity of locally available cereal ingredients. It is a gassy whitish-grey to brown colored beverage with thick consistency and sweet-sour taste. Fermentation of borde has four phases marked by the introduction of ingredients into the fermentation pot at different times. In phase I (primary fermentation), maize grits were mixed with water and left to ferment at ambient temperature in a clean insira for 48-72 h. A portion of the fermented grits from phase I (48 h) was roasted on a mitad into enkuro, a well-roasted granular mass. Fresh malt flour and water were carefully mixed by hand in a smoked insira into a pale brown thick mash. This mixture is called tinsis and it was left to ferment for 24 h. A second portion of the fermented grits from phase I (68 h) was slightly roasted into enkuro, carefully kneaded with mixed flour (wheat, finger millet and teff) and water, and then moulded into stiff dough balls. The dough balls were steamed into gafuma and then broken into pieces. Pieces of cooled gafuma were blended with the fermented tinsis and additional water in the same insira to a thick brown mash called difdif. The difif was then allowed to ferment for 18 h.

The last portion of fermented grits from phase I (72 h)

was added into a pan containing a boiling mixture of whole grains of sorghum and water, further boiled into a very thick porridge with continuous stirring and then cooled. The gelled porridge was added to the fermented difdif, along with a small amount of additional malt. After a thorough mixing, the thick brown mash was sieved through a wonnfit (about 1 mm pore size). The residues were then wet milled using traditional grinding stones and sieved 2 times. The filtrates were pooled and poured back into the same rinsed insira and the fermentation was then continued for 6 h (after the addition of porridge). Actively fermenting effervescent was then ready for consumption. The production of borde was repeated three times at room temperature (20- 23°C) and the results are average of the triplicates. In addition, preliminary experiments were carried out to compare the following: (1) the 48 to 72 h fermentation with 24 to 48 h at Phase I; (2) earthenware pot with plastic, metal and glass jars; (3) substitution of maize grits and sorghum grains with flour; and (4) roasting of enkuro with baking of kita (flat bread).

According to Kebede et al. (2002) report samples of borde from open markets at five localities in southern Ethiopia showed average aerobic mesophilic count (AMC), LAB and yeast counts of 9.9, 10.1, and 8.1 log cfu/g respectively. Enterobacteriaceae were <1 to 3.5 log cfu/g. The pH was 3.92±0.14. During the traditional production of borde with its four phases, the proportions of ingredients and cooking temperature were measured. Development of pH, titratable acidity, microbial load and mash temperature were monitored at 6 h intervals. The initial pH of 6.01 fell to 3.84 at end of Phase I. However, the pH increased at the start of Phase II, III and IV fermentations due to addition of malt and/or unmalted cooked ingredients and then decreased to below pH 4.0 at the end of each phase. During Phase I, EB increased from 5.1 to 7.7 log cfu/g at 24 h, but were not detected after 48 h. AMC, LAB and yeasts increased from their initial 6.5, 5.3 and 4.5 respectively to 10.5, 10.6 and 7.5 log cfu/g at end of Phase I. The AMC of cooked ingredients were 4.6-4.9 log cfu/g, while Enterobacteriaceae, yeasts and LAB were not detected.

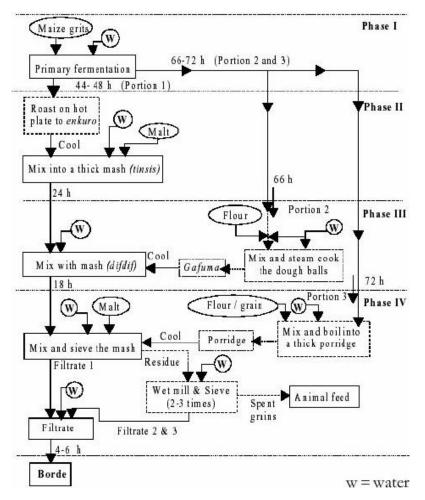


Figure 5. Flow charts of traditional preparation of borde (Kebede et al., 2002).

After mixing the cooked ingredients and malt, the AMC, LAB and yeasts increased from 7.1, 6.3 and 5.4 atPhase II to 10.5, 10.5 and 8.6 log cfu/g in borde, respectively. Enterobacteriaceae decreased from 5.2 to <1 log cfu/g at Phase II and were not detected in borde. The major roles of Phase I, II, III and IV are production of an acidic fermented mass, bulk starter production, main and corrective fermentations, respectively (Figure 5).

