SVOA Microbiology

ISSN: 2634-534X

ScienceVolks Open Access

Research Article

Microbiological Quality Assessment of Milk and Milk Products Along with their Packaging Materials Collected from a Food Industry in the Dhaka Division

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***Received:** August 02, 2021 **Published:** August 23, 2021*

Abstract:

The present study aims to quantify the spoilage causing bacteria from highly nutritious dairy beverages and to also evaluate the air quality at a food processing area. Nowadays, different types of dairy beverages have been becoming increasingly popular in Bangladesh for their better quality, test, and flavor, however, the presence of bacteria including human pathogens is harmful to consumers especially children. A total of six categories of samples were collected in this study. Among them pasteurized milk (n=5), UHT processed milk (n=5), 10 Flavored milk (5 Mango milk and 5 Chocolate milk), 5 Butter, and 5 Cheese samples of the same brand, and different batches were collected from a food factory in the Dhaka division. All the pasteurized milk had a high bacterial load from the range from 2.25×10^2 to 4.69×10^3 CFU·mL^{-1}. All UHT milk samples were free from microorganisms. The range of total viable bacteria count (TVBC) in pasteurized flavored milk was from 5.0×10^1 to 1.8×10^2 CFU·mL^{-1}. The range of TVBC in butter was from 1.90×10^3 to 2.59×10^3 CFU·mL^{-1} and in the cheese sample the count was from 1.22×10^3 to 3.22×10^3 CFU·mL^{-1} Total coliform was absent in all types of liquid milk and milk products. Some butter and cheese samples exhibited a low number of coliform counts. For all the processing areas in the dairy plant, the numbers of mesophilic aerobic bacteria, yeasts, and molds obtained by a one-stage air sampler were within 100 CFU·m^{-3}. Examination of three types of food packages showed that three pasteurized milk packaging poly film and three butter packaging materials exhibited bacterial load range from 1- 5 CFU/ 100 cm^2 below the acceptable limit. All of the examined packages are free from coliforms.

***Keywords:** Dairy products, Microbiological quality, Packaging materials, Air quality*

Introduction

Milk is an ideal food for its nutritious value. Milk contains lactose (sugar), proteins (caseins, whey proteins, and minor proteins), essential amino acids, fats, potassium, magnesium, zinc, calcium, phosphorus, riboflavin, vitamin A, and B12. Over the centuries milk fulfils the human nutrition demand. However, milk is an excellent growth media for microorganisms such as various pathogens (1-5). Along with liquid milk, dairy beverages draw the attraction of consumers and become a popular item on the dining table (6). Pathogens can be present even in freshly drawn milk and can spread further probably due to the improper treatment (7,8). A healthy mammary gland is naturally sterile before initiation of milk secretion and colostrogenesis but after the starting of milking mammary gland comes into contact with the environment and may be contaminated by microorganisms from the teat apex, milking equipment, feed, air, water, soil grass, and other environments (9).

Milk spoilage can be caused by many bacteria. The main pathogens occurring in milk and milk-related products in these times are Shiga toxin-producing *Escherichia coli* (STEC), *Listeria monocytogenes*, *Campylobacter* spp., *Salmonella* spp., and coagulase-positive *Staphylococcus* spp., *Brucella abortus, Bacillus cereus*, *Mycobacterium* spp., and *Clostridium botulinum* (10,11).

Unhygienic conditions can promote milk spoilage by zoonotic pathogens during the handling of raw milk. After collecting the milk, the main concern is trying to keep microbial counts in milk low. Before pasteurization when milk is stored at low temperature milk can be contaminated by psychrophilic Gram-stain-negative bacteria such as proteolytic and lipolytic *Pseudomonas* and *Acinetobacter* species (12-14). After pasteurization, 50% fluid milk can become contaminated by heat-labile bacteria such as *Pseudomonas* spp., coliform, and Gram-stain-positive spore-forming bacteria (14). Contamination in UHT milk is unexpected due to high-temperature treatment but microbial contamination was recorded on the date expire UHT milk (15).

