Nutritional and Microbiological Quality Assessment of Powdered milk of different brands available in retail markets of Bangladesh

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Roll No : 0219/06

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A thesis submitted in the partial fulfillment of the requirements for the degree of Master of Science in Applied Human Nutrition and Dietetics

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AUGUST, 2022

Authorization

I hereby declare that I am the sole author of the thesis. I, Wasifa Anica Hoque, declare that this thesis is submitted in fulfillment of the requirements for the Degree of Master of Science (MS) in Applied Human Nutrition and Dietetics, Department of Applied Food Science and Nutrition, Faculty of Food Science & Technology, Chattogram Veterinary and Animal Sciences University. It is wholly my own work unless otherwise referenced or acknowledged. The document has not been submitted for qualifications at any other academic institution. I, the undersigned, and author of this work, declare that the **electronic copy** of this thesis provided to the CVASU Library, is an accurate copy of the print thesis submitted, within the limits of the technology available.

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DEDICATED TO MY BELOVED PARENTS

AND YOUNGER SISTER

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Abbreviation

%	**:** Percentage
0.1N	**:** 0.1 Normal
AOAC	**:** Association of Official Analytical Chemists
°C	**:** Degree Celcius
BDS	**:** Bangladesh Standards
et al	**:** And his / her associates
etc	**:** Et cetera
FAO	**:** Food and Agricultural Orgnization
gm	**:** Gram
MR	**:** Methyl Red
H_2O_2	**:** Hydrogen peroxide
TSI	**:** Triple Sugar Iron
TVC	**:** Total Viable Bacterial Count
rpm	**:** Rotation Per Minute
E. coli	***:****Escherichia coli*
BSTI	**:** Bangladesh Standards and Testing Institution

Abstract

The current study was designed to assess and evaluate the nutritional and microbial properties of whole powdered milk from 5 different commercial brands (A, B, C, D, E) that are sold in retail outlets in the town of Chattogram. Each brand was taken into account as a separate treatment, and three replications of each sample were obtained for each brand. In the context of the goals of the study, a total of five samples were obtained from a variety of nearby supermarkets. These samples were then subjected to a series of tests to determine the nutritional (percentage of fat, protein, ash, moisture, and acidity), and microbial (total viable count, *E. coli*, and *Staphylococcus aureus*) characteristics of the collected samples to determine their overall quality. Among the brands of powder milk, fat ranged from (31.5 ± 0.86 to 35.67 ± 3.05), protein from (21.91 ± 0.36 to 22.56 ± 0.68), ash from (5.22 ± 0.31 to 5.52 ± 0.05), moisture from (3.01 ± 0.37 to 4.14 ± 0.06), and acidity from (0.13 ± 0.02 to 0.16 ± 0.05). Regarding the nutritional quality, all five brands were of a good quality grade, and there was no noticeable difference between any of them, except the acidity levels for treatment 3, which varied greatly between brands. The total bacterial count in E for treatment 2 was found to be the highest at (7.8×104 cfu/ml), while the total bacterial count in A for treatment 1 was the lowest at (3.3×103 cfu/ml). On the other hand, the presence of *E. coli* was not detected in any of the samples, but *S. aureus* was discovered in a couple of the milk samples. Though no *E. coli* was found, it can be deduced that adequate sanitary precautions were taken during the production of the powdered milk samples and their subsequent storage. In conclusion, it is possible to state that most of the brands of powdered milk possessed the recommended suggested standard in terms of both the powder's nutritional qualities and its microbiological qualities.

Keyword: Powdered milk, assessment, nutritional quality, microbiological quality

Chapter-I: Introduction

For every bodily system's physiological operation, milk includes all the necessary nutrients. According to Byron *et al.* (1974), the average milk composition comprises 87.20% water, 12.80% dry matter (fat 3.70%, protein 3.50%, lactose 4.90%, and ash 0.70%), and 3.70% protein. Calcium, phosphorus, and fat-soluble vitamins are all present in milk in reasonable amounts (A, D, E, and K). It is thus the closest thing to the excellent cuisine that nature has to offer.