#### Shamita fermentation

Shamita is another traditional beverage of Ethiopia, which is low in alcohol content, made by overnight fermentation of mainly roasted barley flour and, consumed as meal-replacement (Ketema et al., 1999). Shamita is a widely consumed beverage in different regions of Ethiopia. It has a thick consistency and most people who cannot afford a reasonable meal consume it as meal replacement. It is produced by fermenting roasted barely overnight. Malt is not

commonly used in shamita fermentation, although local shamita brewers in Addis Ababa use it frequently, and starch is the only principal fermentable carbohydrate.

The microbes liable for fermentation are mostly from back slopping using small amount of shamita from a previous fermentation as well as from the ingredients and equipment. Ready to consume shamita has a high microbial count made up of mostly LAB and yeast. These microorganisms make the product a good source of microbial protein. However, shamita has poor keeping quality because of these high numbers of live microorganisms and becomes too sour about four hours after being ready for consumption (Mogessie and Tetemke, 1995).

According to Anteneh et al. (2011c) study on antagonism of LAB against foodborne pathogens during fermentation and storage of borde and shamita, pure LAB cultures decreased in average the number of test pathogens by 4 log cycles at 24 h during fermentation shamita. And also the mixed LAB cultures decreased the number of pathogens by 5 log units after 24 h of fermentation shamita. Coming to storage of shamita at

|      |      | Lactobacilli         |                     |                            |                     |                     | D:!!                 |
|------|------|----------------------|---------------------|----------------------------|---------------------|---------------------|----------------------|
| Time | рН   | Homo fermentative    | Hetero fermentative | Streptococci               | Micrococci          | Staphylococci       | <i>Bacillus</i> spp. |
| 0    | 5.80 | 6.44×10 <sup>5</sup> | 3.0×10 <sup>4</sup> | < <b>x</b> 10 <sup>2</sup> | 1.5×10 <sup>5</sup> | 2.1×10 <sup>5</sup> | 2.4×10 <sup>4</sup>  |
| 4    | 5.52 | 6.9×10 <sup>6</sup>  | 3.1×106             | < <b>x</b> 10 <sup>2</sup> | 7.2×10 <sup>5</sup> | 2.5×10 <sup>5</sup> | 3.6×10 <sup>4</sup>  |
| 8    | 4.77 | 7.6×10 <sup>6</sup>  | 1.1×108             | < <b>x</b> 10 <sup>2</sup> | 1.1×10 <sup>6</sup> | 5.4×10 <sup>4</sup> | 2.6×10 <sup>4</sup>  |
| 12   | 4.43 | $3.2 \times 10^{7}$  | 1.3×108             | < <b>x</b> 10 <sup>2</sup> | 1.6×10 <sup>6</sup> | 1.9×10 <sup>4</sup> | 2.2×10 <sup>4</sup>  |
| 16   | 4.26 | $4.4 \times 10^{7}$  | 1.8×108             | < <b>x</b> 10 <sup>2</sup> | 2.9×10 <sup>6</sup> | 1.8×10 <sup>4</sup> | $2.1 \times 10^4$    |
| 24   | 4.03 | 1.1×10 <sup>9</sup>  | 2.2×108             | < <b>x</b> 10 <sup>2</sup> | 5.0×10 <sup>4</sup> | 1.4×10 <sup>4</sup> | 4.6×10 <sup>3</sup>  |

Table 5. Changes in counts (cfu/ml) of major microorganisms during shamita fermentation (Ketema et al., 1999).

ambient temperature, the test pathogens were reduced by 4 log units at 12 h and totally eliminated at 24 h. Therefore, they strongly suggest that the isolates are possible candidates for the formulation of starter cultures that can be used to produce safe and bioprotective products.

According Mogessie and Tetemke (1995), report the pH of ready to consume shamita in Awassa town was reported to be 4.2 and the product had high microbial counts (10<sup>6</sup> to 10<sup>7</sup> cfu/ml) consisting mainly LAB and yeasts. In a microbiological study of shamita fermentation, Ketema et al. (1999) reported that all ingredients and the clay jar rinse water had large numbers of aerobic mesophilic bacteria (>10<sup>4</sup> cfu/ml) mainly consisting of *Bacillus* and *Micrococcus* spp. Barley malt contributed most of the LAB and yeasts, which were important to the fermentation. They dominated the fermentation flora reaching final counts of 10<sup>9</sup> and 10<sup>7</sup> cfu/ml, respectively (Table 5).

