

Fermented Ethiopian Dairy Products and Their Common Useful Microorganisms: A Review

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Abstract: Recently emphasis has been given to produce fermented milk with significant quality and improved health characteristics. In Ethiopia, a large proportion of milk is consumed in the fermented form through the application of traditional fermentation methods. The main fermented milk products include *ergo* (sour milk), *ititu* (milk curd), *ayib* (cottage cheese), *neter kibe* (spiced butter), *kibe* (traditional butter), *agwat* (whey) and *arerra* (sour defatted milk). In East and South East part of Ethiopia, fermented (suusac) camel milk is widely consumed, while in North West part, fermented *Metata Ayib* is prepared using traditional cottage cheese with different spices. The objective of this review is to investigate the role of microorganisms in Ethiopian traditional fermented dairy products. With all traditional fermented milk products, lactic acid bacteria (LAB) produce several metabolic products including potential antimicrobial activities, fatty acids, organic acids, hydrogen peroxide, CO₂ and diacetyl. The inhibitory capacity of the acids depends on reduction of pH to levels below the requirement for bacterial growth. Therefore, LAB play a significant role in food fermentation and they have also antagonistic effects against food borne pathogenic microorganisms and help to improve biochemical features of fermented foods. LAB produces specific bacteriocins and proteinoous substances which inhibit the growth of pathogens and food bore pathogens. They are also produces different types of compounds that impart characteristic aroma, color and flavor of fermented foods. In fermented *Metata Ayib*, the combined effect of spices which were incorporated during traditional *Metata Ayib* preparation and LAB with antagonistic activity can reduce the risk of spoilage and pathogenesis.

Key words: Antagonistic activity • Antimicrobial activities • Bacteriocins • Lactic acid • *Metata Ayib*

INTRODUCTION

A wide range of fermented milk products have been produced in different parts of the world in order to preserve and extend shelf life of milk products. The majority of fermented milks of Africa are obtained from cow's milk. Moreover, camel, goat, buffalo, sheep and horse milk are also used in large quantities [1]. The chemical composition of such various mammals' milk is presented on Table 1 [2]. Fermented milk was normally produced by inoculating fresh milk with part of previous fermented batch. This traditional method is still used in some part of societies. Current industrial dairy production process used modern techniques of milk fermentation. In such dairy industries, starter cultures with known characteristics are widely used. Uses of modern techniques have advantages over the traditional methods

for the production of consistent fermented milk products with best qualities [3]. The common bacteria species mostly found in fermented milks are shown on Table 2 [4].

Fermented food products play a significant socio-economic role in the developing world. The importance of traditional fermented foods has been reviewed. These products also contribute to the protein requirements of the indigenous consumers. Lowering the pH of food products through fermentation is a form of food preservation. This is a self-limiting process in that further reduction of pH may be inimical to the producing organisms. As a result, the pH normally stays just below five. Other benefits of fermentation include improvement of food quality through food digestibility to increase essential amino acids, vitamins and protein [5].

Table 1: The chemical composition of milk from various milk producing mammals.

Chemical composition of milk	Species of milk producing mammals						
	Cow	Camel	Goat	Ewe	Buffalo	Mare	Reindeer
Water	87.2	87.6	87.0	80.7	82.8	89.0	63.3
Fat	3.7	5.4	4.3	7.9	7.4	1.6	22.5
Protein	3.5	3.0	3.5	5.2	3.4	2.7	10.3
Lactose	4.9	3.3	4.3	4.8	5.5	6.1	2.5
Ash	0.7	0.7	0.9	0.9	0.8	0.5	1.4
Total solid	12.8	12.4	13	19.3	17.2	11.0	36.7

Source: [1, 2]

Table 2: The most common and significant species of bacteria in fermented milks.

Species of common milk bacteria	Species of milk bacteria
<i>Lactobacillus cldophilus</i>	<i>Leuconostoc dex tranicum</i>
<i>Lactobacillus bulgarcus</i>	<i>Leuconostoc tactis</i>
<i>Lactobacillus cosez</i>	<i>Streptococcus cremoris</i>
<i>Lactobacillus helveticus</i>	<i>Streptococcus lactis</i>
<i>Lactobacillus lactis</i>	<i>Streptococcus lactis subsp. diacetylactis</i>
<i>Leuconostoc cremors</i>	<i>Streptococcus thermophilus</i>

Source: [4]

Fermentation is also known to soften food texture and alter its composition in such a way that it will require minimal energy both in cooking and preservation process. Thus, less fuel will be used for cooking and eliminates the need of preservation as fermentation increases the shelf life of food. These advantages make fermentation a highly desirable technique in rural communities of the third world where resources for cooking and preservation are scarce. Dairy products are one of the most important fermented foods consumed across the world [6].

Milk products like curd and cheese are widely used and consumed in many part of the world and available in market for many generations. There is still an increase demand of consumer due to free from artificial preservatives, high quality natural food and free of contaminating micro-organisms. The significance of fermented milk in nutrition of human is well known in the world and the importance of the products was documented starting from ancient days of civilization. The nutritional and medicinal properties of many fermented foods have been practiced and used by several generations. However, scientific research on the significance of fermented milk products was carried out starting from 1910. Recently, emphasis has been given to produce fermented milk with significant quality and improved health characteristics especially for therapeutic properties of these products. International Dairy

Federation has been defined a fermented milk product as the milk product produced from skimmed milk or not with specific cultures. The micro flora in fermented milk is kept alive until during consumption and may not favour the growth of any pathogenic germs [7].