Apart from, liquid milk, the manufacture of dairy beverages like cheese, butter, and yogurt requires several raw materials primarily milk and water. The use of contaminated raw materials may be hazardous to the final products (16). Moreover, the unawareness of consumers about health issues accelerates the occurrence of foodborne illnesses. On the other hand, the air of food processing areas can contaminate foods with pathogenic or spoilage microorganisms, reducing their quality. Dairy products are particularly susceptible to contamination by airborne microorganisms. The greatest aerosol sources in dairy plants are personnel, floor drains, ventilation systems, and water, when applied under pressure in the cleaning and sanitizing procedures (17).

Most of the people in Dhaka city collected pasteurize milk, UHT milk, and milk product from a super shop rather than the milkman. So, it is necessary to ensure safety as well as continue the clarity of the milk. Based on these facts, this study was carried out to quantify the extent of microorganisms present in different milk, milk products, and processing area.

Materials and Methods

Working place

All the experiments of this work were carried out in the Microbiology lab of a renowned pharmaceutical industry in Bangladesh from November 2020 to January 2021.

Sample Collection

A total of five categories of samples were collected. Among them pasteurized milk (n=5), UHT processed milk (n=5), 10 flavored milk (5 Mango milk and 5 Chocolate milk), 5 Butter and 5 Cheese samples of the same brand and different batches were collected from a specific food and beverage factory in Dhaka division.

Sample processing

For pasteurized milk, 1 mL of the milk sample was taken into 9 mL normal saline which gives 10^{-1} dilution. A vortex machine was used to homogenize the sample. Serial dilutions of samples were made up to 10^{-2} in sterile normal saline. For UHT same procedure was followed. For other milk products, 10 gm sample was homogenized into 90 mL water and 0.1 mL sample was spread on Nutrient agar and MacConkey agar media (1,2).

Enumeration of total viable bacterial count (TVBC) and total coliform count (TCC) by pour plate method

To aseptically transfer liquid milk into a pour plate, one side of a Petri plate lid was raised to allow access to the sample. Then 1 mL of the sample was transferred to the dish and covered immediately with the lid followed by pouring 18-20 mL of sterile agar culture medium. The inoculums and medium are mixed by gentle rotation ten times in one direction and ten times in another direction. The agar was allowed to solidify completely before the plates are inverted for incubation at 37 °C for 24 hours for TCC and 48 hours for TVBC. After incubation, both surface and sub-surface colonies were observed (2).

Enumeration of total viable bacterial count (TVBC) and fungal count by culture settling plate technique

For the culture settling plate technique, open Petri dishes containing 20 ml of culture media (PCA, PDA, VRB, or BPA) were distributed at the processing areas and exposed for about 15 to 30 minutes. The Petri dishes were closed and incubated at 35°C/ 48 h for aerobic plate count, 25°C/ 3-5d for yeasts and molds (17).

Results and Discussion

Milk is an ideal food for human health. The collected pasteurized milk samples, UHT milk, flavored milk, butter, and cheese samples were analyzed for the total viable bacterial count, total coliform count. These findings may be helpful for the governmental regulatory bodies to monitor the quality of the commercial milk products in the market.

Total viable bacteria (TVBC) and Total coliform count (TCC)

The results of bacterial distribution for pasteurized milk and UHT milk are presented in Table 1. All the pasteurized milk had a high bacterial load from the range of 2.17×10^3 to 3.84×10^3 CFU mL^{-1}. The results of bacterial distribution for UHT milk exhibited zero bacterial growth. These samples are free from microorganisms. According to the definition of the UHT process, UHT milk should contain very little or no active bacteria.

The results of flavored milk samples are presented in (Table 2). The pasteurized flavored milk (mango and chocolate) showed total viable bacteria in a range between 2.51×10^3 to 6×10^1 CFU mL^{-1}. The high bacterial load in some of the pasteurized milk may result from defective pasteurization machinery and/or post-pasteurized contamination by workers.

Table 1: Bacterial load of different Pasteurized and UHT milk samples.