In line with this, according to Negasi et al. (2017) study on *in vitro* characteristics of lactic acid bacteria isolated from traditional fermented shamita and kocho for their desirable characteristics as probiotics, the genera *Lactobacillus*, *Leuconostoc*, *Pediococcus* and *Lactococcus* were present in shamita. And also *Lactobacillus* isolates were the most frequently isolated groups from shamita.

#### Keribo fermentation

It is known that 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. Thus, among the various fermented beverages, keribo is traditional fermented beverage produced mainly from barley and sugar in different parts of the country, including Jimma zone. It constitutes a main part of the beverages being served on holidays, wedding ceremony and also as sources of income of

many households. The popularity of this traditional fermented beverage is more reflected among the religious groups and those do not like alcoholic drinks. It has poor keeping quality with distinct characteristic of the deteriorating beverage at the end of 48 h of fermentation (Kebede et al., 2002).

From the traditional Ethiopian fermented beverages, the fermentation processes and microbial dynamics during fermentation of tella (Samuel and Berhanu, 1991), borde (Ketema et al., 1998) and shamita (Ketema et al., 1999) are described. Moreover, the safety consideration of Ethiopian foods and beverages has shown the possibility of isolating some foodborne pathogens from some fermented products. However, there is no scientifically documented information both on the microbiology and safety of keribo preparation.

During keribo preparation, barley was first washed of broken kernels, chaff and extraneous materials. Then the deeply roasted barley is added to boiling water and continued boiling for 10 to 20 min at 65 to 70°C until the ungrounded grain seems to be dissolved. Finally, it is allowed to cool and sieved. To the filtrate, sugar and yeast were added, thereafter, the container is sealed (to create anaerobic conditions) and left to ferment overnight. The final product can then be poured into bottle for sale or for consumption the next day but before consumption, some amount of sugar was added to the fermented product until it become sweet. The preparation process was easy to follow and it took one day to get it fully fermented under optimum temperature (Figure 6).

According to Rashid (2013) report samples of keribo from open markets and households in Jimma zone showed average LAB, AMB, aerobic spore formers (ASF) and yeasts with mean counts of (log cfu m/l)  $2.70 \pm 2.07$ ,  $2.34 \pm 2.37$ ,  $4.96 \pm 2.80$  and  $2.01 \pm 0.60$ , respectively. But the mean counts Enterobacteriaceae, staphylococci and molds 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. L. mesenteroides, identified as the most dominant LAB, were found to be susceptible to

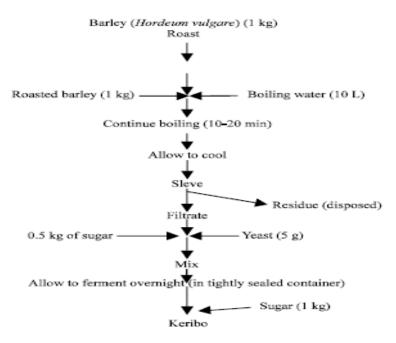


Figure 6. Flow chart of traditional keribo fermentation (Abafita, 2013).

**Table 6.** Microbial count (log cfu m/l) of different microbial groups detected in keribo (Rashid, 2013).

| 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.

penicillin, gentamicin, ampicilin, chloramphenicol, amikacin, bacitracin and norfloxacin but resistant to vancomycin (Table 6).

#### Korefe fermentation

Korefe is the name of the traditional indigenous fermented beverage which is prepared in Begemder Province among the Koumant ethnic group. Dehusked barley is left in water overnight, and after that toasted and milled. And then mixed with water and dried gesho leaves, and fermented in a clay container for two to three months. When the beverage is needed, a small quantity of the mixture is taken, more water is added and after a day of fermentation, the beverage is ready for

Dehusked barley is left in water overnight

Toasted and milled

Mixed with water and dried gesho leaves

Fermented in a clay container for two to three months

Korefe

Figure 7. Flow chart of traditional korefe fermentation.

consumption (Figure 7).