Fermented milk products also serve as the significant delivery vehicles for the growth of probiotic bacteria. The probiotic bacteria are bacteria that associated with fermented dairy products. These probiotic bacteria are bacteria found from fermented milk also associated and found in human body, the gastrointestinal tract, including the mouth, etc. Some member of these microbes have a dual role in transforming milk into a diverse dairy fermented products (cheese, kefir, yoghurt, etc.) and play an important role to and inhibit colonizing bacteria. Dairy products are widely used as major portion of the total functional foods in European market and are currently used as best source of probiotic developments [7].

Traditional Fermented Dairy Products in Ethiopia:

These fermented products have different vernacular names such as *ititu*, *ergo*, *meomata* or *geinto* among the Oromo, Amhara, Wolayta or Sidama people, respectively. The fermentation process is usually carried out using natural wild microorganisms, without using defined starter cultures to initiate fermentation process. In most cases, fermentation of dairy products carried out through the propagation of the initial microorganism, with serious of microbial succession governed by chemical changes and ambient temperatures in the fermenting milk. Traditional dairy fermentation process is conducted with no attempt to control the fermentation processes. In this case, fresh milk is left either at room temperature or kept in a moderate warmer place to facilitate fermentation process. In rural areas, especially among the pastoralists, raw milk is mostly kept in properly smoked container and fermented milk from a previous fermentation uses as source of inoculums. Lactic acid bacteria (LAB) from the inner walls of the container also become established and serve as starter culture. Incubation temperature does have significant role in the lowlands and the quality and taste of the fermented product may be more or less uniform. The fermented dairy product may also be serve as raw material for the production of traditional butter (*qibe*) and butter milk (*arrera*). The butter milk can be further be processed into *Ayib* or traditional cottage cheese and whey (*aguat*) [8]. Some of the associated microorganisms reported from Ethiopian fermented dairy products are presented in Table 3.

Table 3: Associated microorganisms in Ethiopian fermented dairy products.

Fermented Dairy Products	pH	Titration acid	Associated Microorganisms	References
<i>Ergo</i> (sour milk)	4.3	0.88%	<i>Lactococcus garvieae</i>	[9]
			<i>Lactococcus lactis</i> subsp. <i>lactic</i>	
			<i>Lactococcus</i> , <i>Streptococcus</i> , <i>Leuconostoc</i>	[10]
			<i>Lactobacillus</i>	
			Food-borne pathogens	
			<i>Salmonella</i> spp., <i>Bacillus cereus</i>	[11,12,13]
<i>Ititu</i> (concentrated sour milk)	3.65 4	1.92% 0.43*	<i>Lactobacillus casei</i> <i>Lactobacillus plantarum</i>	[14]
			<i>Bacillus cereus</i> , <i>Staphylococcus aureus</i>	[15, *16]
<i>Ayib</i> (Ethiopian cottage cheese)			<i>Microbacterium</i> , <i>Brevibacterium</i> spp	[17]
			<i>Enterobacteriaceae</i> <i>Pseudomonas</i> spp	
			<i>Lactobacillus fermenti</i> <i>Lactobacillus plantarum</i>	
			Grow when added to raw cheese milk	
Camel fermented (<i>suusac</i>) milk	3.65	----	<i>Streptococcus agalactiae</i> , <i>Staphylococcus</i>	[19]
			<i>Streptococcus infantarius</i> , <i>Lactococcus lactis</i> <i>Streptococcus thermophilus</i>	
			<i>S. agalactiae</i> (human pathogen)	
			<i>S. infantarius</i> , <i>Streptococcus bovis</i> / <i>Streptococcus equinus</i> complex	
			<i>S. thermophilus</i> , <i>Lc. lactis</i> <i>Lactobacillus</i> spp	
			<i>Lactobacillus plantarum</i> <i>Lactobacillus curvatus</i> <i>Lactobacillus salivarius</i>	
			<i>Lactococcus raffinolactis</i>	
			<i>Leuconostoc mesenteroides</i> subsp. <i>mesenteroides</i>	[20]
			<i>Lactococcus lactis</i> subsp. <i>cremoris</i> , <i>Enterococcus faecium</i> ,	
			<i>Leuconostoc lactis</i> , <i>Lb. bavaricus</i>	
			<i>Lactobacillus acidophilus</i> , <i>Lb. helveticus</i>	[21]
			Yeast isolates	
			<i>Saccharomyces cerevisiae</i> <i>Candida kefyr</i> ,	[21]
<i>Candida krusei</i> , <i>Candida glabrata</i>				
<i>Geotrichum penicillatum</i> , <i>Candida krusei</i>	[20]			
<i>Rhodotorula mucilaginosa</i>				
Food borne pathogens and opportunistic pathogens	[22]			
<i>Enterococcus</i> spp				
<i>Staphylococcus aureus</i> ,				
<i>Streptococcus agalactiae</i>				
<i>Lactococcus lactis</i> subsp. <i>lactis</i> , <i>Leuconostoc</i>				
<i>Lactobacillus</i> spp., <i>Streptococcus thermophilus</i>				

Ergo (Sour Milk): Ergo is an Ethiopian traditional naturally fermented milk product with more or less the same characteristics to yogurt. It is normally smooth and thick with consistence or uniform appearance and normally has a white color if prepared carefully. This fermented product is usually semi-solid state with a pleasant odor, aroma and taste. It generally constitutes a sour milk product from which other products may be further produced. Depending on the storage temperature, it can be stored for 15 - 20 days and it has been reported that *Lactococcus garvieae* and *Lactococcus lactis* subsp. *lactic* were dominant [9].

