

Research Article

Term paper on the Common contaminants and challenges related to psychotropic microbial spoilage of milk and dairy products in Ethiopia

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Abstract

Milk is known for its high nutritional value and has been increasingly included in human diets. However, the nutritional content of milk, together with its water activity (aw), provides suitable conditions for growth of a multitude of spoilage and pathogenic microorganisms. The contamination and growth of these microorganisms present in raw milk may occur on the farm, during transportation and in the processing plant. They can negatively affect the quality of dairy products. For example, milk containing high numbers of bacteria will have a short shelf life, resulting in off flavours and smells, and also increase fouling in heat exchangers and thus reduce the efficiency of processing equipment. Additionally; milk produced in unhygienic environments using methods that do not follow the principles of good hygienic practices is conducive to microbial contamination. This may increase the exposure of consumers to foodborne pathogens, resulting in foodborne infections. Exposure to foodborne pathogens through consumption of contaminated milk and dairy products is a global problem, which is exacerbated in developing countries like Ethiopia. Some microorganisms, called psychrotrophs, adapt to refrigeration temperatures by synthesizing phospholipids and neutral lipids containing increased proportions of UFA, resulting in a reduction in the melting point of the lipids and also the major technological problems in the dairy industry. The heat-stable enzymes produced by psychrotrophs that can attack milk components. These Microbial contaminants are most and commonly introduced into milk during the milking practice and at subsequent milk processing steps. The objective of this paper is to review common contaminants and chal-

lenges related to psychotropic microbial spoilage of milk and dairy products in Ethiopia.

Keywords: Contaminants; Challenges; Dairy Products and Ethiopia; Microbial Spoilage; Psychotropic Bacteria; Raw Milk

Introduction

A 'dairy product' is a milk-based product that keeps the nutritional benefits of milk while also making it more appealing to customers [1] milk and milk products form a major Part of human food and play a prominent role in the diet Milk and dairy products contain many nutrients, such as protein, vitamins, calcium, phosphorus, magnesium, zinc, etc., which are necessary for healthful living of humans of all age groups and both sex. Milk is known for its high nutritional value and has been increasingly included in human diets [2]. However, the nutritional content of milk, together with its high water activity (aw), provides suitable conditions for growth of a multitude of spoilage and pathogenic microorganisms [3,4].

Some microorganisms, called psychrotrophs, adapt to refrigeration temperatures by synthesizing phospholipids and neutral lipids containing increased proportions of UFA, resulting in a reduction in the melting point of the lipids. This phenomenon serves to maintain their fluidity, thus allowing the continued functionality, solute transport, and secretion of extracellular enzymes [5]. Psychrotrophs are capable of synthesis of phospholipids and lipids, resulting in an increasing proportion of polyunsaturated fatty acids in their cells which protect the bacteria in a refrigerated environment [6]. These bacteria primarily exist in the water and soil. One of the major technological problems in the dairy industry is the heat-stable enzymes produced by psychrotrophs that can attack milk components. Although these bacteria can be killed by pasteurization and UHT processing, heat-stable enzymes produced by these bacteria during milk storage and transportation under refrigeration can remain active after heat treatment.

Raw milk is a fragile ingredient in dairy product manufacture, and the quality of the final product may be affected by many variables associated with raw milk handling. From a microbiological perspective, it is thought that the bacteria, in terms of the numbers and types, present in raw milk at the start of dairy product manufacture, have a big impact on the quality of the final product. Although the combination of refrigeration ($\leq 7^{\circ}\text{C}$), reduced transportation and storage time and heat treatments (72°C , 15 s or 140°C , 1-10 s) of raw milk can significantly extend the shelf life of dairy products, heat-stable bacterial enzymes produced by psychrotrophic bacteria (psychrotrophs) have the potential to harm final dairy products. For example, in ultra-high-temperature (UHT) dairy products, the shelf life is affected mostly by psychrotrophic bacterial proteolytic enzymes [7].

The contamination and growth of these microorganisms present in raw milk may occur on the farm, during transportation and in the processing plant. They can negatively affect the quality of dairy products. For example, milk containing high numbers of bacteria will

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have a short shelf life, resulting in off flavours and smells, and also increase fouling in heat exchangers and thus reduce the efficiency of processing equipment [8]. Additionally, milk produced in unhygienic environments using methods that do not follow the principles of good hygienic practices is conducive to microbial contamination [3]. This may increase the exposure of consumers to foodborne pathogens, resulting in foodborne infections. Exposure to foodborne pathogens through consumption of contaminated milk and dairy products is a global problem, which is exacerbated in developing countries [9].

These heat-stable enzymes can degrade milk proteins and fats, increasing the concentration of free fatty acids and amino acids, resulting in a trace of bitterness, off-flavor and gelation of final products. Milk and dairy products provide unique conditions for the growth of microorganisms. Effectively controlling these microbes is a critical challenge for the dairy industry. The predominant microorganisms leading to product spoilage can vary widely depending on a combination of factors such as the type of product, method of processing, storage temperature, pH, head-space oxygen concentration, and the source of contamination.

