CHAPTER- I

INTRODUCTION

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Toned milk (also called single toned milk) refers to milk obtained by the addition of water and skim milk power to whole milk (De, 1980). Under the Prevention of Food Adulteration (PFA) Act, 1976, toned milk should contain a minimum of 3.00% fat and 8.5% solid not fat. Toned milk can be prepared from the milk of cow, buffalo, or any other milk source. According to Bangladesh Standard Testing Institute (BSTI, 2021) toned milk is the milk obtained by adding water and skimmed milk powder to whole milk where fat content shall be more than 2% but less than 3.5% and SNF should be minimum 9.00%.

Toned milk, a popular dairy product, has gained significant attention in recent years due to its unique composition and potential health benefits. Toned milk is a variant that lies between full-fat milk and skimmed milk in terms of fat content. It undergoes a specific process to reduce the fat content while preserving essential nutrients, making it an appealing option for health-conscious consumers. Toned milk is a minimally altered, yet nutritionally similar to conventional cow's milk. This dairy product has become a staple in many households, contributing to the diversification of available milk options in the market. The creation of toned milk involves blending buffalo milk with skim milk (Veisseyre et al., 1966). Nutritionally, toned milk closely resembles whole cow's milk. In moderation, toned milk emerges as a health-conscious choice suitable for most individuals without dairy allergies. Its production and consumption are predominantly concentrated in India and various regions of Southeast Asia.

The term "toned milk" originates from the process used to create it. Simply diluting whole buffalo milk with water reduces both fat and SNF (Murtaza et al., 2017). However, incorporating skim milk into the mixture "tones up" the SNF to the original level found in pure buffalo milk, which is 9%. Since the resulting product was neither whole milk nor standardized milk, it was aptly named "toned milk." Although coined by the Bombay Milk Scheme, the term is not registered, allowing milk schemes worldwide to adopt it. As a result, "toned milk" is now commonly used in discussions and technical papers across the globe. It is advisable to utilize low-heat spray-dried skim-milk powder for reconstitution, given its superior solubility compared to roller-dried alternatives (Sharma et al., 2012). While small amounts of toned milk can be produced without employing any mechanical mixing device, larger quantities require the use of such devices for effective blending.

Toned milk is typically crafted by blending whole buffalo milk with skim milk and water, resulting in a product that mirrors the nutritional composition of traditional whole cow's milk. Originating in India, this process was devised to enhance the nutritional content of full-cream buffalo milk, aiming to increase its production, accessibility, affordability, and availability. The dilution of buffalo milk with skim milk and water reduces its overall fat content while preserving essential nutrients like calcium and protein (Abd El-Salam et al., 2011).

Toned milk and whole cow's milk are nutritionally similar, with only slight differences in calories, fat, and protein content. Toned milk serves as an excellent reservoir of protein, vitamins, and minerals. When consumed in moderation, it stands out as a highly nutritious option for most individuals. Consistent intake of dairy products, including toned milk, is linked to numerous potential health advantages. These include enhancements in bone mineral density and a lowered risk of chronic conditions such as heart disease and type 2 diabetes (Thorning et al.,2016)

Research has explored the potential health benefits of consuming toned milk. The primary benefits of toned milk lie in its reduced fat percentage, lower calorie content, and rich nutrient profile (Krauss et al.,2000). Toned milk is often preferred in regions with limited milk production or high demand exceeding available capacity. With its lower fat content, toned milk is often recommended for those aiming to manage their weight or reduce overall fat intake (Lichtenstein et al., 2006). The controlled fat levels in toned milk make it a suitable option for individuals with specific dietary restrictions, such as those advised to limit saturated fats. The toning process is designed to retain essential nutrients, ensuring that consumers do not compromise on nutritional value when opting for lower-fat milk. Studies have investigated the effects of toning on the preservation of proteins, vitamins, and minerals, emphasizing the importance of maintaining a well-rounded nutritional profile in toned milk (Gil et al., 2019).

Toned milk is a modified but nutritionally similar variant of traditional cow's milk. Originating in India, this method involves mixing buffalo milk with skim milk, powdered skim milk, and water. This process lowers the fat content, increases the milk supply, and preserves the original amount of non-fat solids. Though the production of toned milk can play a significant role to fulfill the daily demand of milk along with proper nutrition, there is limited research about its quality. As toned milk can be a great source of nutrition, there should be proper evaluation about physical, chemical and microbial quality of toned milk available in market. Toned milk is particularly useful in regions with low milk production or high demand relative to supply. However, the physical, chemical, and microbial qualities of toned milk of various brands in Bangladesh are still yet to know. It is hypothesized that various producers of toned milk in Bangladesh are maintaining the standard of market toned milk

Objective of the research

In Bangladesh, toned milk is produced by Bangladesh Milk Producer's Cooperative Union Limited, Aarong Dairy, Pran Dairy Limited, and Akij Dairy Limited. Introduced to the country in 2021, toned milk is designed to be cost-effective while maintaining nutritional values similar to whole milk, except for a reduced fat content. This makes it an accessible option for low-income individuals seeking to meet their protein needs. Toned milk is particularly beneficial for people with heart disease and high blood pressure. As awareness of healthy eating grows in Bangladesh, toned milk serves as a valuable source of protein and minerals, especially for those aiming to maintain a low-fat diet.

Therefore, this study was conducted to

- Evaluate the physical and chemical properties of toned milk.
- Evaluate the microbial properties of toned milk.
- Determine whether the toned milk produced by the companies are genuinely meeting the quality and standard as they describe.
- Provide a comprehensive understanding of how toned milk measures up to safety and quality standards in Bangladesh

CHAPTER- II

REVIEW OF LITERATURE

CHAPTER II

Review of literature

Milk is recognized as nature's ultimate comprehensive nourishment and stands out as one of the most essential and frequently ingested food items. Milk stands as an essential dietary staple for humans throughout their entire lifespan, providing a well-balanced array of nutrients crucial for optimal health. Its composition closely aligns with an almost ideal proportion of essential elements, earning it the recognition as nature's most comprehensive and complete food source (O'Mahony, 1988). Comprising an intricate blend of fat, protein, carbohydrates, minerals, vitamins, and various other components dispersed in water, milk forms a comprehensive and well-rounded dietary source, encompassing a complete array of nutrients (Kim et al., 1983).

Often hailed as the "ideal sustenance," milk is derived from the mammary glands of periparturient female mammals. It boasts a wealth of essential nutrients, including carbohydrates, proteins, fats, minerals, and vitamins, all dynamically tailored to fulfill the specific developmental requirements of growing newborns (Murphy et al., 2017; Albenzio et al., 2016). Beyond its role in nourishment and hydration, milk plays a crucial part in establishing vital gut micro-flora and priming the immune system in newborn mammals.1 While typically designed as a species-specific bio-fluid for the young of a particular species, humans stand out by consuming milk produced by diverse species, continuing this consumption into adulthood.2 Global milk production is largely dominated by five animal species, with cows contributing 83% of the total, followed by buffaloes at 13%, goats at 2%, sheep at 1%, and camels at 0.4%.(FAO, 2016)

2.1 Economic and nutritional significance

Milk holds great significance due to its unique nutritional value and vital contribution to both human and animal health. It encompasses all essential substances required by organisms in their most easily assimilated form. Rich in high-value proteins such as casein, lactalbumin, and lactoglobulin, it provides crucial amino acids (Popescu & Angle, E., 2009). The fat content in milk serves as an energy source (9.3 kcal/g) with a low melting point (29-34 °C), and its small globules facilitate easy assimilation. (POPESCU, A., & ANGEL, E. (2009). Moreover, milk contains A and D vitamins, playing a special role in the fixation of Calcium and Phosphorus in bones (Kaushik et al., 2014). Compared to other animal-origin foods, milk exhibits low cholesterol levels, making it a favorable choice for a well-rounded and health-conscious diet.

The capacity of humans to extract milk from domesticated animals and incorporate it into their diet throughout adulthood has played a pivotal role in the evolution of agriculture, the establishment of civilized societies, and the creation of numerous widely embraced food items (Diamond J., 2002). The ability to collect and utilize bovine milk has been fundamental to the health, growth, migration, and overall success of the human species for the past 10,000 years. Presently, milk remains one of the most extensively consumed beverages globally, with 811 million tons produced in 2017. It not only serves as the foundation for liquid milk but also acts as a primary ingredient in various dairy products, including flavored milk, ice cream, cheese, butter, yogurt, casein powder, and more (FAO, 2017).