As it is well known, production of ergo is normally carried out by natural fermentation process, as the result different species of microorganisms involved and

contribute a lot to basic final characteristics of ergo. The quality and safety of a fermented product entirely depend on the spices and spices composition of LAB that are involved in the fermentation process [23].

In the time course of ergo fermentation [10], microorganisms such as *Streptococcus*, *Lactobacillus*, *Lactococcus* and *Leuconostoc* are carried out the souring process. They also detected fairly high numbers of micrococci spore formers and coli forms during the first 14 to 16 hours of fermentation. The lactococci species are the most dominant one throughout the fermentation that reached colony counts as high as 10^9 cfu/ml at the end of the fermentation process. At the same time, the aerobic mesophilic bacteria have also shown similar colony counts and the yeast population was increased up to

10^5 cfu/ml at 24 hours. At the end of fermentation, pH value highly reduced and titratable acidity of the fermented product was 0.75%.

The type of microorganisms involved during *ergo* fermentation of raw milk collected from 8 dairy farms at Awassa were entirely depends on various parameters [24]. Initial aerobic mesophilic counts varied between 10^4 and 10^6 cfu/ml among the various fermenting milk samples. Normally *ergo* was formed after 24 hours and the average aerobic mesophilic count was greater than 10^9 cfu/ml. Coliform counts, which were less than $10 - 10^4$ cfu/ml at initiation of fermentation, reached 10^6 CFU/ml within 12 hours but this count decreased markedly thereafter. LAB had initial counts of $<10^4 - 10^6$ cfu/ml and maximum counts ($10^7 - 10^9$ cfu/ml) were attained at 24 hours. Initially, yeasts were below detectable levels but gradually reached colony counts of 10^5 cfu/ml at 24 hours. The pH value of most samples was reduced gradually up to 12 hours and reduced sharply thereafter up to values of 4.3 or below. Average initial titratable acidity of 0.16% was increased to 0.88% at *ergo* formation. To study the effect of container smoking on the microbiological and biochemical qualities of fermenting *ergo* [25], raw milk was allowed to sour naturally at ambient temperatures (25-30°C) in smoked or non-smoked containers. Milk in smoked containers had a lower rate of pH drop and the fermented product had good flavor for a longer time after coagulation. The total count of non-lactic acid bacteria in milk in non-smoked containers reached a high count (greater than 10^8 cfu/ml) within 12 hours, whereas milk in smoked containers required more than 24 hours reaching this level. Similarly, the growth of coli forms and lactic acid bacteria was slow in milk in smoked containers, thus assuring good and slow development of flavor components, safety of finished product and better keeping quality. Lactobacilli dominated the flora of the fermented product in non-smoked containers, while lactococci were not equally dominant in fermented milk in smoked containers.

Findings from various workers indicate that *ergo* is a product obtained by spontaneous fermentation and cannot be defined in terms of its microbiological or biochemical properties. It does not have definite temperature and duration of incubation. Fermentation is carried out at ambient temperatures and precipitation of the casein is usually the sign of completion of fermentation. Consistency and flavor of *ergo* vary within or among households [14].

Considering the possibility of contamination of milk by food-borne pathogens from various sources, several

studies were undertaken to determine the fate of *Salmonella spp.*, *Bacillus cereus*, *Staphylococcus aureus* and *Listeria monocytogenes* during the souring of milk into *ergo* [11-13]. All the test pathogens could grow to levels as high as $10^7 - 10^8$ cfu/ml within 12 hours in fermenting milk. Smoking of containers significantly retarded the growth of the test pathogens, but only until 12 hours. Growth of LAB in souring milk resulted in inhibition of *Salmonella typhimurium* and *Salmonella enteritidis* between 48 and 60 hours of fermentation of milk in non-smoked and smoked containers. *Staphylococcus aureus* and *Bacillus cereus* were inhibited within 24 to 38 hours of fermentation in non-smoked containers and within 24 hours in smoked containers. *Listeria monocytogenes* in fermenting milk of non-smoked containers was inhibited after 48-60 hours, whereas inhibition was observed at 36 hours in smoked containers. It was suggested that the synergistic effect of pH, acids and container smoking were important in the complete inhibition of the test organisms. *Escherichia coli* O157:H7 inoculated in souring milk at initial levels of 10^3 cfu/ml grew to over 10^6 cfu/ml within 24 hours and counts at 72 hours were still at the level of 10^3 cfu/ml. Post-souring inoculation of the pathogen in *ergo*, however, resulted in complete elimination of the pathogen within 6 hours at ambient temperature storage, but it was recovered until 72 hours at refrigeration storage [18].

In most cases, household preparation of *ergo* requires a one-day incubation at ambient temperatures. The milk coagulates within 24 hours and *ergo* is usually consumed preferably at this time due to its good flavor. Longer keeping is not desirable because further drop in pH will result in increased wheying off, which, in turn, results in loss of protein as whey. Observations in these studies have, however, indicated that *Salmonella spp.* and *Listeria monocytogenes* were not inactivated at 24 hours and the count, at this time, ranged between 10^5 and 10^6 cfu/ml for *Salmonella spp.* and 10^3 and 10^4 CFU/ml for *Listeria monocytogenes*. In case of *Staphylococcus aureus* and *Bacillus cereus*, there was either a complete inhibition at 24 hours or the number was below the level required to elucidate enough toxins to cause any gastroenteritis. Despite the general assumption that the low pH in *ergo* controls the proliferation of undesirable microorganisms, the dangers of listeriosis or salmonellosis from fresh *ergo* must not be underestimated. It was, thus, recommended to inoculate boiled milk with a three day old *ergo* to ensure the nutritious quality and wholesomeness of *ergo* [18].