One of the major technological problems in the dairy industry is the heat-stable enzymes produced by psychrotrophs that can attack milk components. Although these bacteria can be killed by pasteurization and UHT processing, heat-stable enzymes produced by these bacteria during milk storage and transportation under refrigeration can remain active after heat treatment. These heat-stable enzymes can degrade milk proteins and fats, increasing the concentration of free fatty acids and amino acids, resulting in a trace of bitterness, off-flavor and gelation of final products. These heat-stable enzymes can resist pasteurization (72°C, 15 s) and even ultra-high temperature (135°C-150°C, 1-10 s). Proteases hydrolyse casein, causing bitterness and gelation of UHT milk. Lipases degrade triglycerides and are associated with flavor defects in cream, butter, cheese, and UHT milk [8].

So these Microbial contaminants are most and commonly introduced into milk during the milking practice and/or at subsequent milk processing steps [10]. For example, the farm environment such as dirty udder exteriors, feces, bedding, and soil in the milking environment and contaminated surfaces of milk handling equipment and utensils (unsanitary design and insufficient cleaning) contribute heavily to contamination during milking [4,11-13]. These problems are seen in countries like Ethiopia where there are a number of challenges in acquiring appropriate milk handling equipment and limited access to clean water [14-16].

The objective of this term paper is to review the Common contaminants and challenges related to psychotropic microbial spoilage of milk and dairy products in Ethiopia and to provide information through a review of previously published peer-reviewed literature, Journal and Researches. Moreover, the information provided in this paper cannot enough but it's nearly enough to inform future intervention areas in the dairy Sectors in the country.

Psychotropic Bacteria Spoilage in Milk and its Products

Raw milk is free of microorganisms when it is in a healthy cow's udder. However, milk starts to become contaminated by many microorganisms when it is released from the teat channel. After secretion, faecal contamination and general dirt from the animal surface and microorganisms in the environment will find their way into the

milk [17]. Under good hygiene conditions, psychrotrophs generally account for 10% of the microbiota of raw milk, but when milk is stored under poor hygienic conditions, the psychrotrophic bacterial population can make up 75% of the total microbial load of raw milk [17].

Fresh raw milk drawn from a cow's udder does not contain detectable numbers of culturable psychrotrophs [6]. However, these populations develop over time during cold storage raw milk. The faster the milk is cooled after milking, the better the quality when it is received from the farm [18]. Both Gram-negative and Gram-positive psychrotrophs have been isolated from raw milk. Gram-positive bacteria found in raw milk include the following genera: *Bacillus*, *Clostridium*, *Corynebacterium*, *Microbacterium*, *Micrococcus*, *Arthrobacter*, *Staphylococcus*, and *Carnobacterium* [6].

Pseudomonas, *Aeromonas*, *Hafnia*, *Acinetobacter*, and *Serratia* are the most commonly found genera in raw refrigerated milk. The majority of Gram-negative psychrotrophs isolated from raw milk are *Pseudomonas* spp., which usually comprise 65-70% of the whole psychrotrophic bacterial population [18].

The longer lag phase at temperatures of 0-7°C, *Bacillus* spp. are less common in raw milk than *Pseudomonas* spp. However, *Bacillus* predominates in marginal cooling temperatures, from 8°C to 10°C. *B. cereus* is an important psychrotrophic pathogenic bacterium in the dairy industry as it produces endospores and survives after heat treatment. The majority of *B. cereus* is not able to degrade lactose but can ferment glucose, fructose, trehalose, N-acetyl glucosamine, and mannose [17].

As described by Zhang [8] Figure 1. Twenty-four fresh raw milk samples collected from different regions across four seasons and six regions were analyzed for PBC and MBC to explore the effect of extended refrigeration on the microbiological composition of raw milk, PBC and MBC of each milk sample stored at 7°C for 5 days were also investigated.

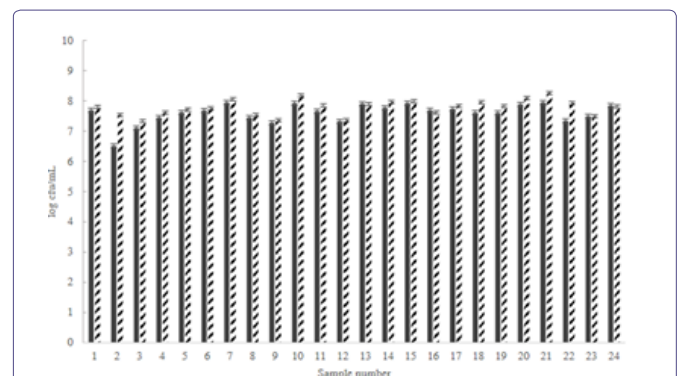


Figure 1: the population of psychotropic and mesophilic bacteria in raw milk after 5 days storage.

Milk is fermented in an open air for quite long time, which is good enough to allow various microbes to grow, quite high number of Irigo producers used refrigerator after the Irigo ferments. The handling of milk and Irigo during transportation, storage and processing were generally poor. This was common particularly for Irigo shops who take milk from multiple farms and those who do not follow strict sanitary practices. The sanitary practices followed at Irigo producers during handling, storage and processing were generally poor. The poor

quality raw milk, unclean and insufficient cleaning of milk equipments was among the most important sources of milk contamination. The milk is generally exposed to different contaminants when it transferred from one container to another, transported to consumers as well as retailers from the production site without cooling facilities, and with no proper milk containers [19].