Consumers consistently seek clean, nutritious food produced and processed with sanitation standards that ensure it's free from harmful pathogens. In meeting this demand, ensuring high-quality milk production becomes imperative. Quality milk refers to milk devoid of pathogenic bacteria and harmful toxins, lacking sediment and foreign substances, possessing good flavor, maintaining normal composition, exhibiting adequate keeping quality, and having low bacterial counts. In Bangladesh, milk production primarily occurs in an unorganized manner, with Goalas typically supplying it to both urban and rural consumers. While there are some pockets of organized milk production such as Milk Vita and established dairy farms, the distribution of this perishable product has not received significant attention regarding hygienic practices. Furthermore, milk serves as an excellent breeding ground for various bacteria. Cousin (1982) highlighted numerous sources of bacterial contamination in milk, including the udder, cow's body, litter, floors, flies, insects, rodents, water sources, milkers, milk utensils, and the surrounding atmosphere. Hence physical quality is prioritized for consumer satisfaction.

Assessing the sensory aspects of milk is crucial across all its applications. Understanding its sensory characteristics is particularly important due to the widespread familiarity with fluid milk and its typical sensory profile. Evaluating the flavor, and ideally the aroma, of raw milk can detect any mishandling or production issues before further processing. Throughout the various stages of commercial milk product processing and preparation, fluid milk may undergo multiple operations at different temperatures. Consequently, evaluating the sensory attributes of the final milk product helps detect any deviations in processing or handling. Often, these quality variations may not be significant on a day-to-day basis but rather manifest as gradual shifts over time, necessitating frequent sensory assessments and comprehensive documentation to effectively address areas of concern (Schiano, 2017).

Considering the substantial economic and nutritional significance of milk, particularly cow's milk, it comes as no surprise that detailed chemical and nutrient analyses of bovine milk have been conducted for many years (Singhal et al.,2017). These analyses encompass extensive studies on milk vitamins(Graulet B., & Girard C. L., 2017).), minerals (Visentin et al., 2018), fats(Van et al., 2015), proteins(Rezaei et al., 2016) and carbohydrate (Garballo et al., 2018). Typically, bovine milk is composed of water (85-87%), fats (3.8-5.5%), proteins (2.9-3.5%), and carbohydrates (5%). At the micronutrient level, bovine milk contains a myriad of bioactive compounds, including vitamins, minerals, biogenic amines, organic acids, nucleotides, oligosaccharides, and immunoglobulins(Fox et al., 1998). The specific nature and relative abundance of these compounds are influenced by various internal and external factors (Palmquist et al., 1993). These factors encompass the metabolic activity within the cow's mammary tissues, overall udder health conditions, the type of feed provided to the cow, the activity and abundance of specific microbes in the cow's ruminal fluid, as well as microbial activity and enzymatic reactions within the raw milk (Hettinga et al., 2009). Milk composition also varies with the cattle breed (e.g., Holstein, Jersey, Brown Swiss), stage of lactation, level of parity, number of viable pregnancies, and undergoes changes during milk quality control and processing procedures following milk collection (Yang et al., 2016; O'Callaghan et al., 2018).

2.2 Bovine milk and Buffalo milk

Bovine milk typically consists of approximately 87.2% water, 3.7% fat, 3.5% protein, 4.9% lactose, and 0.7% minerals, according to research by Haug et al., (2007). The specific nature and relative abundance of these compounds are influenced by numerous internal and external factors (Palmquist et al., 1993). In terms of macro-nutrients, bovine milk typically comprises water (85-87%), fats (3.8-5.5%), proteins (2.9-3.5%), and carbohydrates (5%). On the micro-nutrient level, bovine milk contains a variety of bioactive compounds, such as vitamins, minerals, biogenic amines, organic acids, nucleotides, oligosaccharides, and immunoglobulins (Fox et al., 1998). Lactose content of bovine milk is 4.47 according to Ceballos et al., (2009).

On the other hand, buffalo milk is nearly twice as rich in fat as compared to cow milk and the most important fraction responsible for its high energetic and nutritive value. Varrichio et al., (2007) reported the fact that the fat content has an average value of 8.3% but can also reach upto 15% under normal conditions. (Tonhatiet al., 2011).Buffalomilk exhibits a higher protein content compared to cow milk upto 4.5%- 4.68%, as reported by Ragab et al., (1958), Ganguli (1973) and Ahmad et al., (2008).Buffalo milk boasts a higher concentration of total solids of 10.2% (Roy et al., 1979) when compared to cow milk. The pH of buffalo milk ranges from 6.57 to 6.84 and is not influenced by month, lactation number, or season of calving, but correlated with solid-not-fat and lactose contents (Minieri et al., 1965). Acidity varies from 0.05% to 0.20% (Dharmarajan et al., 1950.) That means these compounds depend on various reasons like location, processing, breeds and so on.

2.3 Market milk

As milk is a highly sought-after food, it has been processed in various ways to meet consumer demand. This processed milk is commonly known as market milk. The production of market milk involves the collection, processing, and distribution of milk from dairy animals, primarily cows. This industry has evolved to meet the growing needs and preferences of consumers, leading to the establishment of a well-developed market for milk and dairy products worldwide. There are various types market milk available throughout the world including Bangladesh. Bangladesh Standard Testing Institute (BSTI) has classified market milk. According to BSTI (2002) market milk are classified as following

1. Unpasteurized raw milk
2. Pasteurized whole milk
3. Toned milk
4. Medium fat milk
5. Low fat milk
6. Skimmed milk
7. UHT treated milk
    - Full fat milk
    - Low fat milk
    - Toned milk
    - Skimmed milk

Food and Drug Administration has classified market milk as following (FDA, 2009):

1. Full fat milk
2. Low fat (2%) milk
3. Low fat (1%) milk
4. Skimmed milk

According to FSSAI, (2016) there are two types of toned milk. They are:

1. Single toned milk

2. Double toned milk

2.4 Invention of toned milk

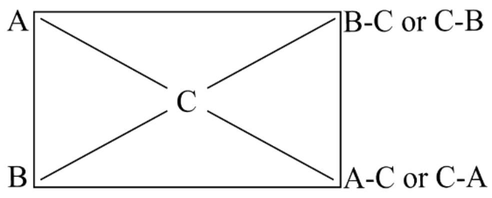
According to Khurody, D. N., (1962) during World War II, the cost of buffalo milk surged significantly in the Bombay area. Addressing the issue required resorting to subsidization since little else could be done. In 1944, a subsidized milk distribution initiative was introduced to benefit women and children. However, extending or maintaining the scheme over an extended period proved unfeasible. In response, the Government Milk Scheme in Bombay introduced novel blended milk in 1946, referred to as "toned milk," leading to a substantial increase in available milk quantities. This also halved the consumer price while providing a nutritious product.

A UNICEF milk initiative in Bombay, known as the World Dairy Scheme, has supplied machinery and dairy equipment that are not readily available in India and are constrained by limited foreign exchange. The State Government of India is committed to match this assistance, aiming to distribute free milk to children or low-income group consumers over a 5-7years period, equivalent in value to 1.5 times the UNICEF aid. UNICEF prioritizes the distribution of low-fat, high-protein milk for this purpose. This milk, with only 1.5% fat and 10% solids-not-fat, is both cost-effective and highly nutritious. This product is known as double toned milk. The preparation method mirrors that of toned milk (Khurody, D. N., 1962)

2.5 Preparation of toned milk

Toned milk is a high - fat milk to which reconstituted non-fat dry milk or skim milk has been added to decrease the fat content of the original milk. The lowering of the fat content produces a larger volume of milk. In the process of toning, it is necessary to determine the fat percentage of the milk to be toned and also to determine the quantity of reconstituted non-fat dry milk or skim milk to be added to lower the fat content of the original milk to the desired level. The simplest method developed for this determination is known as the parallelogram, or "Pearson square" method (Burgwald et al., 1959).

The method may be illustrated as follow:



A = the percent of fat in the milk to be toned.

B = the percent of fat in the reconstituted non-fat dry milk or skim milk to be mixed with A toreduce the fat content of A.

