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Review

A review on lactic acid bacteria in indigenous traditionally fermented camel milk of Ethiopia

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This research review aimed at analysis of lactic acid bacteria (LAB) studies of Ethiopian indigenous traditionally fermented camel milk food products. Lactic acid bacteria (LAB) have a very long history of use in the manufacturing processes of dairy fermented foods. In the eastern part of Ethiopia, farmers and pastoralists produce indigenous traditionally fermented camel milk products including *dhanaan* and *ititu.* Fermentation of *dhanaan* and *ititu* is carried out by mesophilic lactic acid bacteria. Lactic acid bacteria are a broad group of Gram positive organism and are mainly used as a starter strains, predominantly, *Streptococcus thermophilus, Lactobacillus lactis, Lactobacillus helveticus,* and *Lactobacillus delbrueckii subsp. Bulgaricus* are widely used as dairy starter cultures. Genus *lactobacillus*, genus *lactococcus* and genus *entrococcus* bacteria involved in the fermentation of *ititu*. *Lactobacillus salivarius, Lactobacillus plantarum, Lactobacillus delbrueckii subspecies bulgaricus, Lactococcus lactis subspecies lactis,* and *Enterococcus faecalis* are the isolated lactic acid bacteria species from *ititu.* In conclusion, the present research review showed that the potential starter culture of lactic acid bacteria and manufacturing protocol of *ititu* was studied in detailed but there is a gap needs further research for *dhanaan*.

Key words: Indigenous, fermentation, lactic acid bacteria, mesophilic lactic bacteria, dhanaan, and ititu.

INTRODUCTION

Lactic acid bacteria encompass an ecologically diverse group of microorganisms united by formation of lactic acid as their main products of carbohydrate metabolism (Lonvaud-Funel, 2001, Carr *et al*., 2002, Liu, 2003). The lactic acid bacteria can be mainly divided into two groups based on the end-products formed during the fermentation of glucose. Homofermentative lactic acid bacteria such as *Pediococcus, Streptococcus,* and *Lactococcus* produce lactic acid as the sole product of glucose fermentation. Heterofermentative lactic acid bacteria such as *Weissella* and *Leuconostoc* produce equimolar amounts of lactate, CO_2 and ethanol from glucose (Caplice and Fitzgerald, 1999; Jay, 2000; Kuipers *et al*., 2000).

Lactic acid bacteria are a broad group of Gram-positive, non-spore forming, catalase-negative, facultative anaerobic and nutritionally fastidious organism (Axelsson, 1998). They are widespread in soil, vegetables, meat, milk and the human body. Lactic acid bacteria (LAB) consist of a number of bacterial genera within the phylum Firmicutes. The genera *Carnobacterium, Enterococcus, Lactobacillus, Lactococcus, Lactosphaera, Leuconostoc, Melissococcus, Oenococcus, Pediococcus, Streptococcus, Tetragenococcus, Vagococcus* and *Weissella* are recognized as lactic acid bacteria (Ercolini *et al.,* 2001, Stiles and Holzapfel, 1997).

Undoubtedly, the most important application of lactic acid bacteria is their use as starter strains in the manufacture of various fermented dairy products. In particular, *Streptococcus thermophilus, Lactobacillus lactis, Lactobacillus helveticus,* and *Lactobacillus delbrueckii subsp. Bulgaricus* are widely used as dairy starter cultures. *Streptococcus thermophilus* and *Lactobacillus bulgaricus* are the two bacteria required to make yoghurt and *Lactobacillus casei* is frequently found in cheeses. The proper selection and balance of lactic acid bacteria used for starter culture is critical for the manufacture of diary fermented food products with their desirable texture and flavor. Mankind exploited these bacteria for thousands of years for the production of fermented products because of ability to produce desirable changes in taste, flavor, and texture (Derek *et al.,* 2009). In addition, certain lactic acid bacteria strains of the genera *Lactobacillus*, are utilized as health- promoting bacteria (Saxelin et al., 2005), while certain *Lactobacillus* strains are believed to produce bioactive health beneficial peptides from milk proteins (Meisel and Bockelman, 1999, Korhonen and Pihlanto, 2003). Another promising contribution of lactic acid bacteria is to use them as delivery vehicles for molecules with therapeutic value (Nouaille *et al*., 2003). *Lactococcus lactis* is the most extensively studied LAB organism and the second most studied Gram positive bacterium with respect to its genetics, physiology, and molecular biology. Previous reports introduce that genus lactic acid bacteria (LAB) are the dominant bacterial microbiome of fermented milk products which contributes towards the safety, nutritional value, shelf life and acceptability of a wide range of dairy fermented products. Another promising avenue for lactic acid bacteria is to use them as viricidal (Esser *et al.,* 1983) and antitumor (Jay, 1996).