As pointed out by Kwarteng [20] generally, the traditional production of raw milk and dairy products in Africa follows few common stages beginning with animal feed supply, followed by the production of raw milk which may be sold directly to consumers without processing, or further processed into various traditional products by small scale processors. Typically, along the dairy chain in Africa, milk may be consumed as non-pasteurized milk, heated milk or processed into various fermented yo-ghurt-like and cheese-like products.

Microorganism products by small scale processors. Typically, along the dairy chain in Africa, milk may be consumed as non-pasteurized milk, heated milk or processed into various fermented yo-ghurt-like and cheese-like products.

Psychotropic Bacteria and its Challenges to Milk and its Products

Psychotropic microorganisms are cold-loving organisms that like to develop at low temperatures and make up a significant portion of the bacteria found in raw milk. The activity of Psychrotrophs a group of microorganisms (acid-producing, extracellular enzyme-secreting, spore-forming bacteria, etc.) they are most likely to have the greatest impact on product quality [8].

One of the major technological problems in the dairy industry is the heat-stable enzymes produced by psychrotrophs that can attack milk components. Although these bacteria can be killed by pasteurization and UHT processing, heat-stable enzymes produced by these bacteria during milk storage and transportation under refrigeration can remain active after heat treatment. These heat-stable enzymes can degrade milk proteins and fats, increasing the concentration of free fatty acids and amino acids, resulting in a trace of bitterness, off-flavours and gelation of final products.

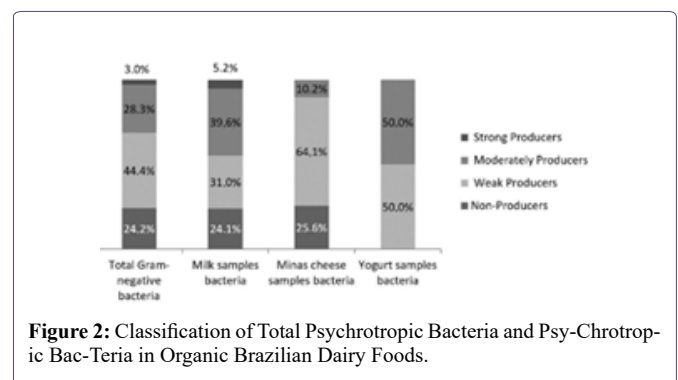
Their presence in processed organic dairy products may be related to the survival of these micro-organisms through conventional heat treatments applied or even incidence of post-pasteurization contamination. *Acinetobacter* is a complex genus whose micro-organisms have been associated with nosocomial infections, predominantly aspiration pneumonia, catheter-associated bacteremia, soft tissue, and urinary tract infections. There are more than 50 species belonging to this genus; however, *Acinetobacter baumannii*, *Acinetobacter calcoaceticus*, and *Acinetobacteriwoffi* are the most frequent species associated with infections [21].

Pseudomonas species are strongly associated with deterioration of milk and other dairy foods [22]. Because it is widely distributed in nature, such as in water and soil, it easily contaminates animal udder and milking equipment and has been used as a model to assess the effects of psychrotrophic bacteria on milk [6,18]. *Serratia* species, such as *Serratia liquefaciens* and *Serratia marcescens*, are also known to deteriorate raw milk, being able to produce thermo resistant enzymes and biofilms [22].

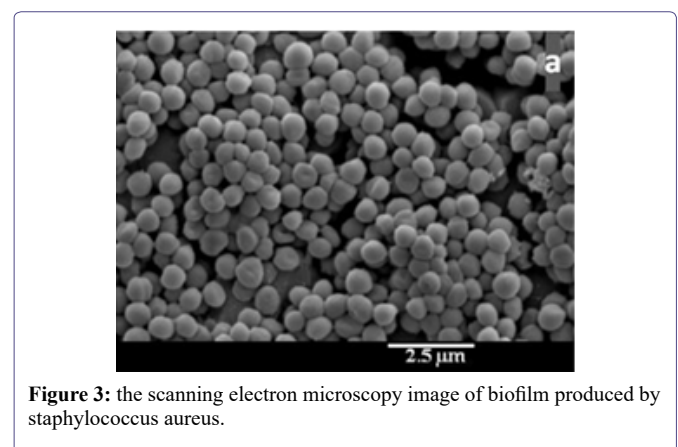
Biofilm Producing Capacity: Biofilms can be characterized as an aggregation of bacteria, algae, fungi and protozoa enclosed in a matrix consisting of a mixture of polymeric compounds, primarily poly-saccharides, generally referred to as extracellular polymeric substance [23]. Many psychrotrophic bacteria secrete exopolysaccharides to form biofilms. These substances facilitate the adhesion and aggregation of the bacteria to the surfaces and, when they adhere irreversibly, biofilm formation is initiated [23 and 24]. Most of the bacteria found in the organic dairy products were classified as non or low biofilm producers (68.7%). However, it is important to note that bacteria that do not produce biofilms can adhere to matrices produced by other bacteria. In the case of milks and processed dairy products, biofilms are a major source of re-contamination [23]. *Pseudomonas aeruginosa* can form biofilms on a variety of surfaces [25].