Ititu (Concentrated Sour Milk): The name *ititu* is used for concentrated fermented milk prepared and consumed by the Borana tribes in southern part of Ethiopia. This pastoral/farmer community prepares *ititu* during the rainy season when milk is available in abundance for later consumption during the drier seasons when fresh milk supply is markedly scanty [14]. The product has good keeping quality and remains acceptable for about two months at ambient temperature (25°C-30°C) and can be stored from about two months [26] to three months [14]. The traditional processing and consumption pattern of *ititu* is well described by Gonfa et al. [14]. It is consumed as side dish with traditional porridge or thin-baked cereal chips. It can also be consumed as food or drink alone. It is considered as one of the special foods and served to much respected guests as well as to weaning-age children and the elderly [25].

During the traditional production of *ititu*, fresh milk is collected in a well-smoked fermenting vessel called *gorfa* [26]. *Gorfa* is woven from fibers of selected plants into a lidded container with a capacity up to three liters. A new *gorfa* is washed with hot water, air dried, rinsed with fresh milk and smoked for a few minutes with splinters of *Acacia nilotica* or other plants. The lid of the *gorfa* is treated with leaves of *Ocimum basilicum* for cleaning and imparting desirable flavor to the product [14, 26]. A small volume of milk (up to 300 ml) is added to the *gorfa* and is allowed to ferment naturally. When the milk coagulates, whey is removed by wooden pipette and an additional volume of fresh milk is added. The process of whey removal and addition of fresh milk is repeated several times until the product is concentrated enough and is ready for consumption. Any mold growth on the surface of the curd is removed.

According to Kassaye et al. [26] report, the total bacterial count was 10^{12} cfu/g, mostly dominated by lactic acid bacteria. Mold and yeast counts were 10^8 cfu/g but coliforms were not isolated and detected. The identified most prevalent LAB in *ititu* were *Lactobacillus casei* and *Lactobacillus plantarum*. Almost the same values LAB (about 10^8 cfu/g) were reported by other researchers [14].

The chemical characteristics of *ititu* were analyzed using randomly collected samples from individual households in Borana region. *Ititu* had an average fat and protein content of 9.05 and 7.17%, respectively. The average pH and titratable acidity (as lactic acid) were 3.65 and 1.92%, respectively [26]. Even the values were varied from sample to sample; *Ititu* was shown high in amount of total amino and free acids in comparison with fresh milk.

Ititu was also rich in amino acids such as alanine, proline, glutamic acid, serine and leucine [26].

Ayib (Ethiopian Cottage Cheese): In Ethiopia, small-holder milk processing is based on sour milk mainly due to high ambient temperatures, consumer's preference and increased keeping quality of sour milk [27]. *Ayib* is a traditional Ethiopian cottage cheese made from sour milk after the fat is removed by churning and produced by slowly heating naturally soured milk until a discrete curd mass develops and floats over the whey. The whey is traditionally known as *aguat*. It is an acidic product. *Ayib* is a well known popular milk product widely consumed by various ethnic groups in different part of the country [23].

Ayib is an important source of nutrients and serves as a staple diet. It may be consumed fresh as side dish, or it may be spiced with hot spices, salt and other herbs [14]. Raw milk is collected in a clay pot and kept in a warm place (about 30°C) for 24 to 48 hours to sour spontaneously. The pH of sour milk is usually about 4. Churning of sour milk is carried out gradually with slow motion of shaking the entire content of the pot until the time of fat is separated from the rest content.

Temperature of *Ayib* preparation could vary between 40 to 70°C without any significant impact on composition and yield. However, temperature above 80°C imparts a cooked flavor to the product [23]. Cooking at 60°C markedly decreased the number of most bacterial groups, yeasts and molds. Heat treatment at the temperature of 70°C is required with a relatively shorter time for the formation of curd and help to reduce various microbial groups at minimum level. Since temperatures higher than 80°C are reported to give the product a cooked flavor [23], heat precipitation of curd at 70°C (pH 4.0) was recommended as it resulted in a less contaminated and more wholesome *Ayib*. After gradual cooling, the curd is recovered from the whey. *Ayib* comprises 79% water, 14.7% protein, 1.8% fat, 0.9% ash and 3.1% soluble milk constituents and the yield should be at least 1 kg of *Ayib* from 8 liters of milk (12.5%) [27]. Fekadu and Abrahamsen [28] analyzed various *Ayib* samples produced by small-holders in three regions of southern Ethiopia and found out that the samples consisted of 80 - 81% moisture, 13.4 - 16% protein, 1.9 - 2.0% fat and 0.75 - 0.87% minerals.

In a study on the microbiological quality of *Ayib* [15], samples collected from an open market in Awassa had counts of mesophilic aerobic bacteria, yeasts and enterococci of 10^8 , 10^7 and 10^7 cfu/g, respectively. Over

60% of the samples had psychotropic count of 10^6 cfu/g and about 55% of the samples were positive for coliforms and fecal coliforms. *Bacillus cereus* and *Staphylococcus aureus* were isolated at varying frequencies but at low levels (10^2 - 10^3 cfu/g). The pH values of the samples varied between 3.3 and 4.6 with about 40% having pH lower than 3.7.