C = the percent of fat desired in the toned milk

By subtracting diagonally, the proportionate amounts of A and B required to be mixed to obtain a mixture containing the desired percentage of fat indicated by C are secured. In other words, AC or C - A (depending on which is the larger) will give the number of kilograms or pounds of B to be taken for the mixture. In a like manner C - B or B - C will show the number of kilogram or pounds of A to be taken for the mixture.

In the preparation of toned milk, strict adherence to hygiene standards is imperative. The cleaning and sterilization of the equipment must meet the same rigorous criteria applied to conventional milk processing plants (Rankin et al., 2017). Additionally, several other crucial factors warrant careful consideration. First and foremost, the water used for powder reconstitution must be inherently devoid of harmful bacteria. Consequently, any water source with questionable quality should be avoided (Beuchat et al., 2013). Furthermore, the powder used in the process must undergo bacteriological testing to ensure its freedom from hazardous bacteria, such as streptococci, staphylococci, and other pathogenic organisms. The selected powder should be of premium quality, characterized by freshness (not exceeding one year old), absence of taints or off-flavors, and a moisture content not exceeding 2.5%. Lastly, any container holding the ingredients must be subjected to stringent hygiene measures to maintain the integrity and safety of the toned milk production process (Patel et al., 2015).

The regulated and systematic incorporation of toned milk into any distribution system also holds the potential to mitigate seasonal or temporary milk shortages. Adverse weather conditions, such as hot summers in warm countries and harsh winters in cold regions, often lead to reduced milk production (Gauly et al., 2013). During such periods, if larger quantities of toned milk could be produced and introduced to the market, it could minimize the impact of shortages. In this context, it is noteworthy that the South Australian Metropolitan Milk Supply Act underwent modification to address emergency shortages of market milk (Harper et al., 2019. This amendment allows for an increase in available milk volume through a "process of toning" using reconstituted skim-milk powder. Under this legal provision, reconstituted skim milk can be blended with whole cows' milk to yield a product containing 3.8% fat and 9% SNF. Such milk has proven to be highly acceptable to consumers and has effectively addressed the challenge of providing affordable, high-quality milk even during periods of natural scarcity (Khurody, D. N., 1962)

2.6 Standards of market milk

Now-a-days, toned milk is commonly produced by mixing buffalo and cow's milk, achieving a composition of approximately 3% fat and 8.5% non-fat milk solids, encompassing milk sugar and proteins. This closely mirrors the content of whole cow's milk (Guo et al., 2010). By reducing the fat content to 3% through the toning process, the available milk quantity is nearly tripled. This is also supported by FSSAI, (2016).

Cows contribute the largest share to the global milk supply, while buffalo milk stands as the second most prominent source (Guo and Hendricks, 2010). Buffalo milk carries a fat content ranging from 7-8%, coupled with a non-fat solids content of 9-10% (Abd El-Salam et al., 2011). This closely aligns with the composition of whole cow's milk, typically featuring 3.25–4% fat and 8.25% SNF (Ceballos et al., 2009). In numerous underdeveloped nations, buffaloes, rather than milk cows, serve as the primary source of milk. Buffalo milk, boasting an average milk fat percentage as high as 7%, is a prevalent choice. In certain regions, the practice involves blending reconstituted nonfat dry milk with this high-fat buffalo milk to create "toned" milk with a reduced fat content. (Burgwald et al.,1959).

Market milk is primarily categorized based on its fat content. As per the guidelines provided by Lee et al., (1998) and the FDA (2008), whole milk is expected to have a fat content of 3.25%, while skim milk should have a fat content ranging from 0 to 0.5%. Reduced-fat milk is required to contain 2% fat, and low-fat milk should contain 1% fat. Bangladesh Standard Testing Institute (BSTI) has specified various type of milk and given specific criteria for specific type of milk. According to BSTI fat% of whole milk, reconstituted milk, low fat milk, skim milk should be 3.5%, 3.5%, min 2%, 0.5% to <2%, 0 to 0.5% respectively (BSTI, 2002).

Table 2.1: According to FDA (2008) fat% of different type of milk

Type of milk	**Fat%**
Whole milk	3.25%
Reduced fat milk	2%
Low fat	1%
Skimmed milk	0- 0.5%

Table 2.2: According to BSTI (2002, 2021) fat% of various type of market milk

Type of milk	**Fat%**
Full fat/ Whole milk	3.5%
Toned milk	Min 2%
Medium fat milk	2.0% to less than3.5%
Low fat	0.5% to less than 2.0%
Skimmed milk	Max 0.5%

In Bangladesh, specific brands are manufacturing various types of market milk, including toned milk. There found some differences in the evaluation of chemical components of market milk in different brands. Research shows a various range of these compounds in market milk available in Bangladesh. The fat content averages for Milk Vita, Tatka, Farm Fresh, Aarong, and RD milk brands were 3.51, 3.06, 3.26, 3.41, and 3.33, respectively. In terms of protein content, the values were 4.07, 4.14, 4.14, 4.03, and 4.10 for Milk Vita, Tatka, Farm Fresh, Aarong, and RD brands, respectively. The corresponding SNF contents were 8.13, 8.54, 8.15, 8.11, and 8.02%, while the total solids were 11.46, 11.58, 11.41, 11.53, and 11.35%, respectively (Saha, S., & Ara, A., 2012).

The investigation according to Karmaker, A,et al., (2020) revealed that raw milk exhibited the highest moisture content at 90.68%, whereas UHT (Ultra High Temperature) milk showed the lowest at 87.60%. The other components were distributed as follows: ash ranged from 0.68% to 0.78%, protein from 3.20% to 3.58%, fat from 3.15% to 3.56%, lactose from 4.35% to 4.62%, acidity from 0.14% to 0.22%, solid not fat from 6.17% to 8.95%, total solid from 9.32% to 12.40%, and specific gravity from 1.026% to 1.034%. On the other hand low fat liquid milk contain less than 2% of fat, 4.3% to 4.5% of lactose, 0.18% to 0.21% of acidity and 4.50% to 4.70% of PH according to Richards et al,. (2016). Skim milk is another type of popular market milk. This type contains lactose of 4.3% to 4.9%, fat of 0.27% to 0.35%, protein of 3.3% to 3.4% found by UlHaq et al., (2013).

All the research findings indicate that the chemical composition of milk varies based on its production and type. Countries have their own standard for each type of milk. In Bangladesh BSTI has set the standards. Though fat% varies on the type of market milk other components remain similar.

Table 2.3: BSTI (2002, 2021) standard for market milk (except fat %)

Parameters	**Value**
Solids Not Fat (SNF)	8.0
Density, g/ml at 15°C	1.028 – 1.036
Lactose %	4.4
Protein%	3.0
Titratable Acidity (lactic acid per 100ml)	0.18

2.7 Standards of toned milk

Toned milk is a special type of market milk which is now available in Bangladesh. This type of market is quite new in Bangladesh. BSTI has specific criteria for toned milk. There is no specific research has found on the quality of toned milk available in Bangladesh. Even no research has found on the specific chemical evaluation of toned milk.

According to Food Safety and Standards Authority of India (FSSAI), 2016 toned milk is classified as:

1. Single toned milk/ toned milk
2. Double toned milk

Table 2.4: Standard has been set by FASSI (2016) for toned milk as below:

Type of toned milk	Fat %	SNF %
Single toned milk/ toned milk	3.0%	8.5%
Double toned milk	1.5%	9.0%

Table 2.5: Criteria for toned milk set by BSTI (2021)

Parameters	**Value**
Fat%	Min 2.0%
SNF%	9.0%

2.8 Microbial Quality

Milk is a nutritionally rich substance, comprising not only the primary milk sugar lactose but also proteins such as caseins, whey proteins, and minor proteins, along with essential amino acids, fats, minerals, and vitamins (Fusco et al., 2020). Due to its rich nutritional content, milk provides a favorable environment for the proliferation of a diverse and abundant array of microorganisms. In addition to its inherent micro biota, various microorganisms originating from the teat canal, udder skin, milking machines, tanks, and storage containers may colonize the milk immediately after milking, reflecting the farm and pasture environment (Addis et al., 2016). From a microbiological standpoint, ensuring the safety and quality of milk is crucial. Therefore, the evaluation of the composition and dynamics of the raw milk micro-biota and its influence on the composition and quality of both the milk and its derived products, from the milking process through transportation, storage, and transformation into dairy products, holds paramount importance (Addis et al., 2016; Quigley et al.,2013). Due to its highly nutritious composition, milk and milk-based foods provide an ideal environment for the extensive growth of various microorganisms. The elevated water activity, moderate pH, and available nutrients within milk are key factors contributing to microbial proliferation. Milk not only acts as a potential carrier for transmitting certain pathogens but also supports the growth, replication, and toxin production of these organisms.