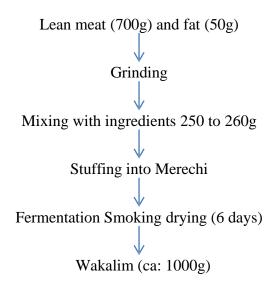


Figure 8. Flow chart for the production of wakalim.

#### TRADITIONAL FERMENTED MEAT PRODUCTS

# Sausage fermentation

Sausages are essential parts of foods in many regions of the world. Coming to our country, Ethiopia, Sausage production has only a recent history. Traditional sausage (wakalim) is the most popular fermented food product in Harari, eastern part of Ethiopia. Its preparation relies on natural fermentation with ingredients as the main source of inocula. The preparation has four-step that includes the preparation of a casing, mincing of meat, stuffing and fermentation. Sausage fermentations are characterized by the succession of microbial groups in the course of fermentation. Although several groups are involved in the initiation of fermentation, only those tolerant to acids and metabolites generated during fermentation survive and dominate the final microflora. In naturally fermenting beef sausage, raw meat yields LAB in low numbers. However, the lactic flora rapidly dominates the fermentation because of the anaerobic environment generated during fermentation (Hammes et al., 1995).

Wakalim is prepared using the following ingredients (g/kg) following traditional techniques: lean meat (700 g), mixed with fat (50 g), salt (20 g), onion (Allium ascalonicum) (160 g), red pepper (Capsicum annuum) (20 g) and 10 g each of Ethiopian cardamom (Aframomum corrorima), black cumin (Nigella sativa), Kemun (Trachyspermum capticum), Ethiopian mustard (Brassica nigra) and garlic (Allium sativum). The ingredients are mixed in a container of 5-kg capacity. About 200 to 250 g of the meat-spice mix is stuffed manually into a prewashed and dried animal casing cut in to 20 cm length, and allowed to ferment at (20 to 25°C) for six days (Figure 8).

Ketema Bacha et al. (2010) reported that wakalim fermentation was dominated by LAB and including staphylococci and members of Enterobacteriaceae. Gram-negative bacteria were under detectable level after day 4 of fermentation. But Staphylococci were detected at low levels (around 4 log cfu/g) until the end of fermentation. Thus, LAB dominated the flora at the end of fermentation. Different species of Lactobacillus and Pediococcus commenced the fermentation and the lactic flora was finally dominated by L. plantarum and P. pentosaceus. The pH of the fermenting wakalim dropped from 5.5 ± 0.22 to 4.1  $\pm$  0.19, while the titratable acidity increased from 0.09 to 0.6% in the course of fermentation. Moreover, moisture content of the fermenting wakalim dropped from 66.5% ± 2.12 to 22.0% ± 0.71 during the 6 days of fermentation.

Assaye and Mogosie (2014) indicated that the mean pH values of retail dry sausages ranged from 6.09 to 6.33 and moisture content values ranged from 35 to 41%. Mean microbial count values (log cfu/g) ranged from 4.87 to 5.18 for AMB, 2.02 to 2.50 for Enterobacteriaceae, 1.73 to 2.24 for coliforms, 2.46 to 3.04 for enterococci, 3.09 to 3.76 for staphylococci, 5.31 to 5.68 for LAB and 3.28 to 3.87 for yeasts. The aerobic mesophilic bacterial flora of retail sliced dry sausages was dominated by Gram-positive bacteria. Salmonella was isolated from two sausage samples. Spoilage of sliced dry sausages, after the vacuum package was opened, was detected within 3 to 4 days during aerobic storage at ambient temperature (22°C on average) and within 12 to 20 days at refrigeration storage (4°C). The storage conditions were intended to reflect what normally would happen in routine food handling in home kitchen environments and food service establishments. Generally, the majority of retail sliced dry sausages showed the presence of high microbial load, which indicated contamination during or after processing of the products (Table 7).