Brand name	Serial number	TVBC (CFU mL^{-1})	TCC (CFU mL^{-1})
Pasteurized milk			
	1	2.90×10^3	Absent
	2	2.97×10^3	Absent
	3	2.17×10^3	Absent
	4	2.59×10^3	Absent
	5	3.84×10^3	Absent
UHT milk			
	1	Nil	Absent
	2	Nil	Absent
	3	Nil	Absent
	4	Nil	Absent
	5	Nil	Absent

TVBC: Total viable bacterial count; TCC: Total coliform count

Table 2: Bacterial load of flavored milk samples.

Flavored milk brand	Serial number	TVBC (CFU mL^{-1})	TCC (CFU mL^{-1})
Farm fresh mango milk (pasteurized)	1	2.51×10^3	Absent
	2	1.17×10^3	Absent
	3	1.57×10^3	Absent
	4	2.10×10^3	Absent
	5	1.57×10^3	Absent
Farm fresh chocolate milk (pasteurized)	1	8×10^1	Absent
	2	6×10^1	Absent
	3	1.8×10^2	Absent
	4	9.9×10^2	Absent
	5	1.5×10^2	Absent

The presence of coliform bacteria such as Escherichia coli in milk is a common indicator of fecal contamination. Coliforms were found only for the three samples of butter. The range of coliform was between 2-3 per mL below10 CFU mL^{-1} (18). On the other hand, coliforms were not present in pasteurizing milk, UHT milk samples, and flavored milk samples. Coliform bacteria are expected to be absent in pasteurized milk because pasteurization temperature prevents their growth. However, TCC may be detected in the pasteurized milk samples due to defects in the pasteurization process, post-pasteurization processes such as from packaging materials, or defects in pipelines (19). In our present, TCC was not found in pasteurize and UHT milk as expected. According to USPHS not 'Grade A' pasteurized milk should contain no more than 10 colonies (20). So, the current study indicates that commercial milk samples available in Bangladesh are of good quality and free of health risks to consumers. In pasteurized milk, the total bacterial count below 10^4 CFU mL^{-1} meets the permissible quality with the range between 10^4 to 10^5 CFU mL^{-1}, whereas samples become unacceptable if bacterial count exceeding 10^5 CFU mL^{-1}UHT products should be sterile according to guidelines of ICMSF (22). Butter is highly perishable. Gram-negative bacteria, yeast, and molds are important spoilage causing microorganisms of butter and can result in surface discoloration and off-flavor. In our present study TCC was found in three butter samples within a range from 2×10^1 to 4×10^1 CFU $mL^{-1}$and three-cheese samples within a range from 9×10^1 to 2.3×10^3 CFU mL^{-1}(Table 3). Previously in some studies, a significant number of *E.coli* and other bacteria is found in butter and cheese samples (21,22). A wide range of microorganisms found for the spoilage and contamination of cheese including *Staphylococcus aureus*, *Salmonella* spp., *Clostridium botulinum* and *Escherichia coli*, indicating that the food was contaminated with pathogenic bacteria (23).

Table 3: Bacterial load of milk products

Brand name	Serial number	TVBC (CFU mL^{-1})	TCC (CFU mL^{-1})
Butter			
	1	1.90×10^3	Absent
	2	1.97×10^3	4×10^1
	3	2.17×10^3	2×10^1
	4	2.59×10^3	Absent
	5	2.84×10^3	3×10^1
Cheese			
	1	2.24×10^3	Absent
	2	3.22×10^3	Absent
	3	1.22×10^3	2.3×10^3
	4	1.82×10^3	Absent
	5	1.42×10^3	Absent

Table 4: Total viable bacterial count (CFU/100 cm^2) on packages manufactured for packaging of different products

Brand name	Serial number	TVBC (CFU mL^{-1})	TCC (CFU mL^{-1})
Pasteurized milk			
	1	2	Absent
	2	3	Absent
	3	5	Absent
	4	-	Absent
	5	-	Absent
UHT milk			
	1	-	Absent
	2	-	Absent
	3	-	Absent
	4	-	Absent
	5	-	Absent
Butter			
	1	-	Absent
	2	-	Absent
	3	1	Absent
	4	2	Absent
	5	2	Absent
Cheese			
	1	Absent	Absent
	2	Absent	Absent
	3	Absent	Absent
	4	Absent	Absent
	5	Absent	Absent