Various pathogenic microorganisms can enter milk and milk products from diverse sources, leading to different types of foodborne illnesses. The microbiological quality of milk and dairy products is influenced by the initial microbial flora of raw milk, processing conditions, and the potential for post-heat treatment contamination (Houghtby et al., 1992). Additionally, contamination risks persist during transport, storage, and various stages of the manufacturing process.

The consumption of raw milk and its derivatives remains a well-recognized risk factor for food borne diseases. In certain countries, particularly those with warmer climates, outbreaks of gastroenteritis are frequently linked to the consumption of raw milk and milk products, such as cheese (De boer et al., 1999).

2.9 Common microorganism and spoilage

Spoilage occurs when microorganisms break down the carbohydrates, proteins, and fats in milk, producing harmful end products. For instance, Lactobacillus or Streptococcus species may ferment lactose to lactic acid and acetic acids, causing the milk to sour (Das et al., 2015). Undesirable microbes, including Gram-negative psychrotrophs, Coliforms, lactic acid bacteria, yeasts, and molds, contribute to the spoilage of dairy products (Ledenbach et al., 2009) Additionally, bacteria with public health implications, such as *Salmonella spp*., *Listeria monocytogenes*, *Campylobacter jejuni*, *Yersinia enterocolitica*, pathogenic strains of *Escherichia coli*, and enterotoxigenic strains of *Staphylococcus aureus*, may be present in milk and dairy products (Kumbhar et al., 2009). Psychrotrophic bacteria, known for producing significant amounts of extracellular hydrolytic enzymes, play a crucial role in determining the shelf life of milk products (Oliveira et al., 2015). Fungal spoilage of milk and its derivatives is characterized by a diverse range of metabolic by-products, resulting in off-odors, flavors, and noticeable changes in color and texture (Pal, M., 2014).

Consumers express a preference for food that is both wholesome and nutritious, produced and processed in a sound and sanitary manner, and free from pathogens. To meet this demand, ensuring the production of high-quality milk is crucial. Quality milk is characterized by normal chemical composition, complete freedom from harmful bacteria and toxic substances, absence of sediment and extraneous materials, a lower degree of titratable acidity, good flavor, adequate preservation quality, and low bacterial counts.

In Bangladesh, milk production often follows non-standardized methods, and it is commonly distributed to consumers by milkmen in both urban and rural areas. While there are limited sources like Milk Vita and established dairy farms providing surplus milk, the hygienic distribution of this perishable product has not received significant attention (Khan et al., 2008). Milk, being an excellent growth medium for bacteria, is susceptible to contamination from various sources, including the udder and body of cows, air-borne dust, litter, floors, flies, insects, rodents, water supply, hands and clothing of milkers, utensils, bottles, and the surrounding atmosphere (Ensminger et al., 1994; Cousin, 1982).

Consequently, milk and dairy products can serve as significant sources of foodborne pathogens (Oliver et al., 2005). Furthermore, the common practice of adulterating milk with water in Bangladesh not only dilutes the milk solids but also introduces the risk of germ contamination, further diminishing its quality. Therefore, it is of utmost importance to deliver such a valuable yet easily perishable food to consumers in a wholesome and unadulterated form.

The nutritional value of milk and its derivatives relies on their cleanliness, purity, and wholesomeness, as highlighted by Nahar et al., (2007). Consequently, there is a need for heightened emphasis on the microbiological aspect of these products. The reduction in food value resulting from the separation of cream and the addition of water to milk is not only a concern. There is also a significant risk associated with the introduction of germs carrying infectious and contagious diseases, particularly in the case of water adulteration (Ghosh and Maharjan, 2002; Barthel, 1910).

Milks susceptibility to bacterial contamination makes it perishable (Kim et al., 1983; OECD, 2005). To address this issue, milk undergoes heat treatment of varying intensities, including pasteurization, sterilization, and ultra-high temperature treatment. Ultra-high temperature (UHT) processing involves heating milk to a temperature of 138°C for a brief duration, effectively eliminating all microbes and deactivating enzymes. This process enhances the milk's shelf life and improves its sensory perception (Bylund, 1995). Pasteurization, employed to prevent milk spoilage, has been a practiced heating process since the early 1900s. This method involves heating raw milk to 161°F for 15 minutes, with the aim of eliminating microorganisms from the milk (Imele et al., 2002). The primary objective of pasteurization is to enhance the storage stability of milk. Government inspection services conduct chemical and microbial quality control to ensure that the milk meets standards for good and safe human consumption.

2.10 Management of microbial quality

The Bangladesh Standards and Testing Institution (BSTI) imposes specific sanitary standards for pasteurized milk (BSTI, 2002).

Table 3.6: Microbial standard

Parameter	**Value**
Total Plate Count, CFU per ml	<30,000
Total coliform Count, CFU per ml	< 10

Microbial quality of toned milk is quite similar to pasteurized market milk. It should contain zero coliform and 30×10^{3}– 50×10^{3}cfu/ml (FASSI, 2015).

Redmond (Redmond, W. A. 2005) defines pasteurization as the process of heating a liquid, especially milk, to a temperature ranging from 55 °C to 70 °C. This method aims to eliminate harmful bacteria without significantly altering the composition, flavor, or nutritive value of the liquid (Redmond, W. A., 2005). According to Gunasekera et al., (2002) pasteurization of milk was introduced as a public health measure to eradicate human pathogens and diminish the activities of spoilage microorganisms. The viability of bacteria in milk following heat treatments can be evaluated using three different indicators:

(i) colony forming units (CFU) on plate count agar,

(ii) de novo expression of a gfp reporter gene, and

(iii) membrane integrity based on propidium iodide exclusion (Gunasekera et al., 2002).

The standard plate count (SPC), used to determine the total number of bacteria in a specified amount of milk (typically one milliliter), is a crucial parameter for milk grading.An effective indicator for monitoring the sanitary conditions throughout the production, collection, and handling of raw milk is the "total" bacterial count, also known as the standard plate count (SPC). According to Fernandes, R., (2009)The SPC is determined by plating (or employing equivalent procedures) on a standardized plate count agar, followed by aerobic incubation for 2 or 3 days at 32°C or 30°C, respectively. Microorganisms that fail to form colonies are not included in the count. The SPC, while not revealing the source(s) of bacterial contamination or identifying production deficiencies leading to high counts, serves to highlight changes in the production, collection, handling, and storage environment. Supplementary microbial assessments for psychotropic or thermoduric bacteria, spore-forming bacteria, streptococci, and coliforms can aid in identifying sanitary deficiencies.

SPC values for raw milk can vary from less than 10000 cfu/ml, indicating minimal contamination during production, to exceeding 1 x 10^7 cfu/ml. The microorganisms present may originate from one or a combination of the three main contamination sources previously identified. Consequently, elevated initial SPC values (e.g., >100,000cfu/ml) indicate serious deficiencies in production hygiene, while SPC values below 20,000 ml^1 reflect good sanitary practices (International Dairy Federation, 1974). In many countries, the standard for Grade A (or Grade 1) raw milk is an SPC of less than 1×10^5cfu/ml for milk intended for heat treatment before consumption. Additionally, the coliform plate count is widely utilized to ascertain the total number of coliforms present in one milliliter of a milk sample.

