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Review on Characteristics of Probiotics from Ethiopian Traditional Fermented Dairy Products

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Abstract

Fermentation is an ancient widely practiced technology and fermented foods are an essential part of diets in all parts of the world. Traditional fermentation is an ancient food preservation technology, although, it is still part of the cultural norm usually being practiced at a local or household level among indigenous communities in Africa and most of the developing world. Based on its rich cultural diversity, a wide variety of fermented foods and beverages are consumed in Ethiopia from different raw materials such as cereals, ensete (false banana), honey, milk, etc. Fermented dairy products are products that can be produced via fermentation of lactose by microorganisms especially by lactic acid bacteria (LAB). Emergence of new pathogens, the resurgence of old ones, and development of antibiotic resistance have caused an increase in research on a group of LAB known as probiotics that could be potentially used to combat bacterial infections.

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Introduction

Fermentation is an ancient widely practiced technology and fermented foods are an essential part of diets in all parts of the world (Akalu *et al.,* 2017). The fermentation processes associated with the different products makes the raw materials not only more palatable but also extends the shelf life of the product. Fermentation can improve the nutritional quality of some products due to some desirable changes such as increased nutrient bioavailability and synthesis of some nutrients. Further, fermented foods often reported to be more microbiologically stable and safe and health-promoting compared to unfermented foods (Protus, 2016).

Traditional fermentation is an ancient food preservation technology, although, it is still part of the cultural norm usually being practiced at a local or household level among indigenous communities in Africa and most of the developing world (Pundir *et al.,* 2013). Food fermentation covers a wide range of microbial and enzymatic processing of food and ingredients to achieve desirable characteristics such as prolonged shelf-life, improved safety, attractive flavor, nutritional enrichment, and promotion of health (Holzapfel, 2002).

Ethiopia is one of the countries where a variety of fermented, nonfermented foods and beverages are produced and consumed from a wide range of raw materials using traditional techniques. The most known are:1) breads, injera (pancake like flat bread, difo-dabo, and ambasha; 2) beverages: a) alcoholic: tella (local beer), areki (liquor) and tej; b) nonalcoholic: borde, shamita, bukre, cheka, merissa, korefe and non-fermented foods:kita, guenfo, atmit, kinche, nifro, kollo, besso, chicko, shiro,and ashuck,etc (Tiruha *et al.,* 2014).

Based on its rich cultural diversity, a wide variety of fermented foods and beverages are consumed in Ethiopia from different raw materials such as cereals, ensete (false banana), honey, milk, etc. (Mogessie, 2006).

Fermented dairy products are products that can be produced via fermentation of lactose by microorganisms especially by lactic acid bacteria (LAB). When examining worldwide, various dairy products which are different in name but similar in content can be found and those products are an important part of human diet (Nilsson *et al.,* 2006; Hugenholtz, 2013).

Historically, the major use of food fermentation by LAB has been to prevent spoilage. However, the emergence of new pathogens, the resurgence of old ones, and development of antibiotic resistance have caused an increase in research on a group of LAB known as probiotics that could be potentially used to combat bacterial infections (Aijaz and Tariq, 2012).

Probiotics are defined as live microorganisms which when administered in adequate amounts confer a health benefit on the host (FAO/WHO, 2002). Probiotics have recently emerged as the most powerful food grade microbial agents comprise the ability to express numerous health promoting functions of considerable commercial value and therapeutic potential (Jain *et al.,* 2017).

Milk and milk products play a very important role in feeding the rural and urban population of Ethiopia and have a high nutrition value and is daily produced, sold for cash or readily processed (Belete *et al.,* 2010). Dairy production depends mainly on indigenous livestock genetic resources; more specifically on Cow, goats, camels and sheep (CSA, 2009). Cow has the largest contribution (81.2%) of the total national annual milk output, followed by goats (7.9%), camels (6.3%) and sheep (4.6%). In rural area cow is a common domestic animal so that cow milk can be potential source for probiotic bacteria. Cow Milk is a pale liquid produced by the mammary glands of cow. It is the primary source of nutrition for infant mammals before they are able to digest other types of food (Misganaw and Teketay, 2016).

Ethiopia holds large potential for dairy development. These dairies, especially yoghurt and cheese are popular and their antagonistic effect against pathogens is important. Accordingly the present study will be conducted to screen out best probiotics from dairy products and to evaluate their antagonist effect against some human pathogens.

Fermentation of dairy Products

Fermented food can be define as food substrate being employed by edible microorganisms with their enzymes function to hydrolyze the respective substrates along with desirable changes of flavors, aroma, and texture (Balqis *et al.,* 2013).

Since ancient times, dairy products have been part of human diet. These serve as good source of calcium, vitamin D, proteins and other essential nutrients. Various fermented dairy products are prepared using different microbial strains. Microbes ferment the carbohydrates present in milk, which is mainly lactose to lactic acid and some other products. The acid precipitates the proteins in the milk; therefore fermented products are usually of thicker consistency than milk. The high acidity and low pH hinders the growth of other bacteria including pathogens. The fermentation of milk provided a simple way to increase its shelf-life while improving its safety. Humans learned to control fermentation processes from the initial accidental events in fermentation (Anil and Nikita, 2017).