# TRADITIONAL FERMENTED CONDIMENTS

#### Awaze fermentation

It is known that, fermented food, beverage and condiment products are commonly produced throughout the world. Some fermented products produce strong flavor such that the product is not consumed alone, but is added as a condiment to make the food more tasty and enjoyable. In general, different countries of Africa protein-rich food ingredients are often fermented to make condiments. Siljo, awaze and data are among the traditional fermented condiments in Ethiopia and are consumed with other items on the basis of their desired aromas and flavors. Therefore, these condiments result from the microbial fermentations of vegetable-spice mixtures (Hesseltine, 1980).

| Microbial around     | Log cfu/g (Mean±SD     |                        |                         |  |  |  |
|----------------------|------------------------|------------------------|-------------------------|--|--|--|
| Microbial groups     | Processor 1            | Processor 2            | Processor 3             |  |  |  |
| Aerobic mesophiles   | 5.43±1.21 <sup>a</sup> | 4.82±0.83 <sup>a</sup> | 4.54±0.91 <sup>a</sup>  |  |  |  |
| Enterobacteriaceae   | 3.44±1.46 <sup>a</sup> | 1.89±1.01 <sup>b</sup> | 2.17±1.14 <sup>ab</sup> |  |  |  |
| Coliforms            | 3.33±1.45 <sup>a</sup> | 1.56±0.92 <sup>b</sup> | 1.82±1.14 <sup>b</sup>  |  |  |  |
| Enterococci          | 3.53±1.22 <sup>a</sup> | 2.37±1.26 <sup>b</sup> | 1.78±1.09 <sup>ab</sup> |  |  |  |
| Staphylococci        | 3.72±2.18 <sup>a</sup> | 2.89±1.69 <sup>a</sup> | 2.66±1.58 <sup>a</sup>  |  |  |  |
| Lactic acid bacteria | 6.19±1.89 <sup>a</sup> | 5.98±1.59 <sup>a</sup> | 4.86±1.23 <sup>a</sup>  |  |  |  |

**Table 7.** Microbial counts of retail sliced pork dry sausages from three producers (Assaye and Mogosie, 2014).

Means in rows followed by the same letters are not significantly different (P > 0.05). SD: Standard deviation.

4.37±0.59<sup>a</sup>

The main substrates in awaze are red sweet pepper (C. annum), garlic (Allium ursinum) and ginger (Zingiber officinale) with which some proportions of different spices are added. Awaze is commonly known in the north and central Ethiopia and is often consumed with sliced raw or roasted meat and other traditional pancakes. While the microbiology and biochemical properties of several other traditional Ethiopian fermented foods and beverages have been studied (Gashe, 1985, 1987; Sahle and Gashe, 1991; Ashenafi and Mehari, 1995; Bacha et al., 1998), there are no reports on the fermentation of awaze, indigenous Ethiopian condiment.

Yeast

However, a study on fermentation of awaze indicated that the aerobic mesophilic microflora of the ingredients of awaze was dominated by Bacillus spp. (1.1×10<sup>6</sup> cfu/g) and LAB (4.5×104 cfu/g). The counts of AMB dropped during the fermentation period. LAB reached the maximum count of 5.9×10<sup>9</sup> cfu/g at day 4 and the count remained >10<sup>8</sup> cfu/g throughout the fermentation. The heterofermentative LAB dominated until day thereafter, the homolactics dominated the fermentation. Yeasts appeared at day 6 and increased to 2.5×10<sup>6</sup> cfu/g. Hence, fermentation of awaze was accompanied by declining pH and increasing titratable acidity. In addition to this, Salmonella Typhimurium was repressed during the fermentation within 48 h. But awaze had low initial contents of available protein and reducing sugars and did not show marked differences throughout fermentation (Ahmed et al., 2001).

On the other hand, Asnake and Mogessie (2010) studied that LAB were enumerated and isolated from traditional fermented awaze. According them, a total of 87 LAB strains were isolated from awaze sample. Therefore, the isolates were grouped to different genera with their respective number: Lactobacillus (52), Leuconostoc (1), Pediococcus (27) and Lactococcus (7). In line with this, based on their glucose fermentation profile, the isolates were grouped as homofermentative and heterofermentative. However, the count of LAB for an awaze sample was (9.8 log

cfu/g).

#### Fermentation of siljo

3.91±0.78<sup>a</sup>

Siljo is one of the traditional fermented condiments of Ethiopia made up of safflower (Carthamus tinctorius) extract and faba bean (Vicia faba) flour (Mogosie and Tetemke, 1995). The black mustard powder, which is added after cooking the mixture of the safflower and faba bean, helps as source of starter microorganisms (Mogosie and Tetemke, 1995; Zewdie et al., 1995). The fermented product has protein and fat content of 28 and 25%, respectively, with improved protein availability and concentration as a result of fermentation (Mogosie and Tetemke, 1995). The heating step in siljo may be essential in decreasing the level of contamination, but addition of plant materials, for flavoring purposes, to the heated gruel during the process of fermentation, the frequency of serving, and hygienic quality of handlers are factors that contribute to the exposure of siljo to pathogens. Siljo is consumed usually during the long fasting periods when people consume no fatty food of animal origin that may prevent the proliferation of the pathogens (Shin et al., 2002).