Coliforms are regarded as 'indicator organisms' due to their presence in food signaling potential contamination. Coliform bacteria encompass organisms like *Escherichia coli* and *Enterobacteraerogenes*, both naturally occurring in the large intestine (Barton, L. L., & Northup, D. E., 2011). Their presence in milk indicates potential fecal contamination. Unsatisfactory handling post-pasteurization can lead to such contamination. *E. coli*, especially the enteropathogenic type, is a significant foodborne pathogen capable of causing diarrhea and severe complications, including fatalities (Barton, L. L., & Northup, D. E., 2011). Approximately 18,000 individuals in northern Illinois and nearby states experienced severe gastrointestinal illness in 1985 due to *Salmonella typhimurium* infection (Bergquist, L. M., &Pogosian B., 2000). The outbreak was traced back to a dairy plant operated by a major grocery store chain, where a defective valve in the pasteurizer led to improper pasteurization and the presence of Salmonella, resulting in serious gastrointestinal complications (Bergquist, L. M., &Pogosian, B., 2000). The standard for coliforms in "grade A" milk should not surpass 10 cfu/ml (Frazier &Westhoft., 1958).

So far, some researchers have been done in Bangladesh on microbial quality of milk available in Bangladesh. According to (Hossain et al., 2011) raw milk samples exhibited a substantial bacterial load, ranging from 1.75×10^6 to 1.22×10^8cfu/ml. The primary cause of this high bacterial count is often attributed to inadequate cleaning of the milking system. Factors contributing to the elevated bacterial count include the milking of dirty udders, maintenance of an unclean milking and housing environment, and the failure to promptly cool milk to temperatures below 40°F. The TVBC (total viable bacterial count) in pasteurized milk samples ranged from 7.5×10^{7} to 1.24×10^{8}cfu/ml, significantly exceeding the recommendations set by BSTI. The coliform count in the raw milk samples varied from 4.5×10^{3} to 2.03×10^{6}cfu/ml and in pasteurized milk it was zero. But another research shows a significant number of coliform in pasteurized market milk containing more than10 cfu/ml. It also shows a higher range of total bacterial count from 5.4×10^{4} to 6.8×10^{4}(Saha, S., &Ara, A., 2012).

2.11 Conclusion

So far enough literary work has not been found on quality assessment of toned milk in Bangladesh. There is no resource found on toned milk that is produced in commercial milk plant of Bangladesh. Their Chemical quality has been set at a level but how far the companies are maintaining is not examined. Similarly microbial quality of toned milk available in Bangladesh is not evaluated. That is why consumer's preference along with public health significance is yet to come in light. So far various researches had been shown the Chemical and microbial quality of other kinds of market milk available in local market of Bangladesh. This literary work will be on count in evaluation of Physical, chemical and microbial quality of toned milk available in Bangladesh.

CHAPTER- III

MATERIALS AND METHODS

CHAPTER III

Materials and Methods

3.1 Sample collection, sample designing and location of the study

In Bangladesh, milk is commonly distributed through two methods. In the first scenario, farmers transport milk in open containers and directly sell it in the market without undergoing any processing or packaging. Alternatively, in a different approach, milk companies gather milk from farmers or dairy farms, subject it to pasteurization or UHT treatment, and subsequently package the processed milk. This packaged milk is then made available for purchase in stores under designated brand names. In this study pasteurized toned milk samples were collected from various shops and transported the samples from shops to laboratory by a cool box. Samples were chosen randomly on the basis of batch and date of production. Total 18 samples were collected. Among the samples there were 6 samples each of three rewound brands of Bangladesh namely as Farm Fresh, Aarong and Milk Vita. Six samples of Milk Vita were tagged as M_1, M_2, M_3, M_4, M_5, M_6. Six samples of Farm Fresh and Aarong tagged as F_1 to F_6 and A_1 to A_6 respectively. Samples were preserved for short time at4^0C temperature. All the quality tests were performed in Dairy Science and Poultry Research and Training Centre (PRTC), CVASU.

3.2 Organoleptic Tests

The assessment of milk products involves a sensory examination using the sight and smell to evaluate and document overall quality. This initial and fundamental test serves as the primary method for assessing the characteristics of milk and its various products. However, it is essential to supplement this test with additional laboratory analyses. The sight and smell evaluation (color and appearance, odor, flavour, body) is conducted promptly after opening the packets according to FSSI and ILRI, (2020) and Deka et al., (2020).

3.2.1. Taste Panel Score

A team of experts assessed the sensory quality of each raw milk sample through organoleptic evaluation using score board recommended by Bureau of Indian Standard (BIS). Tested milk was graded according to quality measures as suggested by BIS.

Fig 1: Sensory Evaluation

Table 3.1: BIS recommended score board for physical evaluation of toned milk

Characteristics	**Scores**
Color and appearance	10
Odor	20
Flavour	40
Body	30
Total	100

Table 3.2: BIS recommended evaluation of quality and grading of toned milk:

Quality of milk	**Scores obtained**	**Grade of milk**
Excellent	>90	**A**
Good	80 - 89	**B**
Fair	60 -79	**C**
Poor	<59	**D**

3.2.2. Determination of Specific Gravity

Specific gravity was determined by the conventional method using lactometer described in FSSAI and ILRI, (2020) and Deka et al., (2020).

Procedure:

Mix the milk sample thoroughly

↓

Pour it into a dry cylinder which enables the lactometer to float without touching slides

↓

let the lactometer into the cylinder

↓

Allow the lactometer to remain steady in the milk

↓

Take the reading from the lactometer as son as it becomes stationary(with in 30 seconds)

↓

Note the corrected lactometer reading(CLR)

Fig 2: Lactometer reading for determining specific gravity

Calculation:

Corrected Lactometer Reading (CLR) = Lactometer reading ± (temperature of milk - 60) ×0.1

Specific gravity = (CLR/1000) + 1

3.3 Chemical evaluation

3.3.1 Fat percentage determination

Fat% was determined by Traditional approach employing the Gerber centrifuge according to FSSAI and ILRI, (2020) and Deka et al., (2020).

Procedure:

Transfer 10 ml of sulfuric acid into a butyrometer using a 10ml acid pipette

↓

Fill the 10.75 ml pipette with milk and deliver the sample into butyrometer

↓

Add 1 ml of amyl alcohol using a 1ml pipette and close

↓

Put the butyrometer in the centrifuge, placing two butyrometers diametrically opposite, centrifuge at maximum speed for 4 minutes

↓

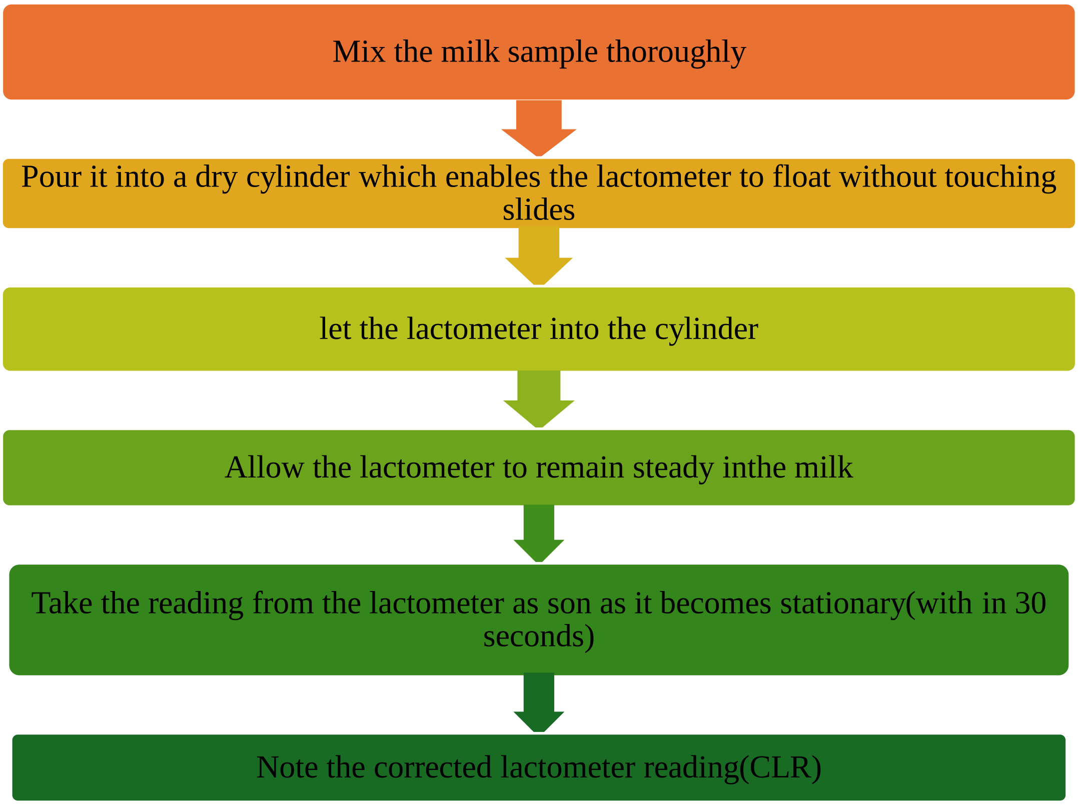
Bring lower end of fat coloum using stopper to a main graduation mark by slightly withdrawing stopper

Fig 3: Measurement of fat column

3.3.2. SNF percentage and Total solids (TS) percentage determination

SNF refers to the non-fat solid components in milk, excluding the fat content. It was determined by the conventional method using lactometer described in FSSAI and ILRI, (2020), Deka et al., (2020) and Jagdish, P., &Neeraj, M. (2008).