In traditional *Ayib* making, the milk itself may have a high initial count of microorganisms and further processing may result in increase in counts. However, during preparation of *Ayib*, the high initial count of microorganism in milk, which increases during the fermentation process, is shown to decrease by the combined action of cooking and low pH. So *Ayib* is supposed to have a lower microbial load after heating. The presence of high microbial load of ready-to-consume *Ayib* is believed 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 [23].

Further analysis of *Ayib* micro flora showed that bacterial and yeast counts did not correlate with pH value of *Ayib* samples [17]. However *Ayib* samples with pH greater 4.0 contained more bacterial groups than those with pH less than 4.0. The Gram-positive rods dominated the aerobic mesophilic bacterial flora, *Microbacterium* and *Brevibacterium spp.* being the most abundant. *Enterobacteriaceae* and *Pseudomonas spp.* constituted the bulk of the Gram-negative rods. The count of LAB was around 10^6 cfu/g and *Lactobacillus fermenti* and *Lactobacillus plantarum* dominated the flora.

Although the low pH of *Ayib* prevents the growth of many food-borne pathogens, higher numbers of LAB and yeasts are not desirable in *Ayib*. A much lower pH due to the activity of LAB may result in a too sour product with a low sensory quality. The proteolytic activity of certain LAB and yeasts may also impart *Ayib* with uncharacteristic flavors. Thus, appropriate temperature of curd-cooking coupled with the low pH of the product should make *Ayib* a safe and nutritious product with an improved keeping quality.

In a study on microbiological safety of *Ayib* sold in an open market, *Bacillus cereus* and *Staphylococcus aureus* were isolated at varying frequencies but at low numbers (10^2 - 10^3 cfu/g) [15]. *Listeria monocytogenes* was, however, not encountered in any of the samples. The survival and growth of *Salmonella* during the making of *Ayib* were studied by Mohammed *et al.* [29]. *Salmonella typhimurium*, *Salmonella enteritidis* and *Salmonella*

infantis were able to grow when added to raw cheese milk, but none was able to survive the heating process at the prevailing low pH. When the *Salmonella* test strains were added after heating, they were able to survive for over 24 hours. They disappeared only after three days, by which time palatability had deteriorated. Mekonnen and Mogessie [18] showed that the count of *E. coli* O157:H7 increased during milk souring for *Ayib* processing, but the count immediately after curd-cooking was below detectable limits, although the pathogen was recovered after enrichment. It was completely eliminated 24 hours after curd-cooking. When *E. coli* O157:H7 was inoculated into steam-treated *Ayib* at low initial inoculation level and maintained at ambient temperatures, it was eliminated within a day. At higher initial inoculum level and ambient temperature storage, however, *E. coli* O157:H7 was detectable by enrichment until day 9 (Table 6). At refrigeration storage, decrease in counts was gradual and counts at day 9 were $>10^4$ cfu/ml.

In summary, the growth and survival of microbial pathogens in cheese usually depend on many factors, including variations in pH, a_w , salts, the presence of competing microbiota and the temperature and biochemical changes during ripening. The microbiological quality of milk and the good manufacturing practices will also contribute to safety of the final product; especially in cheeses where milk is not pasteurized [30].

Qibe (Traditional Ethiopian Butter): Qibe is a traditional Ethiopian butter which is made from *ergo* and not from cream [31]. It has a white to yellowish color, depending on age and is semi-solid at room temperature. It has a typical diacetyl taste and flavor when fresh, but extended storage at ambient temperatures results in putridity and rancidity. *Qibe*, without further processing, is used for hairdressing and as a skin cosmetic mainly by women. A small amount of the fresh form is traditionally fed to infants of weaning age. Generally, *qibe* is used in the diet after processing into *nitir qibe* (traditional ghee), by heating it to boiling after selected types of spices are added to it. *Nitir qibe* is basically used for the preparation of stews made of legumes or meat, which are eaten with *enjerra*, fermented pancake-like bread. In Addis Ababa, where consumption of *qibe* is believed to be high, over 54% of milk is converted to *qibe* [32].

Qibe is produced by churning *ergo* in traditional utensils with a volume of 20-25 liters. Milk for churning is accumulated over several days in the utensil and allowed to sour into *ergo*. Traditionally, *qibe* production is the

responsibility of women and the processing of 20-25 liters of *ergo* needs 1 to 4 hours of churning time and about 1 kg of *qibe* is produced [33]. The curd is broken by agitation before churning starts. Agitation of churn is carried out by rocking the churn placed on the ground forwards and backwards, or by suspending it from a tripod or doorpost or shaking it on a person's lap [34, 35]. This process results in the formation of fat granules which will coalesce into larger grains towards the end of the churning time. Final rotating of the churn on its base would lump the fat grains together into *qibe* which is then skimmed off. *Qibe* has 17.2% moisture, 1.3% protein, 81.2% fat, 0.1% carbohydrate, 0.2% ash, 0.024% calcium and 0.0015% iron [36].

Camel Raw and Fermented (Suusac) Milk: The arid and semi-arid part of East Africa land is estimated to have 11 million camels which accounts for 58% of the world camel population. Camels in this portion of the world produce over 2/3rd camel milk (1.3 million tonnes per year) [37]. Ethiopia is estimated to have the third herd of camel in the world after Somalia and Sudan in the order. Apart from camels from Afar region and Northwest border part of Ethiopia, Somali Region has an estimated population of more than two million herds. This region provides milk for the society in the region as well as neighboring countries such as Somaliland and Djibouti on a daily basis.