As reported by Mangalea [26], *Serratia* species and *Burkholderia pseudomallei* are known to produce biofilms. Bacterial biofilms make bacteria less susceptible to the antimicrobial agents, more resistant to sanitation, environmental changes, and dehydration/dissection [23].

According to Rabêlo [27] figure 2. Classification of total psychrotrophic bacteria and psychrotrophic bacteria (%) in organic Brazilian dairy foods (milk, minas cheese and yogurt samples) related to their capacity of biofilm production. Showed that 68 (68.7%) of the Gram-negative bacteria isolates were non-producers or low bio-film former, 28 (28.3%) were medium biofilm formers and only 3 (3.0%) were high bio-film producers.



As described by khelissa [28], scanning electron microscopy image of biofilm produced by *staphylococcus aureus* cip 4.83 on 316l stainless steel after 24 h incubation at 37°C (Figure 3).



Capacity to Produce Extracellular Enzymes: Psychrotrophic bacteria (psychrotrophs), can produce heat-stable enzymes (proteases and lipases) before the heat treatment. These heat-stable enzymes can remain active following the heat treatment and then hydrolyse the milk proteins and fats during storage, leading to the spoilage of final products. The specific composition of the raw milk microbiota can influence on the quality and shelf life of final dairy products in the market [8].

The psychrotrophic bacteria isolated from organic milk and other dairy foods showed high production capacity of enzymes (proteases, lecithins, and lipases), which may alter the sensory, nutritional, and technological characteristics of the products [18].

In pasteurized whole milk there was a higher incidence of lecithin producing isolates (52%), followed by proteases (34.5%), and lipases (3.5%). In Minas Frescal cheese, there was a higher incidence of protease isolates (34.5%), followed by lecithinase (26%), and lipase (5%) producers. Finally, only lecithinase producers (50%) were iso-lated from yogurts. Overall, the type of processing to which the milk is subjected and the process conditions influence the type of micro-organism found and, consequently, the type of enzymes produced [27].

Thermo resistant proteases present in milk and dairy products can act in the hydrol-ysis of several types of casein resulting in color changes, with development of grayish coloration. In addition, during cheese processing, proteases increase the destabiliza-tion of casein and compromise micelles coagulation, resulting in cheeses with lower consistency and yield [29].Prolonged storage of raw milk under re-frigeration favours the growth of psychrotrophs and the heat-stable protease and li-pase produced by those psychrotrophs potentially play a major role in the spoilage of dairy products [8].

As described by Ribeiro [5] Table 1.Identification of spoilage mi-croorganisms (n = 141) among the total psychrotrophic microbiota (n = 295) with proteolytic and lipolytic potential at 35 and 7°C of the strains from high microbiological quality Bra-zilian refrigerated raw milk.

16S rDNA clustering identification	Total		Proteolytic		Lipolytic		Proteolytic and lipolytic	
	No.	%	35°C/48 h, no. (%)	7°C/10 d, no. (%)	35°C/48 h, no. (%)	7°C/10 d, no. (%)	35°C/48 h, no. (%)	7°C/10 d, no. (%)
<i>Lactococcus lactis</i>	35	24.5	20 (57.1)	18 (51.4)	11 (29.9)	6 (17.1)	4 (11.4)	3 (8.6)
<i>Enterobacter kobei</i>	22	15.4	5 (22.7)	2 (9.1)	9 (40.9)	7 (31.9)	8 (36.4)	3 (13.6)
<i>Acetivibrio urinaequi</i>	12	8.4	3 (25)	3 (25)	6 (50)	1 (8.3)	6 (50)	1 (8.3)
<i>Acetivibrio lacticif</i>	9	6.3	2 (22.2)	2 (22.2)	7 (77.8)	8 (88.9)	2 (22.2)	2 (22.2)
<i>Koribacter phosui</i>	8	5.6	4 (50)	3 (37.5)	1 (12.5)	1 (12.5)	1 (12.5)	6 (75)
<i>Serratia ureolytica</i>	7	4.9	5 (71.4)	1 (14.3)	2 (28.6)	1 (14.3)	1 (14.3)	1 (14.3)
<i>Bacillus licheniformis</i>	7	4.9	2 (28.6)	4 (57.1)	2 (28.6)	1 (14.3)	1 (14.3)	1 (14.3)
<i>Staphylococcus epidermidis</i>	6	5.6	4 (66.6)	2 (33.3)	1 (16.7)	1 (16.7)	2 (33.3)	2 (33.3)
<i>Macrococcus caseolyticus</i>	5	3.5	2 (40)	2 (40)	1 (20)	1 (20)	2 (40)	1 (20)
<i>Staphylococcus parvulus</i>	4	2.8	2 (50)	3 (75)	1 (25)	1 (25)	1 (25)	1 (25)
<i>Enterococcus faecium</i>	3	1.4	1 (33.3)	2 (66.7)	1 (33.3)	1 (33.3)	1 (33.3)	1 (33.3)
<i>Acetivibrio phosui</i>	2	1.4	1 (50)	1 (50)	1 (50)	1 (50)	1 (50)	1 (50)
<i>Enterococcus faecalis</i>	2	1.4	1 (50)	1 (50)	1 (50)	1 (50)	1 (50)	1 (50)
<i>Stenotrophomonas rhizophila</i>	2	1.4	1 (50)	1 (50)	1 (50)	1 (50)	1 (50)	1 (50)
<i>Acetivibrio lacticif</i>	1	0.7	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)
<i>Acetivibrio spp.</i>	1	0.7	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)
<i>Bacillus cereus</i>	1	0.7	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)
<i>Bacillus pumilus</i>	1	0.7	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)
<i>Enterococcus hermanniensis</i>	1	0.7	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)
<i>Pantoea spp.</i>	1	0.7	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)
<i>Staphylococcus warneri</i>	1	0.7	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)
Total	141	100	45 (31.9)	29 (20.6)	53 (37.6)	38 (27)	43 (30.5)	21 (14.9)