Procedure:



Calculation of SNF%: According to Indian Standard Institution Formula used by Jagdish, P., &Neeraj, M. (2008).

SNF% = (CLR / 4) + 0.2F + 0.6

Here,

CLR = Corrected Lactometer Reading

F = Fat percentage in the milk sample.

Calculation of TS%: According to Troyes formula described in Jagdish &Neeraj (2008).

TS% = SNF% + fat%

3.3.3. Determination of acidity percentage

Acidity percentage of milk was determined by titration according to FSSAI (2016).

Procedure

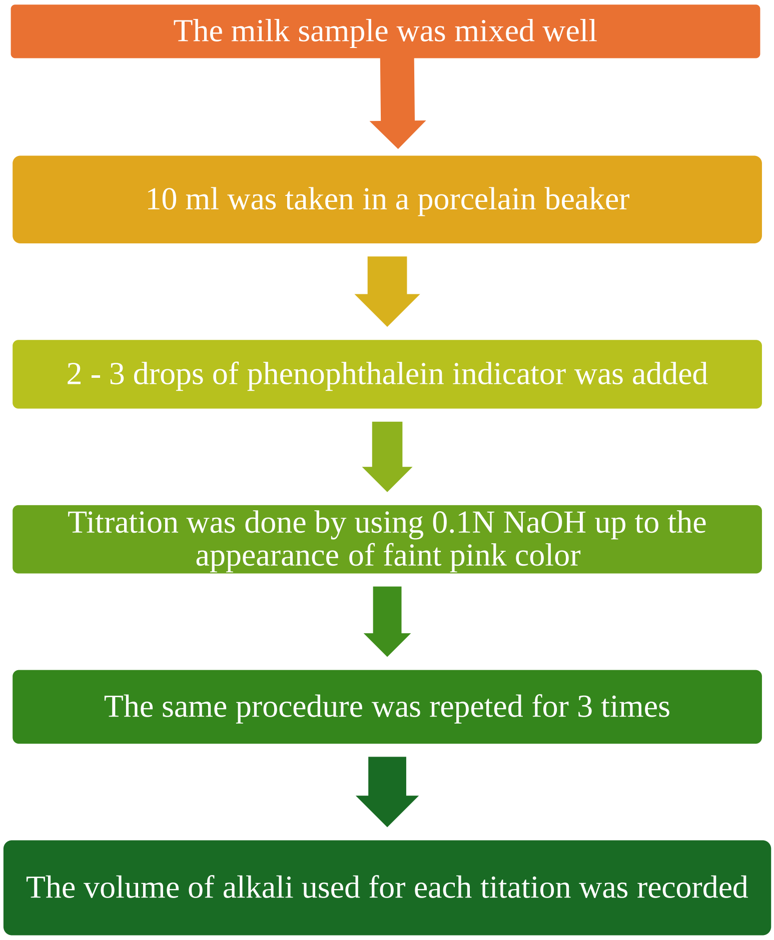


Fig 4: Titration for acidity determination

Calculation:

Acidity % = (ml of alkali used × normality of NaOH × 0.09 ×100)/ml of milk sample

3.3.4. Determination of Protein and Casein percentage

Protein and casein were determined by Aldehyde method/ formal titration method (Pyne, G. T., 1932) as described in milk and milk product testing manualof the Madras Veterinary College.

Procedure:

10 ml of milk sample was taken in a conical flask

↓

0.4 ml potassium oxalate added and mixed then kept rest the mixture for two minites

↓

2 - 3 drops of phenophthalein indicator was added

↓

Titration was done by using 0.1N NaOH up to the appearance of faint pink color

↓

2 ml of formaldehyde solution was added and kept in rest for 30 minuted

↓

again titration was done by the same procedure

↓

amount of alkali used was recorded

Calculation:

Protein%= Titrate value×1.7

Casein%= Titrate value× 1.32

3.3.5. Determination of Lactose percentage

Lactose was determined by Bock's method (Gänzle et al., 2008)as described in the milk and milk products testing manual of the Madras Veterinary College.

Procedure:

20 ml of milk was taken in a 100 ml of volumetric flask

↓

12 ml of 10% sodium tungstate and 12ml of 2/3N sulphuric acid was added

↓

the mixture was made upto the mark using distilled water

↓

the mixture was filtrate and filtrate was taken in a burette

↓

in a seperate 250 ml conical flask 25 ml of benedict solution was taken

↓

lactose in the filtratr was determined by titrating it with boiling benedict solution upto the appearance of white precipitation with straw yellow color

Fig 5: filtration for lactose determination

Calculation:

Calculation of the above titration method is done bearing in mind that 25 ml of Benedict reagent is completely reduced by 0.067 grams of lactose.

Lactose% = (0.067× 100× 100) / (titrate value× 20% of specific gravity)

3.4 Microbial evaluation

3.4.1. Total viable count

Total plate count results indicate the quantity of colonies capable of developing under specified physical and chemical conditions, encompassing factors like atmosphere, temperature, pH, nutrient availability, and the presence of growth-inhibiting agents. Colonies represent clusters of viable microbial cells, making direct comparisons with direct counts unfeasible. Plate counts tend to underestimate microbial presence as they may exclude dormant, viable but non-culturable, and non-culturable microorganisms

This test was conducted by following the method recommended by APHA (2004) and FSSAI (2016).

Procedure:

Mark the tubes and petri dishes for batch no., sample no., parameter, dilution etc.

↓

Pipette out 10 or 11 mL of sample from sample bottle into 90 or 99 mL of diluent bottle (1:10 dilution

↓

Pipette out 1 mL of diluted sample from 1:10 dilution bottle into 9 mL of diluent tube

↓

Pipette out 1 mL from each dilution into respectively marked dilution plates

↓

Subsequently go for further dilutions if required by pipetting out from previous

↓

Pour the PCA medium into the plates and allow it to solidify

↓

Incubate the plates at 35 ± 2°C for 48–72 hrs

↓

Then count the colony using colony counter

Fig 6: TVC count

3.4.2. Coliform count

Coliform bacteria, which may be present in dairy products processed under unsanitary conditions, were investigated in this study. The coliform count of milk was determined according to APHA (2004) and FSSAI (2016) using MacConkey's agar. The process involved preparing a serial dilution of the sample and inoculating a sterilePetridis with 1 ml of the necessary dilutions in duplicate. Subsequently, each plate received 10-15 milliliters of previously melted MacConkey's agar cooled to 45°C, and thorough mixing ensued. The agar was allowed to solidify, followed by the addition of a second layer of three to four milliliters of medium over the hardened surface. The dishes were inverted and incubated for 24 hours at 37°C in an incubation chamber. After the incubation period, positive test results were identified by the presence of dark red colonies measuring at least 0.5mm.