According to FAO (2009), there are about 22 million camels in the World. Of this, 19.58 million are believed to be one-humped dromedary camels (Camelus dromedarius) while the remaining 2.42 million are two-humped bacterian camels (Camelus bactrianus). More than 60% of the dromedary camel population is concentrated in the four North East African countries viz. Somalia, Sudan, Ethiopia and Kenya (FAO, 2004). Ethiopia is estimated to have the third largest camel herd in the world after Somalia and Sudan (FAO, 2008). According to CSA (2010), Ethiopia which possesses about 2.4 million camels ranks second in camel milk production in the world next to Somalia. Pastoralists consume camel milk either in its raw state without heat treatment or after it turns sour (Seifu, 2007). Fermentation appears to be the only means of preserving camel milk under warm conditions (Farah, 1993; Mohamed et al., 1990). Camel milk has critical importance for treatment of various communicable and non-communicable diseases and it is superior nutritionally and medicinally than other milk species (Rao M.B *et al.,* 1970). In India, camel milk is used therapeutically against dropsy, jaundice, problems of the spleen, tuberculosis, asthma, anemia, piles and diabetes (Rao M.B *et al,* 1970).

The production of Ethiopian indigenous traditional fermented camel milk food products is still a household art and in most households no attempt is made to control the fermentation process and products in generally have poor qualities and do not meet the acceptable quality requirements. In Ethiopia, study on microbiology and nutritional quality of traditionally fermented camel milk food products was started in 1991. Most of the works are addressed on microbiological quality and chemical composition of traditionally fermented food products and basically concern on isolation, characterization, and development of starter culture, and processing of traditional fermented foods in order to improve the spontaneous traditional fermentation by controlled fermentation using mesophilic lactic acid bacteria. The objective of this research review is limited to analysis of bacteriological studies conducted by various researchers on fermentation of Ethiopian indigenous traditional fermented camel milk food products.

Traditional Fermented Camel Milk Products

There are various traditional fermented camel milk products that are produced in different parts of the world by camel herders (Abdelgadir *et al.,* 1998; Abdel Rahman *et al.,* 2009; Hassan *et al.,* 2008; Lore *et al.,* 2005; Yagil, 1982). *Suusac* and *garris* are fermented camel milk products in Kenya, Somalia and Sudan (Abdelgadir *et al.,* 1998; Lore *et al.,* 2005). In Ethiopia, Pastoralists produce different fermented camel milk products such as *dhanaan* which is produced by pastoralists in Somali Region (Seifu, 2007) and *ititu* is produced in the Kereyu area of the Oromia Region in the eastern part of Ethiopia. These two products are Ethiopian indigenous traditionally fermented camel milk food products.

Pastoralists in eastern Ethiopia produce traditional fermented camel milk by placing fresh camel milk in a clean and smoked container, wrapping the container with a piece of cloth, and keeping it in a warm (ambient temperature 25^{o}-30°C) place for about 12-24 hrs to allow spontaneous fermentation to take place (Seifu, 2007). Fermentation is initiated by the natural microorganisms of the milk without using commercial starter cultures or by back sloping technique (adding small amounts of previously fermented milk as a starter into fresh camel milk). In most urban homes, no attempt is made to control the fermentation. Due to spontaneous nature of the fermentation, this traditional method results in a product with varying taste and flavor often of poor hygienic quality.