Table 1: identification of spoilage microorganisms (n = 141) among the total psy-chotropic microbiota (n = 295) with proteolytic and lipolytic potential at 35 and 7°C of the strains from high microbiological quality brazilian refrigerated raw milk.

In Table 1, it is possible to observe that the species *E. kobei* (18.6%), *S. ureilytica* (16.7%), *A.urinaequi* (14%), and *L. lac-tis* (9.3%) predominated among the microor-ganisms that present-ed simultaneous proteolytic and lipolytic activity at mesophilic incubation. Among the 45 isolates that showed only proteolytic

activity, the most fre-quently observed species were *L. lactis* (44.4%), *E. kobei* (11.1%), *B. licheniforis* (11.1%), *K.gibsonii* (8.9%) and *M. caseolyticus* (8.9%), and of the 53 purely lipolytic isolates, *L. lactis* (20.8%), *E. kobei* (17%), *A. lwoffii* (13.2%), *A. urinaequi* (11.3%), and *S.epidermidis* (7.5%) predominated. Considering the deteriora-tion potential at refrig-eration temperature, the predominant species were *S.ureilytica* (75%), *A. urinaequi* (13.6%), and *L. lactis* (8.6%) for simultaneous proteolytic and lipolytic activity, *L. lac-tis* (51.4%) and *A. urinaequi* (25%) among the proteolytic psychrotrophs, and *A. lwoffii* (88.9%) and *E. kobei* (31.9%) among the lipolytic. The 88 proteolytic isolates at mesophilic incubation were tested for alkaline metalloprotease production potential, and 15 (17%) showed a positive PCR reaction for the *aprX* gene. Of these, 5 (33.3%) were identified as *S. ureilytica*, 5 (33.3%) as *E. kobei*, 2 (13.3%) as *Pseudomonas* spp., 1 (6.7%) as *A. urinaequi*, 1 (6.7%) as *Y. enterocolitica*, and 1 (6.7%) as *Stenotropho-monasrhizophila*. According to sample results were obtained at 7 °C, but slightly fewer isolates belonging to each genus showed spoilage potential. A total of 403 isolates showed the ability to produce at least one type of enzyme.

As Figure 4 out that the proteolytic activity of the predominant psychrotrophic bacteria is shown in Figure 5. The range of proteolysis was 1.23–13.02 h–1 · ml–1 at 28 °C and 0.42–5.72 h–1 · ml–1 at 7 °C.

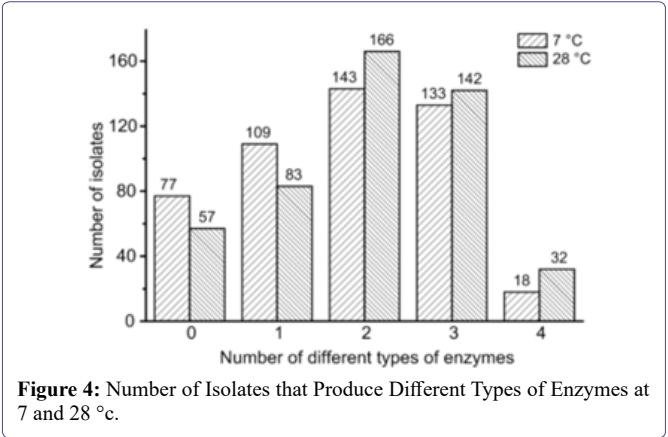


Figure 4: Number of Isolates that Produce Different Types of Enzymes at 7 and 28 °C.