3.5 Statistical Analysis

The data were stored into Microsoft Excel 2010, and subsequently, data analysis was performed using R statistical software version 4.3.3. To assess the distribution of the data, a Shapiro test was employed. Parameters exhibiting a normal distribution underwent one-way ANOVA testing, while parameters deviating from normal distribution were analyzed using the nonparametric Kruskal-Wallis test. Following ANOVA, Tukey's test was applied as a post hoc test, whereas after the Kruskal-Wallis test, Bonferroni test was conducted as a post hoc test where there was a significant difference. A significance level of $P \leq 0.05$ was considered to determine the significance of the mean differences

CHAPTER- IV

RESULTS

CHAPTER IV

Results

4.1Physicalparametersanalysis

Table 4.1 Sensory evaluation of studied toned milk

Breand	Parameters (mean±SD)				Total (mean)	Quality	Grade
	Color and appearance	Odor	Flavour	Body			
Aarong	8.33±0.58	17.67±2.52	35.00±0.00	27.67±2.52	88.67	Good	B
Farm Fresh	8.00±1.00	15.00±2.65	27.00±6.81	22.67±11.02	73.33	Fair	C
Milk Vita	9.00±0.00	14.67±4.16	32.00±2.51	26.00±5.29	82.00	Good	B
P value	0.21	0.51	0.08	0.95	0.36	-	-

Score of color and appearance of milk vita was higher than Aarong and Farm Fresh where farm fresh had the lowest value (table 4.1). There was no significant difference among them (p value 0.21). In case of odor Aarong toned milk had higher score than the other two brands. There is no significant difference among them (p value 0.51). Aarong toned milk has a high score value for both flavour and body but there is no significant difference among them (p value 0.08, 0.95). Total score of Aarong toned milk for sensory evaluation by expert panel is 88.67 giving it grade B and good quality whereas scores for farm fresh and milk vita are 73.33 and 82.00 giving them grade C and B respectively. Though there are no significant differences among them in their total scores (p value 0.36) (table 4.1). Result shows that the Aarong toned milk had a better score than other two whereas farm fresh had the lowest scores in expert panel.

4.2: Chemical Analysis

Table 4.2: Chemical analysis of toned milk

Parameters	Brands (mean±SD)			Anova (p value)	Kruskalwallis (p value)
	Aarong	Farm Fresh	Milk Vita		
Specific gravity	1.031±0.0011[a]	1.030± 0.0005[b]	1.031±0.0006[a]	_	<0.001
Acidity%	0.174±0.008	0.177±0.008	0.174±0.011	_	0.46
Casein%	2.601±0.163	2.519±0.088	2.531±0.145	_	0.46
Protein%	3.348±0.194	3.244±0.114	3.320±0.183	_	0.31
Fat%	1.991±0.079[a]	1.892±0.090[b]	1.925±0.062[ab]	_	0.02
SNF%	8.974±0.229[a]	8.529±0.144[b]	8.935±0.129[a]	<0.001	_
TS%	12.174±0.229[a]	11.729±0.144[b]	12.135±0.129[a]	<0.001	_
Lactose%	4.45±0.067	4.93±0.069	4.96±0.10	_	0.94

Different superscript letters in the same row differ significantly (p <0.05)

The specific gravity of Farm fresh toned milk was significantly lower than the Aarong and Milk Vita toned milk (P< 0.001) (Table 4.2). The acidity, casein and protein of Aarong, Farm Fresh and Milk Vita toned milk were not significant (P value >0.31). The fat of Aarong toned milk was significantly higher than Farm Fresh toned milk. The SNF of Aarong and Milk Vita toned milk were significantly higher than the Farm Fresh toned milk (p<0.001). The TS of Aarong and Milk Vita toned milk were significantly higher than the Farm Fresh toned milk (p <0.001). The Lactose of Aarong, Farm Fresh and Milk Vita toned milk were not significant (p value 0.94) (Table 4.2).

4.3: Microbial Analysis

Table 4.3: Microbial analyses

Brands	TVC (Total Viable Count) (mean±SD)	Coliform Count
Aarong	37750±2094.36[b]	0
Farm fresh	36167±2124.89[b]	0
Milk vita	39667±1302.68[a]	0
P- value (ANOVA)	<0.001	-

Different superscript letters in the same row differ significantly ($p < 0.05$)

The Total viable count of bacteria of Farm Fresh toned milk was significantly lower than the Milk Vita toned milk ($p<0.001$) (Table 4.3). The coliform count was nil in all toned milk samples from the available brands.

4.4: Comparison with BSTI standards

Table 4.4: Comparison with BSTI standards

Parameters	Standards (BSTI, 2021)	Brands (mean)		
		Aarong	Farm Fresh	Milk Vita
Specific gravity	1.028 –1.036	1.031	1.030	1.031
Acidity%	0.18	0.174	0.177	0.174
Casein%	-	2.601	2.519	2.531
Protein%	3.0	3.348	3.244	3.320
Fat%	Min 2%	1.991	1.892	1.925
SNF%	9.00%	8.974	8.529	8.935
TS%	-	12.174	11.729	12.135
Lactose%	4.4	4.45	4.93	4.96
TVC (cfu/ml)	<30000	37750	36167	39667
Coliform count/ml	<10	0	0	0

BSTI has established criteria for toned milk, focusing on selected parameters such as fat content, solids-not-fat (SNF), total viable count (TVC), and coliform count. As per the BSTI 2021 guidelines, the fat percentage in tested toned milk from all three companies (mean <2) fell below the standard (min 2%) (Table 4.4). Similarly, the SNF content of toned milk of all three companies (mean <8.93) were below the standard (9.0%) set by BSTI (2021). The Acidity of all sampled toned milk (mean <1.77) was also below BSTI (2021) standard point (0.18). On the contrary the protein content of all three companied toned milk (mean >3.2) had met the standard value (>3.0). The lactose content of examined toned milk (mean >4.4) was above the set value (4.4%). Conversely, the total viable count exceeded the BSTI standard (mean >36166). However, the coliform count in the toned milk from the three companies met the standard (Table 4.4).

CHAPTER-V

DISCUSSION

CHAPTER V

Discussion

This research involved analyzing a total of 18 samples of toned milk from three companies (Aarong, Milk Vita, and Farm Fresh) to assess their physical (color and appearance, odor, flavour, body), chemical (specific gravity, acidity, casein, protein, fat, SNF, TS, lactose), and microbial (TVC, coliform count) qualities. All parameters were compared against BSTI standards. Due to limited scientific research on the quality evaluation of toned milk, there is limited literature available for comparison purposes.

5.1 Physical evaluation

Tested toned milk of Aarong and Milk Vita was good quality with a score of total 86.67 and 82.00 and graded as B whereas Farm Fresh toned milk was graded as C with a score of 73.33 and marked as fair quality. Farm Fresh toned milk was lack in flavour according to expert panel thus had a low score in flavour. On the contrary, Aarong toned milk higher flavour, color and body score than other two which ranked it at first place in physical evaluation. According to Fenton et al., (1968) market milk should be free from any foreign particles, sediment, unpleasant odor and abnormality for consumption. All three toned milk were free from extender materials which indicated the acceptance for consuming. Though, the flavour varied giving a low recognition for Farm Fresh which may be caused by improper processing and handling of milk or preservation of raw milk in inappropriate condition (Zucali et al., 2016).

Specific gravity

Specific gravity of toned milk of the three companies had met the BSTI, (2021) standard (1.028 – 1.036). Hence, all three categorized sample were very close to each other in case of specific gravity where Aarang and Milk Vita toned milk had specific gravity of 1.031 and Farm Fresh toned milk had 1.030 which is slightly lower than the previous two. Specific gravity serves as a crucial indicator of milk quality, reflecting factors such as fat and other solids content and temperature. At 15°C it varies from 1.020 – 1.038 (Sharp, P. F., & Hart, R. G., 1936). The finding of this study indicates that the toned milk was good quality as its value is within the acceptable limit. In low fat pasteurized milk specific gravity was found 1.022 – 1.032 (Sánchez et al., 2010). This study finely matches with the findings indicating sample toned milk had undergone proper standardization or the original milk was a good quality milk.

5.2 Chemical quality

Acidity

The acidity percentage serves as an indicator of milk's freshness and bacterial presence. According to Popescu and Angel, (2009), top-notch milk should exhibit an acidity level below 0.14%. On the other hand, BSTI, (2021) has allows a highest level of 0.18% acidity for toned milk. In this study Acidity% of Aarong and Milk Vita toned milk had found 0.174 while acidity of Farm Fresh had found 0.177 which is lower than the other two. Though the difference among the acidity was not significant (p = 0.46). The acidity% of all the toned milk was under the highest-level as set by BSTI,(2021). Although the acidity level exceeds the threshold of 0.14% suggested by Popescu and Angel (2009), this could be attributed to the pasteurization and processing of milk in industries. Measuring the acid content in milk is crucial for assessing its quality, with acidity playing a significant role in taste. Elevated acidity levels in milk can be attributed to factors such as its age and bacterial activity (O'Mahony, 1988). The extent of bacterial presence and the storage temperature are key determinants affecting the formation of acidity (Hossain et al., 2011). As the Acidity of the sampled toned milk was under the utmost level it can be said that the quality of the milk was reasonably good.