Ititu

Ititu is produced by the Kereyu area of the Oromia Region in the eastern part of Ethiopia (Eyassu, 2012). These pastoral communities in this area produce *ititu* when fresh milk is available during the rainy season (Almaz Gonfa *et al.,* 2001).

The product has good nutritional quality and shelf life for about two months at ambient temperature (25^{o}C-30^{o}C). The traditional fermentation process and consumption pattern of *ititu* is well described by (Almaz Gonfa *et al.,* 2001). 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.

Table 1. Lactic acid bacteria species isolated from the traditional fermented camel milk *ititu.*

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

Source: Eyassu (2007).

Manufacturing Protocol of Ititu

Farmers and pastoralists in Kereyu area of the Oromia Region traditionally ferment *ititu* from camel milk. During the traditional fermentation of *ititu*, fresh milk is collected in a well smoked fermenting vessel called gorfa (Kassaye *et al.,* 1991). Gorfa is woven from fibers of selected plants into a lidded container with a capacity up to three litters. 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 (Kassaye et al., 1991; Almaz Gonfa et al., 2001). 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.

Bacteriological Quality of Ititu

Eyassu (2012) made a study on isolation of lactic acid bacteria involved in the fermentation of *ititu* and reported that genus *lactobacillus*, genus *lactococcus* and genus *entrococcus* carried out the souring process. *Lactobacillus* species was the dominant genus and comprised of (58%) of the total lactic acid bacteria isolates (Table 1). Similar observations in composition and diversity of lactic acid bacteria isolated from traditional fermented milk of cows and camel were reported by (Gonfa *et al.,* 1999) from Ethiopia.

Lactobacillus salivarius, Lactobacillus plantarum and *Lactobacillus delbrueckii subspecies bulgaricus, Lactococcus lactis subspecies lactis,* and *Enterococcus faecalis* are the isolated lactic acid bacteria species from *Ititu. Lactobacillus salivarius* was a relatively fast acid producer bringing the initial pH of the skim milk medium to the target/final value of 4.6 before 48 hrs of incubation followed by *Lactobacillus plantarum* and *Lactobacillus delbrueckii subspecies bulgaricus.* Similar observations in composition and diversity of lactic acid bacteria isolated from traditional fermented cow milk from Borona tribes in southern Ethiopia were reported by (Kassaye *et al.,* 1991), the total bacterial count was 10^{12} cfu/g, mainly dominated by lactic acid bacteria. Genus *Lactobacillus* (*Lactobacillus casei* and *Lactobacillus plantarum*) are the dominant lactic acid bacteria species.

Kassaye et al. (1991) studied chemical and microbiological characteristics of *ititu* randomly collected from individual households in Borana region. *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. Most of these values varied markedly among samples, though. *Ititu* had increased contents of free and total amino acids when compared to fresh whole milk and was rich in amino acids such as glutamic acid, alanine, proline, leucine and serine (Kassaye *et al.,* 1991). In a study on farm-made fermented milk in southern Ethiopia, (Fekadu Beyene and Abrahamsen., 1997) reported that *ititu* had 3.3 - 3.7% fat, 3.3 - 3.6% protein and 3.3 - 3.5% lactose. (Kassaye *et al.,* 1991) further reported that the total bacterial count was 10^{12} cfu/g, mainly dominated by lactic acid bacteria.

Dhanaan

Pastoralists in the areas of Shinile and Jigjiga zones of eastern part of Ethiopia produce naturally fermented sour milk called *dhanaan* (Seifu, 2007). *Dhanaan* has better nutritional quality and shelf life (up to five months) as compared to *ititu.* The traditional fermentation process, consumption pattern and bacteriological characteristics of *dhanaan* are not well described.

Dhanaan is made by placing fresh camel milk in a clean/smoked container, wrapping the container with a piece of cloth and keeping it in a warm (ambient tempera-

Table 2. Amino acid content of ititu.