Milk from camel plays an important role to provide nutrition for of pastoral communities. Recent research is focused on camel milk for its medical application to treat several ailments such as autoimmune diseases, juvenile diabetes and allergies. According to Kassaye [38] chemical composition of raw camel milk was 14.63% total solids, 3.29% crude protein, 5.98% fat, 0.73% ash, 0.13% phosphorus and 0.10% calcium, while fermented milk was 20.87% total solids, 7.17% crude protein, 9.05% fat, 0.74% ash, 0.16% phosphorus and 0.09% calcium. The pH value of raw milk was reduced from 6.7 to 3.65 while amount of lactic acid increased from 0.18 to 1.92%.

Camel milk is traditionally consumed in East Africa including Ethiopia in the form of raw or as a spontaneously fermented product called *suusac* [19]. *Suusac* production in Ethiopia, Kenya and Somalia is the same since camel milk is widely used by pastoral communities living at the boundaries of these neighbour countries. *Suusac* is well known fermented camel milk that widely prepared and consumed by the pastoralist societies living in Somalia and Kenya. It is prepared through fermentation of raw fresh camel milk by the help

smoked container (gourd) at room temperature (26 - 29°C) for 1 - 2 days [20]. The microbial profile of unfermented and fermented camel milk was studied in Kenya and Somalia [19].

A total of 59 samples were collected from unfermented raw milk and *suusac* of Kenya and Somalia to determine the microbial diversity of predominant LAB using MRS and M17 agar. From raw milk market chains and *suusac*, the total micro flora were \log_{10}^6 and \log_{10}^8 cfu/ml, respectively [19]. The predominant LAB in unfermented products were *Streptococcus agalactiae* and *Staphylococcus* spp. whereas in *suusac* contained *Streptococcus infantarius* subsp. *infantarius* as predominant species followed by *Lactococcus lactis* subsp. *lactis*, *Streptococcus thermophilus* and lactobacilli. In the investigated samples, *S. agalactiae* was a major human pathogen associated with neonatal sepsis and a clear health risk. Among commensal inhabitants of the gastrointestinal tract of mammals, *S. infantarius* was reported, which is considered as a member of the *Streptococcus bovis/ Streptococcus equinus* complex (SBSEC). Moreover, SBSEC is associated with several animal and human infections including endocarditis, bacteremia and colonic or non-colonic cancer. Therefore, *S. infantarius* was considered as potential health risks for consumer. In conclusion, strains of *S. thermophilus*, *Lc. lactis* and *Lactobacillus* spp. could be used as subsequent starter culture application. Using such starter culture can avoid animal and human infection and also help to produce consistent quality *suusac* product [19].

According to Lore et al. [20] reports, a total of 45 LAB and 30 yeast isolates were identified from Kenyan traditional fermented camel milk. The isolated species of LAB were *Lactobacillus plantarum*, *Lactobacillus curvatus*, *Lactobacillus salivarius*, *Lactococcus raffinolactis* and *Leuconostoc mesenteroides* susp. *mesenteroides*. At the same time, a number of yeast isolates such as *Geotrichum penicillatum*, *Candida krusei* and *Rhodotorula mucilaginosa* were identified. Another type of study was conducted by Shuangqaan and Miyamoto [21] at nomadic families in the Inner Mongolia Region. In this study the identified LAB were *Lactococcus lactis* susp. *Cremoris*, *Enterococcus faecium*, *Leuconostoc lactis*, *Lb. bavaricus* and *Lactobacillus acidophilus*, *Lb. helveticus*. Yeasts isolated and identified in this study were *Saccharomyces cerevisiae*, *Candida kefir*, *Candida krusei* and *Candida glabrata*.

Table 4: Spices used in the preparation of *Metata Ayib* [39].

Scientific name	Common name	Vernacular name (Amharic)	Part of the plant used
<i>Brassica nigra</i>	Mustard	Senafitch	Seed
<i>Coriandrum sativum</i>	Coriander	Dimbillael	Seed
<i>Zingiber officinale</i>	Ginger	Zingebil	Rhizomes
<i>Allium sativum</i>	Garlic	Netchishinkurt	Bulbs
<i>Ocimum basilium</i>	Basil	Zekakibe (Basobila)	Seed
<i>Ruta graveolence</i>	Rue	Tenadam	Seed

With regard to hygiene of camel milk, high counts of well known food borne pathogens and opportunistic pathogens such as *Enterococcus* spp, *Staphylococcus aureus* and *Streptococcus agalactiae* were isolated and identified. The potential solutions to improve quality and safety camel fermented milk (suusac), use of adapted starter cultures such as *Lactococcus lactis* susp. *lactis*, *Leuconostoc* spp. *Lactobacillus* spp. and *Streptococcus thermophilus* were recommended. To avoid risks associated to hygiene, coordinated interventions such as appropriate medical diagnosis, treatment and separation of infected animals are significant [22].

Metata Ayib: Metata Ayib is a traditional fermented milk product prepared from cottage cheese that widely consumed in Northwest Ethiopia. Production of *Metata Ayib* is carried out using traditional cottage cheese with different spices through spontaneous fermentation for 20 days. *Metata Ayib* has a long shelf life up to one year in semi-solid form but more than ten years in dry form. In contrast *Ayib* has a shelf life of only a few days. The property of *Metata Ayib* has not been fully understood and characterized [16].