Since milk is full of nutritious products, its fermented products are also having their popularity among consumers (Gonfa *et al.,* 2001). It must be noted that a group of useful bacteria are involving in production of milk products where a unique ration of each of them lead to produce a special products with aroma and antibacterial compounds etc. Varying the micro flora of fermented dairy products from region to region is arising from climatic environment and being eminent of healthy products. Nutritionally, milks contain properties by which plays important role in the controls of diseases (Shortt *et al.,* 2004). In fermentation process, microorganisms change organic compounds and convert them into desirable products such as lactic acid, ethanol, and CO_2. In this regards fermentation caused to create more delicious beverage and food. Acidity of milk activates enzymes with ability of curdling and culturing milk to produce butter and cheese so findings showed that fermented foods and drinks has a long history (Javad *et al.,* 2016).

Milk products also serve as the important delivery vehicles for probiotic bacteria. The probiotic bacteria have a long history of association with dairy products. This is because some of the same bacteria that are

associated with fermented dairy products also make their homes in different sites on the human body, including the mouth, the gastrointestinal tract etc. Some of these microbes, therefore, can play a dual role in transforming milk into a diverse array of fermented dairy products (yoghurt, cheese, kefir, etc.), and contributing to the important role of colonizing bacteria. In fact, in some countries, dairy products, represent a major portion of the total functional foods market, and are at the forefront of probiotic developments (Panesar, 2011).

Traditional Fermented Dairy Products in Ethiopia

Livestock keeping is an ancient tradition of rural areas of Ethiopia. The size and diversity of livestock resources have become vital to sustenance of rural life and in fact the largely agrarian economy of the country.

Cattle constitute the predominant element of livestock wealth in Ethiopia both in the agricultural high lands and pastoral and agro-pastoral low lands, and hence the proportional contribution to the national economy is considered to be high (Yayeh *et al.,* 2017). Livestock provide food in the form of meat and milk, non-food items such as draft power, manure and transport services as inputs into food crop production, fuel for cooking

Various milk products are consumed in the fermented form in Ethiopia. The fermentation is usually carried out by the micro flora, with no defined starter cultures used to initiate it. Most of the time, this is made possible through the proliferation of the initial milk flora, with microbial succession determined by ambient temperatures and chemical changes in the fermenting milk.

No attempt is made to control the fermentation process in most urban homes. Raw milk is kept in a warmer place or left at ambient temperatures to ferment. Among pastoralists of rural areas, raw milk is usually kept in a well-smoked container and milk from a previous fermentation serves as source of inoculums.

Milk is used for rearing calves and children, and the surplus is soured for making different fermented milk products. The major ones include: *Ergo*, *Ayib*, butter (three types of butter can be distinguished namely *Lega*, *Mekakelegna* and *Besal*, which refer to fresh, semi-rancid and rancid), *Nitir kibie* (melted butter or ghee), *Arrera* (defatted sour milk, a by-product of butter-making and a raw material for *Ayib*-making) and *Aguat* (whey) (Zelalem, 2012).

Ergo (sour milk)

Ergo is produced from raw milk of cattle in all parts of Ethiopia by smallholder farmers. It is also made from milk of goats and camels in the lowland regions in relatively small amounts. As the major fermented dairy product, ergo is popular and is consumed in all parts of the country and by every member of the family. It is known by many different names by the many ethnic groups in the country. Ergo is considered as a special food which serves as a basis for further processing and it is particularly used as a nutritional support to sick people, children and to pregnant and lactating mothers (Muluken *et al.,* 2016).

Different groups of microorganisms may participate in and contribute to the final characteristics of *ergo*, as a natural fermentation. The safety and wholesomeness of a fermented product may depend on the types of LAB that are involved in the fermentation process (Anteneh *et al.,* 2011).

Microorganisms involved in the culturing of "*Ergo*" were studied by Almaz *et al.,* 1999) and reported that S*treptococcus*, *Lactobacillus, Lactococcus* and *Leuconostoc* are carried out the souring process. The most dominant species throughout the fermentation are *lactococci* that reached colony counts as high as 10^9 CFU/ml at the end of the fermentation process. 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%.

Findings from the various workers indicate that *ergo* is a product obtained by spontaneous fermentation and cannot be defined in terms of its biochemical or microbiological 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 (Mogessie, 2006).

Ititu (Concentrated sour milk)

Concentrated fermented milk product known as *ititu in Afaan Oromo is* prepared and consumed by the Borana tribes in southern part of Ethiopia. The pastoral/farmer community prepares this product during the rainy season when milk is available in huge amount for later

consumption during the drier seasons when fresh milk supply is markedly scanty. The product remains acceptable for about two months at ambient temperature (25-30 ºC) and has a good keeping quality and can be stored from about two months to three months. 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 (Almaz *et al.,* 2001; cited in Mogessie, 2006).

While traditional preparation of *ititu,* fresh milk is collected in a well-smoked container called gorfa. Gorfa is woven from fibers of selected plants into a lidded vessel 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.