Dry milk, often known as powder milk, is a product made by removing the water and fat from whole milk. The fat percentage of powdered whole milk is typically between 1 and 2 percent, while that of powdered partly skim milk is between 1.5 and 2.5%. Under no circumstances should the water percentage in dry milk surpass 5%. Two steps are taken to remove the water from the milk. Drying comes after the initial stage of concentration via vacuum evaporation. In the evaporator, 90% of the water in the milk is removed, while only 10% is removed in the spray drier (Robinson, 1994a). Roller or drum processing and spray drying are the two main methods used to create powdered milk. The freeze drying system and the form mat system are further systems. Equipment that was recently developed with combinations of these basic procedures are developed has been discovered (FAO/WHO 1973). The goal of contemporary technology is to minimize improving the loss of nutritional value, and microbiological quality and enhance the milk powder's rehydration capabilities. A high-quality product for the food industry is now produced using optimal design, predictive process and product models, and enhanced automation (Jong and Verdurmen, 2001).

Dry milk is therefore preferable in terms of affordability and practicality. Due to its concentrated source of numerous key components, powdered milk has advantages (Hall and Hendrick, 1966). The quality of the raw milk used to make the milk powder determines its quality, and the shelf life can be increased beyond six months when stored at room temperature. Due to a lack of fresh milk, the usage of milk powder is growing daily in both rural and urban areas, as well as in metropolitan areas (Lapar *et al*., 2010). Also, powder milk (whole and non-fat) is used by flour millers and cheese processors to make ice cream, baby food, bread goods, confections, and sausages. The majority of baby food in Bangladesh is made from whole milk and half-cream

powdered milk that is sold in tin containers. These are also used to prepare a variety of different sweetmeats and for recovering patients. According to business leaders in the sector, the aforementioned data shows that Bangladesh has virtually doubled its imports of powdered milk due to rising domestic and industrial use (Belewu *et al.*, 2009).

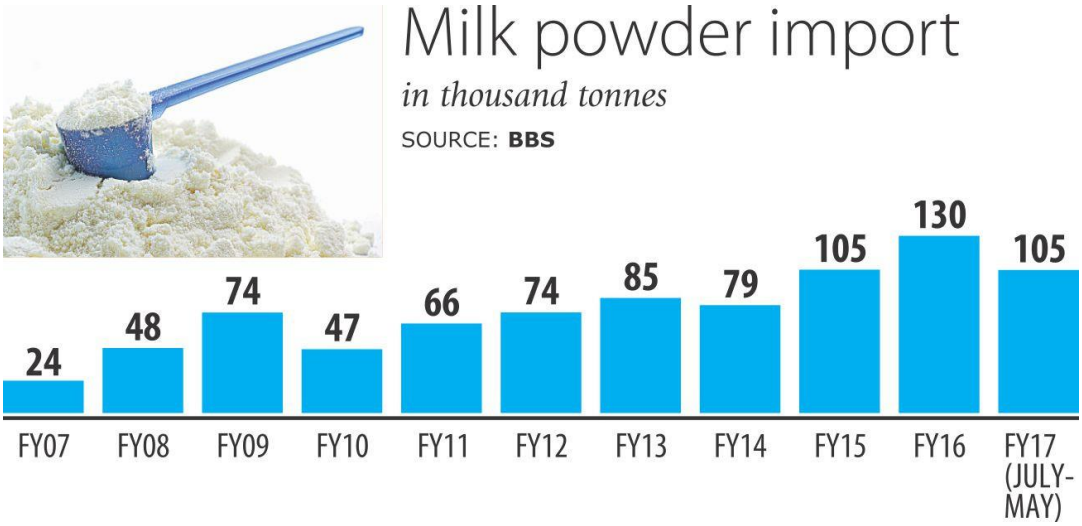


Figure 1: Annual import of powdered milk in Bangladesh till 2015-16 (BBS)