According to Eyasu [16] report, *Metata Ayib* water, protein, fat and ash content were 42.3 ± 5.1 , 43 ± 6.9 , 28.7 ± 8.4 and 3.2 ± 0.65 g/100 g, respectively. The average pH values and titratable acidity (lactic acid) of samples were 4.0 ± 0.1 and $10.43 \pm 0.07\%$, respectively. The analyzed *Metata Ayib* samples had a total bacterial count was in the ranging from 2.5×10^5 - 6.9×10^7 cfu/g. At the same time yeast and mould colony count was also in ranging from 1.2×10^2 - 6.1×10^3 cfu/g. However, coliforms were not detected. *Metata Ayib* was found to be a novel indigenous dairy product with better microbiological quality in comparison with traditional cottage cheese *Ayib*. Fermented *Metata Ayib* is locally prepared from different spices (Table 4): *Brassica nigra*, *Coriandrum sativum*, *Zingiber officinale*, *Allium sativum*, *Ocimum basilium* and *Ruta graveolence* [39].

Dairy products prepared in poor hygienic conditions create a great health problem to consumers. Despite the fact that *Metata Ayib* is produced under non-hygienic environment with a high possibility of contamination, the combined effect of spices which were incorporated during traditional *Metata Ayib* preparation and the lactic acid bacteria with antagonistic activity can reduce the risk of spoilage and pathogenesis [39].

According to Tsehai et al. [39] study, ginger and garlic extracts have exhibited wide spectrum of antimicrobial properties. Therefore, as it was determined in the study that spices decrease and inhibit the growth of contaminants but favor lactic acid bacteria. The use of spices was generally decreased the chances of microbial food poisoning and thereby increase the food shelf life. In other words, use of spices (garlic/ginger) in diet can reduce the risk of food contamination, protect the consumer from different food borne diseases, improve their health status and combat with the food borne diseases. This investigation was also indicated that the culture filtrates of lactic acid bacteria isolated from *Metata Ayib* exhibited antimicrobial activity against four pathogenic test bacteria; *S. aureus* (ATCC2923), *S. pneumonia* (ATCC49619), *Shigella* and *E. coli* (ATCC2592). Lactic acid bacteria isolates have been shown a strong antagonistic activity against pathogenic and food spoilage microorganisms and play a vital role in the preservation of *Metata Ayib*.

Dominant Useful Microorganisms in Dairy Products

Lactic Acid Bacteria: Lactic acid bacteria (LAB) comprise a diverse group of Gram positive, non-spore forming cocci, cocco-bacilli or rods. In most cases they are anaerobic, microaerophilic or aerotolerant in their oxygen demands. Even though some members of LAB produce pseudocatalase when grown at low concentrations of sugar, they are generally oxidase and catalase negative. They are chemo-organotrophic and grow in complex media. LAB in general are non-pathogenic to man and animals. Many species of LAB are recognized as safe for

human being and are extensively used in the production of dairy products. They need simple fermentable carbohydrates as best source of energy as well as for growth and production of lactic acid [40].

The lactic acid bacteria consist of several genera, which include *Streptococcus*, *Enterococcus*, *Lactococcus*, *Leuconostoc*, *Lactobacillus* and *Pediococcus*. Based on similarities in physiology, metabolism and nutritional needs, these genera are grouped together. A main similarity of such bacteria members is that all produce lactic acid as a main sole end product during the fermentation of sugars [41]. Species LAB belong to *Streptococcus* and *Leuconostoc* produce the small amount of acid in comparison to others; while the homofermentative species of *Lactobacillus* produce the highest quantities of lactic acid. Heterofermentative *Leuconostoc* and *Lactobacillus* species convert glucose to about 50% lactic acid, 25% acetic acid and ethyl alcohol and 25% carbon dioxide. This is important in leavening and in flavour development of certain bread like fermented foods [42].

Role of LAB in Food Preservation: LAB are widely utilized to produce fermented foods with good quality nutrition value, aroma and flavour as well as with safe metabolic activities. During the growth of LAB in foods, they use and metabolize sugars for the production of different metabolites and organic acids [43]. In common dairy fermented products like yogurt, mainly lactic acid are produced by the help of starter culture bacteria to avoid the growth of undesirable micro-organisms introduced from the environment. Despite improved manufacturing facilities and implementation of effective process control procedures such as Hazard Analysis and Critical Control Point (HACCP) in the food industries, the number of food borne illnesses has increased [44].

Currently consumers produce quality food substances with the use of limited chemical preservatives. As a result, societies tend to focus to preserve food substances through the application of selected LAB due to their safe and sound association with human fermented foods. LAB produced several metabolic products with potential antimicrobial activities, fatty acids, including organic acids, hydrogen peroxide and diacetyl. However, special attention has given on the ability of LAB to produce specific bacteriocins and proteinoous substances which inhibit the growth of pathogens, such as *Bacillus spp.*, *Listeria*, *Clostridium*, *Enterococcus spp.* and *Staphylococcus*. Therefore, they increase and enhance the shelf life of the food [45].

Lactic acid bacteria were likely the prime agents in producing soured (fermented) milk and dairy products although yeasts are also involved. They are normally found in the udder of cow and introduced into the raw milk of healthy animals [30]. The primary characteristic of LAB is the production of different acids, especially lactic acid. The inhibitory capacity of the acids depends on reduction of pH to levels below the requirement for bacterial growth. Lactic acid has unique sensory qualities, antimicrobial properties and has a sour and pleasant taste [30].