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 (Kassaye *et al.,* 1991; Almaz *et al.,* 2001).

Ethiopian cottage cheese

Cheese is an ancient, traditional food and is an important dairy product; Humans have been making and eating cheese since prehistoric times. Purpose for preparation is to gather the interest of the people, because it is nutritious food, excellent source of fat, has high calorific value, good source of protein and rich source of vitamin A, D, Ca and P (Bezaye, 2010)

Ayib in Amharic is a traditional Ethiopian cottage cheese produced by slowly heating naturally soured milk until a distinct curd mass forms and floats over the whey. *Ayib* is a popular milk product consumed by the various ethnic groups in the country (Anteneh *et al.,* 2011). Temperature of *ayib* preparation could vary between 40 to 70 °C without any significant impact on composition and yield. But, temperature beyond 80°C imparts a cooked flavour to the product. Curd preparation at 70°C produced a wholesome product with low microbial count (Mogessie, 1990). 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 (Anteneh *et al.,* 2011). 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 (Anteneh *et al.,* 2011).

Based on the study of the microbiological quality of *ayib* (Mogessie, 1990), 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. Above 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.

More over analysis of *ayib* microbiota showed that yeast and bacterial counts did not correlate with pH value of *ayib* samples. However *ayib* samples with pH >4.0 contained more bacterial groups than those with pH <4.0. The Gram-positive rods dominated the aerobic mesophilic bacterial flora, *Microbacterium* and *Brevibacterium spp*. being the most abundant. *Enterobacteriacea*e and *Pseudomonas spp*. constituted the bulk of the Gramnegative rods. Lactic acid bacteria count was around 10^6 CFU/g and *Lactobacillus fermenti* and *Lactobacillus plantarum* dominated the flora (Mogessie, 1994).

Traditional Ethiopian 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.

Table.1 Associated microorganisms in Ethiopian fermented dairy products

Fermented Dairy Products	pH	Titrable acid	Associated Microorganisms	References
Ergo (sour milk)	4.3	0.88	*Lactococcus garvieae*	Esayas, *et al.,* 2008
			Lactococcus lactic sub spp. Lactic Lactococcus, Streptococcus, Leuconostoc	
			Lactobacillus Food	
			Lactobacillus Food	Mogessie, 1994a.
			Salmonella spp., Bacillus cereus	
			Staphylococcus aureus, Listeria monocytogene	
			After 48 hours of fermentation, the colony count highly reduced	
Ititu (concentrated sour milk)	3.45	1.92%	*Lactobacillus casei Lactobacillus plantarum*	Gonfa *et al.,* 2001
	4	0.43*	*Bacillus cereus, Staphylococcus aureus*	Eyassu, 2013.
Ayib (Ethiopian cottage cheese)			*Microbacterium, Brevibacterium spp*	Mogessie, A., 1994b
			Enterobacteriaceae Pseudomonas spp	
			Lactobacillusfermenti Lactobacillus plantarum	
			Salmonella typhimurium, *Salmonella enteritidis Salmonella infantis*	Mekonnen, T. and A. Mogessie, 2005

The moisture content of the traditional dairy product, butter 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).

Antagonistic Effects of probiotics

Generally, the Lactic Acid Bacteria LAB are the most implicated of the probiotic organisms with respect to intestinal bacterial colonization, particularly those of the genera *Lactobacilli* and Bifidobacteria, which stakes out their territory by secreting acids, thereby creating an environment which is inhospitable to disease-causing bacteria. *Lactobacilli* change the oxidation-reduction potential through its production of metabolites by making the environment less conducive for organisms requiring oxygen. This action contributes to the overall inhibiting effect of these probiotic bacteria (Olotu, 2007).

Probiotic prophylaxes and therapies are gaining wider acceptance as more scientific data emerge regarding the interaction between pathogen and beneficial microbes in the human intestinal tract and molecular mechanisms of probiotics' action. Probiotic bacteria which confer beneficial effect for the host and have pronounced antagonistic activity against these pathogens are expected to present a clear alternative in the prevention and treatment of foodborne infections (Moore *et al.,* 2013).

One of the most frequent health claims for probiotics concerns the putative reduction and prevention of infectious disease in the GIT. The effect of probiotic strains depends on their ability to survive during passage through the stomach, as well as their ability to persist and compete with pathogens in GIT (Hutt *et al.,* 2006). In order to test the suppression of different pathogens by

probiotic bacteria, it is necessary to consider their distinct environmental conditions in GIT.

In conclusion, a greater portion of the population of developing nations depends on agriculture. Livestock keeping is an ancient tradition of rural areas of Ethiopia. Cattle constitute the predominant element of livestock wealth in Ethiopia both in the agricultural high lands and pastoral and agro-pastoral low lands. Dairy products in Ethiopia such as sour milk, concentrated sour milk, Ethiopian cottage cheese contains important bacteria in different concentration. Lactic Acid Bacteria LAB are the most implicated of the probiotic organisms with respect to intestinal bacterial colonization, particularly those of the genera *Lactobacilli* and Bifidobacteria, have antagonistic to disease-causing bacteria.

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