Microbiological evaluation of the food packaging materials

Packaging serves as a major defense against hazardous elements. However, interactions between packaging material and food can give rise to potential threats e.g., penetration of microorganisms, insects, and rodents through packages and chemical reactions with a food product. Examination of three types of food packages in the present study (Table 4) showed that three pasteurized milk packaging poly film and three butter packaging materials exhibited bacterial load range from 1- 5 CFU/ 100 cm2 below the acceptable limit. All of the examined packages are free from coliforms. Microbiological contamination of packaging materials is most likely to occur during manufacturing, transportation, storage condition, and at the time of handling (24). Most of the paper and packaging plants have a quality system that is based on the ISO 9000 series of standards. Good manufacturing practice (GMP) plays a key role in quality control and product safety assurance. By building up a hygiene and safety management system (HACCP program), GMP monitored microbial risk management in the paper and packaging industry. The HACCP principles have been internationally accepted and approved by, e.g., the Codex Alimentarius Commission (25-27). The Food and Drug Administration (FDA) standard (FDA, 1991) also states that microbial numbers should not exceed 1 CFU cm^2 or 250 CFU g^1. The total numbers of yeasts, molds, and bacteria accepted must be below, and no pathogenic bacteria, including enterobacteria and *Escherichia coli*, should be detected (28).

Microbiological evaluation of air quality

Table 5 shows the microbial numbers obtained by culture settling plate techniques. The highest aerobic count (95 $CFU.m^{-3}$) was recorded in the butter processing area and the lowest count (57 $CFU.m^{-3}$) was recorded in the milk pasteurization area. Yeast and mold count was recorded within a range from 42 to 87 $CFU.m^{-3}$. According to the APHA guideline, both bacterial and fungal counts adopted the recommended limit below 100 $CFU.m^{-3}$. Apart from, APHA, there are other recommendations for microbiological counts in the air at food processing areas. Kang and Frank, 1989, recommended 180-360 $CFU.m^{-3}$ of air for mesophilic aerobic bacteria and 70-430 $CFU.m^{-3}$ for yeasts and molds (29). The present study supported some previous research which gives more accuracy to our study (30,31).

Table 5: Number of aerobic mesophilic bacteria and yeast mold count by culture settling plates technique.

	Aerobic bacterial count	Yeast and mold count
Processing area	$CFU.m^3.\ week^{-1}$	$CFU.m^3.\ week^{-1}$
Milk pasteurized	57	42
Butter processing room	95	66
Milk packaging room	84	87
Cheese processing room	89	59

Table 6: Microbiological limits for milk and milk products

Milk and Milk products	TVBC	TCC	Yeast and mold	Method of test
Pasteurized milk	3×10^4 CFU mL^{-1}	less than 10	Nill	EAS 68-1 EAS 68-2
UHT milk	10	0	Nill	EAS 68
Butter	10^4	Absent in 1 gm	10 per gm	ISO 11866
Cheese	Not found	10 CFU/gm	10 CFU/gm	ISO 4832 ISO 6611

Conclusion

In summary, it can be concluded that different types of milk and milk products collected from the food industry are of high quality. It is highly expected that these products should be free from disease-causing microorganisms. A proper monitoring system should be followed from the raw material process to finish products packaging. Every step is equally important for a final purified product. It is recommended that government and relevant stakeholders should be aware of food safety issues of dairy beverages to avoid any possible foodborne illness.

Conflict of Interest

The authors declare no conflict of interest.

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Citation: Nur IT, Ghosh BK, Urmi JN, Akter D, Ema EI . "Microbiological Quality Assessment of Milk and Milk Products Along with their Packaging Materials Collected from a Food Industry in the Dhaka Division". SVOA Microbiology 2:2 (2021) Pages 19-25.