Amino acid	Day 0		Day 28	
	Free	Total	Free	Total
Alanine	0.29	9.06	1.74	12.65
Arginine	0.06	10.75	0.55	14.73
Aspartic	0.45	20.96	0.77	26.61
Cystine	0.00	1.81	0.00	2.16
Glutamine	1.23	67.96	2.45	85.27
Glycine	0.19	5.06	0.36	6.89
Histidine	0.06	8.22	0.16	10.85
Isoleucine	0.08	15.11	0.36	19.08
Leucine	0.22	28.45	1.29	40.33
Lysine	0.24	24.88	0.69	33.26
Methionine	0.05	8.85	0.18	11.90
Phenylalanine	0.14	14.67	0.64	19.48
Proline	0.08	29.81	1.66	36.64
Serine	0.24	15.46	0.97	20.64
Threonine	0.13	12.37	0.55	15.81
Tyrosine	0.09	14.87	0.57	21.60
Valine	0.13	17.51	0.54	22.18

Source: Kassaye *et al*., (1991)

ture) place for about 12-24h to allow spontaneous fermentation. *Dhanaan* have a shelf life of about 5 months. 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 made 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 (Abdelgadir *et al.,* 1998).

No information has been reported about the bacterial characteristics, manufacturing protocols and potentials of the fermented camel milk product *dhanaan* produced in Ethiopian. Pastoralists make *dhanaan* from camel milk because they believe 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 quenches thirst. Although, the farmers and pastoralists in eastern Ethiopia are widely using *dhanaan* for nutritional and medicinal benefits, there was no study conducted regarding the scientific microbiological evaluation as far as Ethiopia is concerned.

Dhanaan is made by spontaneous fermentation without using 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 hrs to obtain *dhanaan* (Seifu, 2007). Kenyan researchers showed that the quality of *susac*, fermented camel milk, 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). Isolation and identification of microorganisms that are responsible for the fermentation and production of the indigenous fermented camel milk product, *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; otherwise the fermentation process doesn't take place. This suggests that the microorganisms responsible for souring or fermentation of camel milk are probably *thermophilic* anaerobic types.

CONCLUSION

In Ethiopia, a significant amount of camel milk is consumed in the fermented form and the fermented product has different vernacular names. In the eastern part of Ethiopia farmers and pastoralists produce indigenous traditionally fermented camel milk products such as *dhanaan* and *ititu*. Fermentation of *dhanaan* and *ititu* is carried out by mesophilic lactic acid bacteria used as starter cultures. Lactic acid bacteria (LAB) have a very long history of use in the manufacturing processes of dairy fermented foods and a great deal of effort was made to investigate and manipulate the role of lactic acid bacteria in these processes.

Lactic acid bacteria are a broad group of Gram-positive organism and are mainly used as a starter strains, particularly, *Streptococcus thermophilus, Lactobacillus lactis,* *Lactobacillus helveticus,* and *Lactobacillus delbrueckii subsp. Bulgaricus* are widely used as dairy starter cultures. Genus *lactobacillus*, genus *lactococcus* and genus *entrococcus* bacteria involved in the fermentation of *ititu*. *Lactobacillus salivarius, Lactobacillus plantarum* and *Lactobacillus delbrueckii subspecies bulgaricus, Lactococcus lactis subspecies lactis,* and *Enterococcus faecalis* are the isolated lactic acid bacteria species from *ititu. Lactobacillus salivarius, Lactobacillus plantarum* and *Lactobacillus delbrueckii subspecies bulgaricus* are relatively fast acid producers respectively. However, *Dhanaan* is made by spontaneous fermentation without using a starter culture. In conclusion, the present review showed that the potential starter culture of lactic acid bacteria and manufacturing protocol of *ititu* was studied in detailed but No information has been reported about the bacterial characteristics, manufacturing protocols and potentials of *dhanaan* which needs further research as far as Ethiopia is more concerned.

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