LAB use carbohydrate fermentative path way and produce lactic acid. Lactic acid production leads decrease in pH. Fermentation of sugars that cause leading to pH decrease is important for clotting of milk. Beside, increasing acidity initiates following desirable reactions and changes such as whey expulsion. This is because there is a correlation between pH value and whey expulsion from curd of milk. Additionally, acid production has beneficial effect on formation of texture, aroma and flavor [46].

Proteolysis is an important event that occurs during cheese ripening. The lactic acid bacteria use the polypeptides. These polypeptides are generated by milk clotting enzymes and by bacterial cell-wall proteins. Rennet which is the milk clotting enzyme is responsible for casein degradation. Because of the casein degradation peptides are produced which are transported into the cell. In the cell, peptidases continue degradation to produce smaller peptides and amino acids. It has been known that amino acid composition plays an essential role in the aroma of cheese [47].

The quality of cheese and other fermented food products is dependent on the ability of flavor and aroma production of microorganisms which include LAB. Flavor compounds produced by LAB can be divided into two groups; the compounds in fermented milk and the compounds present mostly in matured cheese.

First group consists of organic acids such as lactic acid and acetic acid, which are produced by *L. lactis* sp. *lactis* and *L. lactis* sp. *cremoris*. Second group consists of acetaldehyde, diacetyl, acetoin and 2-3 butylene-glycol which are produced by *L. lactis* sp. *lactis* biovar diacetylactis and *Leuconostoc* species from citrate present in milk. It has been reported that these aroma compounds might be produced to avoid to pyruvate accumulation in the cell. Moreover, improved knowledge of proteolysis and peptidolysis in cheese, analysis on enzymatic systems of LAB and evaluation of different strains, will provide better understanding between flavor development and starter activity [48].

LAB has been used as natural preservatives because of their antimicrobial capacity. Through fermentation products: Antimicrobial activity can be exerted through the reduction of pH or production of organic acids (lactic acid, acetic acid), CO₂, reuterin, diacetyl, 2-pyrrorelidone and 5-carboxylic acid (PCA) [49]. Effective starter culture activity can prevent the pathogen and contaminant growth that may occur during cheese making process.

Through Bacteriocins: Bacteriocins can be defined as protein antibiotics of relatively high molecular weight and mainly affecting the same or closely related species. It is known that LAB are generally regarded as safe microorganisms and so are their bacteriocins. Thus, these bacteriocins can potentially be used to control the growth of spoilage and pathogenic organisms in food [50]. Fourteen strains of lactococcal with a potential of production of bacteriocin have been widely used successfully as starter cultures for the preparation of safety and quality cheese.

Antagonistic Effects of LAB on Some Food Borne Pathogens: Fermentation biotechnology is one of the well known oldest methods for food preservation. Fermentation processes promote the development of safe food production and thereby help to prevent food borne pathogens and the outgrowth of spoilage bacteria [51]. Lactic acid bacteria play significant role in food fermentation and they have antagonistic effects against food borne pathogenic microorganisms and help to improve biochemical features of fermented food. They have enzyme systems to utilize carbohydrates for the production of organic acids such as lactic acid or acetic acid. Most of food borne contaminants and pathogenic organisms are sensitive to these acids since they have a potential to reduce pH value of fermented food products. They also have a potential to produce antibacterial substances such as hydrogen peroxide, diacetyl, bacteriocines and CO₂ which can play part in the antagonism food borne and pathogenic microorganisms [52]. Lactic acid bacteria also produces different types of compounds that impart characteristic aroma, color, flavor and test of fermented foods [53].

Role of Spices in Food Preservation: Spices have been widely used for many centuries in different part of the world to enhance aroma and flavor of food substances. Spices were widely used and applied for treatment of clinical ailments and food preservation and they can also affect antibiotic resistance bacterial pathogens [54].

Natural substances possessing antimicrobial and antioxidant characteristics have been used by consumers and provide a number of health advantages. Some herbs and spices are currently used as valuable substances for their medicinal effects and antimicrobial activities in addition to their fragrance and flavor qualities. The extracts of large number of plant species have become accepted at this time and the attempts made to characterize their bioactive compounds have wide range of acceptance in food processing and pharmaceutical applications [54].

Antimicrobial activities of wide range of plant extracts have many applications, including pharmaceuticals, alternative medicines, raw and processed food preservation and natural therapies [55]. Many studies have reported that spices phenolic compounds and herbs considerably contribute a lot to their pharmaceutical and antioxidant properties [56]. The spices have a special flavour and aroma that are derived from compounds which are commonly known as secondary metabolites or phytochemicals. The phytochemicals are mostly antimicrobial substances found in the spices which are potentially repel harmful organisms and attracting benefits; they also widely used as photoprotectants and responds to environmental changes friendly. A numbers of classes of phytochemicals including the flavonoids, isoflavones and anthocyanins are found to be associated with most of the spices [54].

The polyphenolic substances play a wide range of biological activities such as anti-inflammatory, antibacterial, hepatoprotective, antiallergic, antithrombotic, antiviral, anticarcinogenic and vasodilatory and cardioprotective effects [57]. Such huge essential functions have been associated with their antioxidant activity through several mechanisms such as reducing agents, free radical scavengers, quenchers of the formation of singlet oxygen, complexers of pro-oxidant metals and stimulating the antioxidative defense enzymes activities [58].

Different types of food packaging in combination with different storage techniques can be used in order to extend the shelf-life of foods. One of the most significant technological techniques lagely needed during the time of storage is maintaining and preservation of food from contamination, microbial spoilage and proliferation of food borne pathogenic microorganisms [59, 60, 61].

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