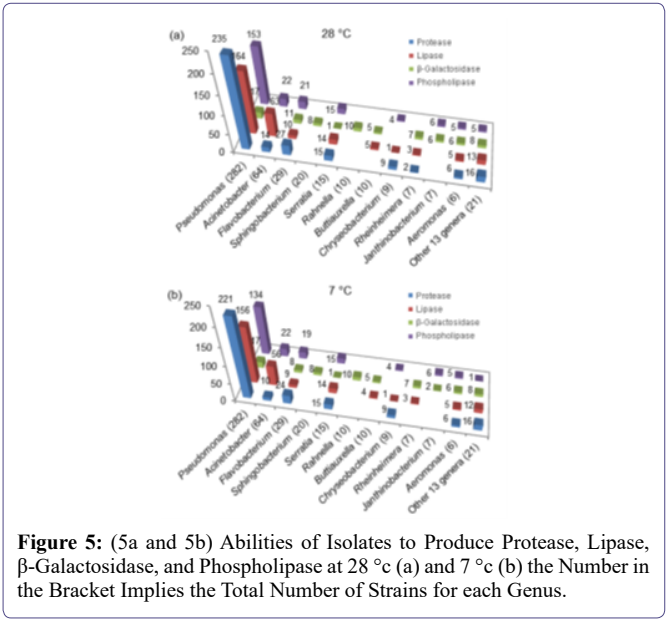
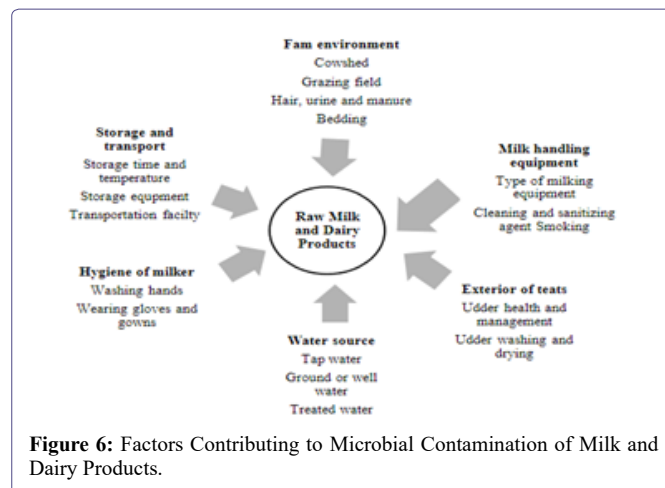


Figure 5: (5a and 5b) Abilities of Isolates to Produce Protease, Lipase, β-Galactosidase, and Phospholipase at 28 °C (a) and 7 °C (b) the Number in the Bracket Implies the Total Number of Strains for each Genus.

Source of Microbial Contamination of Milk and Dairy Products

Microbial contamination of milk and dairy products can originate from various sources [30]. These sources of microbial contamination include animal health, farm management and environmental factor, milking and milk handling practice, milk handling equipment and sanitary practices, milk storage and transport, and water source (Figure 6).



The higher total aerobic bacterial count observed in the study may be attributed to the initial contamination of milk samples either from of the cow, milker's hand, milking areas and container itself. On the other hand, high bacteria count observed in milk samples collected from informal merchant and retail shop could probably be due to further contamination of the milk during transportation, extremely high transportation temperature, the use of poorly cleaned milk containers, lack of and improper cooling systems at milk vending areas and poor personnel hygiene. The higher count indicates substandard hygienic conditions practiced during milking and subsequent handling. This implies that the sanitary conditions in which milk has been produced and handled are substandard subjecting the product to microbial contamination and multiplication. [31].

The following discussion provides further detail on the Source of microbial contamination of milk and dairy products [32].

Animal Health: From a healthy animal, raw milk is expected to harbour no pathogens at the point of collection. However, this is seldom the case. Generally, pathogenic microorganisms can contaminate raw milk in two ways. First, endogenous contamination occurs when milk is contaminated by a direct transfer of pathogens from the blood (systemic infection) of an infected animal into the milk, or via an infection in the udder. The second means by which fresh milk can be contaminated, known as exogenous contamination, occurs where milk is contaminated during or after collection by animal faeces, the exterior of the udder and teats, the skin, and other environmental sources [20].

Farm Management and Environmental Factors: The production of safe milk begins with the implementation of good hygienic practices on-farm, which is an effective first step in reducing milk contamination [33,34]. Farm management includes preventing cows from grazing in unhygienic pasture and living in sheds that are not cleaned

on a regular basis [33]. Exposure of cow's udder to environment contaminated with feces or debris is a major source of microbial contamination of milk [10]. Additionally, irregular cleaning of the milking areas and animal sheds contributes to cross-contamination of milk in household dairy farms [33].

A study conducted by Mitiku [35] in Haramaya district, reported that all cow-sheds (100%) included in their study were not constructed in a way that would facilitate drainage of farm waste, including animal feces and urine. The report also indicated that cowsheds did not use proper bedding materials like sand bedding for the animals to prevent dairy cow udders from becoming soiled. Similar studies revealed that 81% and 83% of the evaluated households did not use any bedding material in Jimma and Sidama Zones Respectively [36,37]. According to the coliform counts in milk samples is reduced when cows are housed in an environment with sand bedding as compared to straw or saw-dust bedding. Thus, clean and dry bedding condition is important to reduce microbial contamination of milk [38].

Bethlehem and Shimels [39] reported that 52% of the farms included in their study did not have a separate milking cowshed. In this regard, lack of comprehensive and uniform hygienic procedures to be followed by producers has posed a challenge to implement and use new procedures and research findings in the dairy sector of Ethiopia [40]. In general, a proper and clean housing environment is a pre-requisite to produce milk of acceptable quality and safety as it can significantly reduce risk factor of mastitis and other pathogenic microbes like *Listeria monocytogenes* [11].