Protein

The protein content of cow milk has been reported to vary from 3.22% to 3.92 % (Ramasamy et al.,1999, Lingathurai et al., 2009). Results of this study showed that the protein content of toned milk was above the threshold level of 3.0% set by BSTI,(2021). The protein content of Aarong toned milk is the highest having 3.34 whereas Farm Fresh toned milk had the lowest value of 3.244. Thus, result obtained for all toned milk samples were met up the standard protein content as per BSTI, (2021). Though the difference of protein content among the tested toned milk was not significant (p value 0.31). Proper level of protein content indicates the good quality of milk. The original milk must have contained high protein as pasteurization can cause decrease of protein in some extent (Franzoi et al., 2022).

Fat

In the current study, the average values of fat content observed for the three brands toned milk were generally below the minimum level of 2% (BSTI, 2021). The fat of Aarong toned milk was significantly higher than Farm fresh toned milk. Farm Fresh had the lowest level of fat (1.89%) whereas Aarong toned milk had the highest fat content (1.99%) which is very close to standard. The reduction of fat level in the toned milk may be the result of starting milk with a lower-than-normal fat level (Santos and Fonseca, 2001) or may also be caused by the withdrawal of fat from the original milk that were used for pasteurization or any alteration of proper ration. Burgwald, L. H., &Strobel, D. R., (1959) found 1% fat in double toned milk and <2% fat in single toned milk. The single toned milk fat content matches the findings of this study.

Solids-Not-Fat

There were significant differences in solids not fat contents among the different brands of toned milk. Aarong toned milk boasted the highest SNF content among the three brands (8.97%), nearing the BSTI (2021) standard. Farm Fresh exhibited the lowest SNF content (8.529%). The Food and Drug Administration (FDA) mandates a minimum SNF content of 8.25% for toned milk (Graf, 1976). The results of this study align closely with the FDA standard. FASSI, (2016) established an SNF standard of 8.5%, which is also upheld by this study. Conversely, BSTI, (2021) set the SNF standard at 9%, a benchmark not met by the SNF contents of the sampled toned milk of three companies. The slightly lower SNF content might be attributed to standardization or lower SNF content in the original milk, or it could be due to the loss of solids content during pasteurization. The addition of water dilutes milk, reducing its total solids content.

Lactose

The lactose content in cow's milk varies depending on the breed of the cow (Fox et al., 2015). Cerbulis and Farrell Jr., (1975) discovered that milk must contain at least 4.2% lactose for any manufacturing process. According to BSTI, (2021), the standard for lactose content in toned milk is set at 4.4%. Samples of all the three brands (Aarong, Farm Fresh, and Milk Vita) met this target with values of 4.45%, 4.93%, and 4.96% lactose, respectively. Among them, Milk Vita's toned milk exhibited the highest lactose content, while Aarong's had the lowest. There is no significant difference of lactose content in the tested toned milk (p value 0.94). Research indicates that low lactose levels signify inadequate energy intake and underfeeding. Additionally, the lactation stage influences lactose levels. During early to mid-lactation, lactose levels typically exceed 4.5%, while herds on a high nutritional plane may reach 4.6-4.9%. Towards late lactation, these levels typically drop below 4.5% (Kittivachra et al., 2007). Having the lactose value above standard indicated the good quality of milk. The original milk from which toned milk was produced may be from cows at mid lactation or high lactose producing breed. The results of this study also endorsed the efficacy of a high-quality nutrition regimen.

5.3 Microbial quality

Total viable count

All the samples had higher total viable count than standard where Milk Vita toned milk showed highest value of 39,666 cfu/ml and Farm Fresh showed lowest value of 36,166 cfu/ml.According to Reta, M. A., & Addis, A. H., (2015) total bacterial count for grade A pasteurized should not exceed 20,000cfu/ml. Food Safety and Standards Authority of India (FSSAI) set the microbial standard at maximum 30,000 cfu/ml. Total plate count can vary in a range of 13,000-18,000 cfu/ml on the basis of pasteurization process and proper temperature maintenance (Anderson et al., 2011). Though BSTI, (2021) has set a standard of relatively higher value at maximum 30,000cfu/ml, none of the tested toned milk could accomplish the criteria. High bacteria counts in pasteurized milks could be attributed to several factors, including malfunctioning pasteurization equipment, bacteria surviving the pasteurization process, and contamination after pasteurization due to substandard processing and handling practices, or inadequate hygiene maintained by employees involved. Anderson and Stone, (1955) also noted that pasteurized milk could become contaminated due to the poor bacteriological quality of the milk itself and insufficient cleanliness of the processing plant. Pasteurized milk's short durability over extended periods can be attributed to inadequate time-temperature management, unsuitable storage conditions, adulteration, and contamination (Woldemariam&Asres, 2017). The elevated microbial count found in tested toned milk could result from unsatisfactory sanitary conditions within the milk plant or inadequate pasteurization. It may also be due to incorrect temperature control during preservation.

Coliform count

Coliform count is especially associated with the level of hygiene during production and subsequent handling since they are mainly of fecal origin (Van den Berg& J. C. T., 1988). Coliforms do not survive at pasteurization temperature and their presence in the pasteurized milks indicates recontamination after pasteurization (Tola et al., 2007). If the coliform count in any milk surpasses a certain threshold, such as exceeding ten coliform organisms per milliliter of pasteurized milk, it signifies that the milk was processed under improper procedures (Walstra, P., 1999). BSTI, (2021) has established a standard of maximum ten coliform counts. This study revealed no presence of coliforms in any of the tested toned milk samples from the three companies, aligning precisely with the BSTI standard. This absence indicates no fecal or post-pasteurization contamination. According to Frazier and Westhoft, (1958), the coliform standard for "grade A" milk should not surpass 10 cfu/ml. However, research conducted by Saha and Ara, (2012) found coliform counts in pasteurized milk ranging from 10 to 14 cfu/ml. That milk may be subjected to fecal contamination or improper pasteurization or post pasteurization contamination.

CHAPTER- VI

CONCLUSION

CHAPTER VI

Conclusion

Aarong, Farm Fresh and Milk Vita all three brands have described having fat % of >2% in their toned milk. But this research had found fat% of 1.99%, 1.89% and 1.92% respectively. This means all the findings are below their own description. Aarong prescribed in the label 5.13% lactose but this study found only 4.45% lactose. Milk Vita and Farm Fresh had exceeded their own standard of 4.45% lactose at 4.93% and 4.96% respectively. All the three brandshad shown in their product label protein of 3.8%. But none of them had fulfilled the criteria where the toned milk of these brands contained 3.32 to 3.34% protein. In case of microbial quality all three companies toned milk is of good quality of having zero coliform but slightly alarming in total viable count (TVC) having more than 30,000 cfu/ml. On the other hand, according to expert taste panel these three companies toned milk was good to fair quality.So, the manufacturers should try to maintain the chemical standards and physical quality of their toned milk as they are slightly behind the recommendation. They also should more careful about microbial quality maintenance as it has public health significance.

CHAPTER- VII

REFERENCES

CHAPTER VII

References

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Biography

I am PapryChakrabortty, daughter of JayantoChakrabotrry and ProtimaChakrabortty. I passed my Secondary School Certificates (SSC) examination from Ahammad Uddin Shah Shishu Niketan School and College, Gaibandha in 2013 and Higher SecondaryCertificate (HSC) examination from the same institution in 2015.I have completed my graduation on Doctor of Veterinary Medicine (DVM) fromChattogram Veterinary and Animal Sciences University (CVASU) in 2020. Currently,I am a Master of Science (MS) candidate in Dairy Science under the Department of Dairy and Poultry Science, Faculty of Veterinary Medicine, Chattogram Veterinary and Animal Sciences University (CVASU).I intended to pursue my future research on dairy product marketing and dairy microbiology.