3.34±1.28<sup>a</sup>

During the natural fermentations, the type of fermenting flora is determined by the initial flora of the ingredients. Thus, different workers have reported diverse microorganisms to be liable for the fermentation of siljo (Zewdie et al., 1995; Mogosie and Tetemke, 1995). In preparation of siljo, a volume of 1600 ml of siljo was made from safflower (*Carthamus tinctorius*), faba bean (*Vicia faba*) and black mustard powder. This was divided into 4 screw-capped bottles, each containing 400 ml of the gruel. The gruel was left to ferment naturally at ambient temperature. At around 32 h of fermentation, peeled garlic, ginger, Ethiopian caraway and rue leaves, about 2 g each, were added into each bottle and the fermentation was allowed to continue at ambient temperature.

According to Eden and Mogessie (2005) report siljo was made to ferment naturally and the count of LAB reached 9.9 log cfu/ml on day 5. The pH dropped from an initial value of 5.8-4.65 during this time. The lactic acid flora was dominated by Leuconostoc spp. At ambient temperature storage (18 to 22°C), the product spoiled on day 16. The spoilage was caused by Bacillus spp. At refrigerated storage (4°C), however. the count of Bacillus spp. was below detectable limits (<1 log cfu/ml) until the end of experiment on day 16. When Salmonella Typhimurium DT 104 was inoculated into the fermenting gruel at low initial levels, the count decreased steadily and the test strain was not detected by enrichment on day 5. At higher initial inoculum level (5.5 log cfu/ml), complete elimination was observed on day 7. In a non-fermenting control gruel, count of the test strain increased by about 3 log units on day 7.

#### **Datta fermentation**

There are many traditional condiments in different parts of the world produced by microbial fermentations. Such traditional condiments are used as taste enhancers in traditional dishes. The majority of these fermentations are accompanied by certain biochemical changes of nutritional importance (Hesseltine and Wang, 1980). Datta is among the traditional fermented condiments mainly in the southern parts of Ethiopia and are consumed with other items on the basis of their desirable aromas and flavors. It is results from the microbial fermentations of vegetable-spice mixtures. But the major substrate in the making of datta is the small chili pepper (C. frutescenc) at its green stage. Datta was also prepared following traditional methods. The small green pepper together with its seeds was carefully washed and cut into pieces. Garlic and ginger, in small proportion, were peeled, washed and cut into small pieces. The pepper, garlic and ginger were mixed with small amounts of fresh sweet basil and seeds of rue. The mixed ingredients were manually wet-milled on a flat smooth traditional stone-mill into a greenish paste. It was then transferred into a 500 ml screw-cap bottle to ferment at ambient temperature (20 to 25°C).

According to Ahmed et al. (2001) study in datta fermentation, the count of AMB remained unchanged during the fermentation. LAB initiated the fermentation at a level of 7.1×10<sup>4</sup> cfu/g and reached 1.2×10<sup>9</sup> cfu/g at day 7. The homolactic LAB started and dominated the fermentation for the first 2 days and the heterolactics took over thereafter. Datta fermentations were accompanied by declining pH and increasing titratable acidity. *Salmonella* Typhimurium was repressed during both fermentations within 48 h. Datta had low initial contents of available protein and reducing sugars and did not show marked differences throughout the fermentation.

#### CONCLUSION

Fermentation is one of the best efficient techniques of producing and preserving foods. It is fairly a low-energy requiring conservation technology that improves shelf life of food products. In cases fermentation is important to obtain a certain food, the microorganisms present on the raw ingredients or in the containers spontaneously take care of the process. In most of these products the fermentation is spontaneous and involves different microorganisms.

Generally, clear understanding of the procedures involved in the process of making of the traditional fermented food and beverage products could help to design mechanism for production of an industrially based finished product so as to develop the keeping quality of the products. And it has the advantage of reducing wastage during processing, which is significant at household level. In line with this, eating fermented foods has a beneficial health effect for human beings as well as animals.

#### **CONFLICT OF INTERESTS**

The authors have not declared any conflict of interests.

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