Milking and Milk Handling Practices: Milking and milk handling practices have significant effects on the quality and safety of milk and milk products [39]. Fufa [10] reported that udder washing before milking is not widely practiced by Ethiopian dairy farmers. Of the 70 participants surveyed in their study, 26% did not wash udders prior to milking and only 30% of them used separate drying towels or cloths between milked cows to dry udders after washing. This data is based on selected sub-cities of the country's capital, Addis Ababa, and it is the authors' belief that this issue is magnified more in rural parts of the country where farmers typically do not avoid milking cows that show signs of infections, and where improper hand washing and handling of milk is common.

In similar studies conducted in the cities of Gonder, Harrarghe and Dangila, 72, 99, and 94% of the participants, respectively, were not regularly washing cows' udders and teats before and after milking cows, unless the udder was contaminated with manure [35,39 and 41]. Other studies also revealed that among the participants who practiced regular washing of cows' udders, more than 80% failed to dry the washed udder using a dry and clean towel or a cloth [41].

As the milkers' moves from one cow to the next, without washing and disinfecting their hands, they can potentially transfer pathogenic microorganisms between animals in the herd. If the milker is sick, she/he can transmit disease through milk handling [42].

Bethlehem and Shimels [39] reported that out of 60 randomly selected dairy farmers included in their study, 19 (32%) did not practice hand-washing prior to milking. In many instances, where hand-washing practices were in place, only water was used to wash hands. Proper hand washing both before and after milking should be practiced among dairy farmers by using water and soap, which can significantly reduce the microbial load on hands and therefore reduce the risk of milk contamination [43].

Milk Handling Equipment and Water Source Used for Sanitation: Equipment used for milk handling, storage, and transportation has an effect on the safety and quality of milk and is a major source of microbial contamination [40]. In Ethiopia, the majority of the farmers use plastic containers, clay pots, and bottle gourds to carry milk, which are difficult to thoroughly clean due to their shape and narrow opening.

In Ethiopia, the main sources of water for sanitary activities associated with milk handling equipment include rivers or spring water, ponds, rain water, ground or well water and tap water [42]. Water from these sources is typically used without further treatment [10 and 42]. Furthermore, the use of poor-quality contaminated tap water can also lead to introduction of pathogenic bacteria into the milk production chain [11,12].

Milk Storage, Transport and Cold Chain: Poor storage and transportation conditions can further facilitate the contamination of milk from milk handling equipment. Raw milk can only be kept for hours without storage at an appropriate temperature (4°C) before it deteriorates in both quality and safety [40].

However, such storage facilities are not readily available in Ethiopia, particularly in rural areas and cooling systems are not feasible due to lack of the required dairy in-frastructure and unstable power supply [42]. Transportation used for the delivery of milk can also influence the quality and safety of milk. Animal-drawn carts, motor bicycles, three-wheel drive vehicles (Bajaj), four-wheel-drive vehicles, or public transportation are among the methods used as a means to deliver milk to collection centers or selling points by dairy farmers in Ethiopia [40].

The time it takes to transport or deliver milk to collection centers is another factor that affects its quality and safety. According to Eyasu [43], samples from dairy farmers that had more than a 30 min travel time to the collection center had a 5.6 times higher risk of contamination with *Staphylococcus aureus* when compared to farmers that had less than 30 min of travel time to the collection centers.

In rural areas of the country, placing the milk in containers at cool (windy) places or in a cool water and electrical or solar operating bulk cooling tanks can be used to cool milk at the farm level [11]. These alternatives allow harvested milk to be stored longer and maintain its quality and safety. Even though not well known or practiced; the use of preservatives like lacto-peroxidase has been recently used to prolong milk shelf life [40].

Hygienic Conditions at Marketplaces: Milk and dairy products are marketed in formal and informal marketing systems [16]. In Ethiopia, Informal marketing systems are widely observed in traditional open markets and at the household level, in which limitations on infrastructure, proper packaging, storage and transportation equipment are present. Market access in a pastoral production system is particularly limited, which has led to a majority of the produced milk to be sold through informal market settings [32]. The hygienic conditions of the informal markets are not monitored or sustainably maintained [32]. According to Berhe table 2. Hygienic practices of dairy farmers in the Tigray region at 2017 G.C [44].

Generally, according to Berhe [44]. From the 96 dairy farmers who practiced cleaning of utensils, 44.4% (43/96) used cold water and soap for washing utensils while 13.5% (13/96) used only cold water.

No.	Description of variable	Frequency	Percent
1	Cleaning of utensils		
	Cold water	13	13.5
	Soap and cold water	43	44.8
	Soap and hot water	39	40.6
	Only hot water	1	1.0
2	Hand washing		
	Cold water	18	18.8
	Soap and cold water	66	68.8
	Soap and hot water	10	10.4
	Only hot water	2	2.1
3	Udder washing		
	Cold water	46	47.9
	Soap and cold water	9	9.4
	Soap and hot water	24	25
	Only hot water	17	17.7
4	Containers used for transportation of milk		
	Wide necked aluminum vessel	3	3.03
	Narrow necked aluminum vessel	0	0
	Narrow necked plastic vessel	69	69.7
	Wide neck plastic vessel	24	25.0
5	Means of milk transport		
	Cars	11	11.5
	Bicycle	16	16.7
	Bajaj or Motorcycle	14	14.6
	On foot	55	57.3
6	Sources of water		
	Tap water	66	68.8
	Wells	16	16.7
	Ponds and streams	14	14.6
7	Milk storage container		
	Plastic container	92	94.8
	Stainless steel container	2	2.1
	Aluminum container	2	2.1

Table 2: Hygienic Practices of Dairy Farmers in the Tigray Region at 2017 G.C.

All of the interviewed dairy farmers reported that they washed hands before milking and 68.8% (66/96) used cold water and soap for washing their hands. The udders of lactating cows were washed with cold water by 46.2% of the farmers. All dairy farmers reported that they filtered the milk after milking and before selling to consumers.

To transport milk to their customers, 69.7% of the dairy farmers reported that they used narrow necked plastic vessels and 25.0% used wide neck plastic vessels. Transporting the milk to market was done mainly on foot (57.3%) and bicycles (16.7%). The source of drinking water for the farms was mainly tap water (68.8%) followed by wells (16.7%) (Table1).

As described by [30] (Table 3) Participants of this study were also asked about the hygienic practices they follow in the process of milk production.

Low dairy house cleaning practices as well as dirty environments, and also milkers poor personal hygiene in most smallholder dairy farmers, have implications on sources of pathogens for mastitis and other diseases. The finding of this term paper is in line with the previous reports by Mesfin and Bethlehem and Shemles in Arusha, Tanga, Sidama, and Gondar, respectively. In the different study, there is no proper concrete drainage system that makes cleaning of the farms

Variables		Study area			Overall (N=120)
		Merasakebele 02 (n=40)	Abiyotitie (n=40)	WoldiaKarkura (n=40)	
Milking frequency					
	Twice a day	87.5% (35)	92.5% (37)	95% (38)	81.7% (110)
	3 times a day	12.5% (5)	7.5% (3)	5% (2)	8.3% (10)
Milking utensils used for milking					
	Plastic	90% (36)	87.5% (35)	75% (30)	84% (101)
	Pot	10% (4)	12.5% (5)	25% (10)	16% (19)
Cleaning frequency of milking utensils					
Udder washing before milking	Daily	95% (38)	35% (16)	80% (32)	70% (84)
	3 times a week	5% (2)	65% (26)	20% (8)	30% (36)
	Yes	35% (14)	47.5% (19)	55%(22)	45.8% (55)
	No	65% (26)	52.5% (21)	45%(18)	54.2% (65)
Hand washing practice before milking					
	Yes	45% (18)	35% (14)	27.5% (11)	35.8% (43)
	No	55% (22)	65% (26)	72.5% (29)	64.2% (77)
Teat (udder) drying habit or practice before washing					
	Yes	40% (16)	30% (12)	35% (14)	35% (42)
	No	60% (24)	70% (28)	65% (26)	65% (78)

Table 3: Milking and Hygienic Practices Followed by Producers in the Study Areas, 2017 (n=120).

Table 3: Milking and Hygienic Practices Followed by Producers in the Study Areas, 2017 (n=120).

very dif-ficult and increases the probability of microbial contamination of milk which is in agreement with the reports by [37].

All the smallholder dairy farms in the study area uses hand milking in which milking is done without washing of hands before milking and between milking of different dairy cows in the same barn and probably increase microbial contamination of milk. However, it is important since cow's udder and teat could have direct contact with the ground, urine, dung and feed refusals while resting, and this contributes the possible contamination of milk [30].

Conclusion

Milk is highly nutritious as well as pH-neutral, providing the ideal conditions for mi-crobal growth. Raw milk is usually stored at cold temperatures, e.g., about 4 _C be-fore processing to reduce the growth of most bacteria. However, psychrotrophic bac-teria can proliferate and contribute to spoilage of ultrahigh temperature (UHT) treated and sterilized milk and other dairy products with a long Shelf life due to their ability to produce extracellular heat resistant enzymes Worldwide, species of *Pseudomonas*, with the ability to produce these spoilage enzymes, are the most common contami-nants isolated from cold raw milk although other genera such as *Serratia* are also reported as important milk spoilers, while for others more research is needed on the heat resistance of the spoilage enzymes produced. The residual activity of extracellu-lar enzymes after high heat treatment may lead to technological problems (off flavors, physico-chemical instability) during the shelf life of milk and dairy products.

The psychrotrophic bacterial contamination of milk and dairy product is one of the key factors determining the quality of processed dairy products due to the heat-stable enzymes produced by these bacteria. The quality and safety of milk and dairy prod-ucts are global concerns, particularly in developing countries like Ethiopia. The con-tamination may result from infected or sick animals, unhygienic conditions and prac-tices in milking and milk handling, unhygienic milking equipment and poor quality of water. The safety and quality of milk is highly affected by unhygienic practices in dif-ferent stages of milk production. Reduced quantity and quality of milk production has been a challenge for the dairy sector in Ethiopia, resulting in a significant economic and social impact. Moreover, lack of knowledge and skills (awareness) for hygienic production and processing of milk and dairy products are the major concerns for the dairy industry in the country.

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