Due to their extended shelf life, simplicity of storage, and adaptability, dairy powders are a widely used product. Due to their high nutritious content, milk and dairy products are loved by people all over the world. Dairy powders can be used as a component in a variety of dishes, such as soups and sauces, confectionery, and fortifying other dairy products (Karam *et al*., 2013; Sharma *et al*., 2012), nutritional supplements for athletes, newborn formula, and foods for health recovery (Gill *et al*., 2001; Lagrange *et al*., 2015). Additionally, dry milk has the benefit of longer shelf life, requiring less storage space, and having lower shipping costs than liquid milk (Robert *et al*., 2015). However, the increasing production could put the dairy industry at risk for both safety and financial loss when managing microbial loads in dairy powders. When stored without refrigeration, powdered milk has a substantially longer shelf life. Compared to other solids, much less storage space is needed. It is possible to distribute goods to nations, especially those where it is impractical to produce perishable dairy products due to harsh environmental circumstances.

Due to the paucity of studies on the quality of powdered milk in Bangladesh, customers have little knowledge of higher-quality options (Kajal *et al.*, 2012). As far as I'm aware, virtually little research has been done in our nation on high-quality powdered milk (Uddin *et al.*, 2011).

Taking into account the aforementioned information, the present study was conducted for the chemical and microbiological analysis of different brands of powdered milk in Bangladesh to meet the following objectives-

1. To evaluate the nutritional and microbiological properties of different brands of powdered milk available in Bangladesh.
2. To isolate and identify any harmful bacteria present in the powdered milk sample.
3. To compare the quality to industry standards and the values listed on the labels of each sample.

Chapter - II: Review of literature

2.1 Definition of milk

The nutritive fluid released by a mammary gland in a female mammal is known as milk. One of the defining traits of animals is the female's capacity to produce milk. Colostrum, the first milk produced during breastfeeding, provides the newborn with the mother's antibodies. In both the mother and the newborn, it can lower the risk of several disorders (USAD National Nutrient Data Base for Standard References, 2005).

All species' milks are complex biological fluids with a wide range of diverse ingredients and distinct physical properties (Robinson and Phil, 1985).

2.2 Milk Nutrition

According to the USAD National Nutrient Data Base for standard references from 2005, milk is a good source of high-quality protein, calcium, Vitamins A and D, riboflavin, other B vitamins, and phosphorus.

2.3 Powdered milk

One way to preserve food is to dry it, which prevents bacteria from having access to the water they need to develop (Bylund, 1995).

Due to its low moisture content, powdered milk has a far longer shelf life than liquid milk and does not require refrigeration (USAD National Nutrient Data Base for standard references, 2005)

2.4 History

Osip Krichevsky, a Russian, created powdered milk for the first time in 1802. Several emerging nations have an abundance of it since the expenses of storage and transportation are lower. It is preferred by survivalists and other people who require nonperishable, simple-to-prepare consumables, as it is nonperishable like other dry foods (USAD National Nutrient Data Base for standard references, 2005).

2.5 Uses of dried milk

It is initially created as a baby meal, powdered milk. Every sector of the food industry that produces goods with regular milk now uses it. In the production of candies and ice cream, it is utilized in the baking business (Eckles and Combs, 1980).

2.6 Processing of powdered milk:

Neither whole milk nor skim milk is pasteurized before being utilized to make milk powder, according to Rosenthal (1991). In tubular heat exchangers, the milk is preheated before being dried. The season (which influences the stability of milk protein) and the required qualities for the finished powder product determine the preheating temperature. In order to increase the concentration of total solids, the heated milk is directed through an evaporator. The efficiency of the machinery and the quantity of heat that can be applied without destroying milk protein, he added, determine the solids concentration that can be achieved.

It is concentrated, pushed to the drying chamber's atomizer, and then the milk is released as a fine, fog-like mist into the fast circulating hot air system, where each drop of the mist instantaneously evaporates. Milk powder spills into the chamber's bottom, where it is removed (Rosenthal, 1991). With the help of the hot air system, the finer milk powder particles are removed from the chamber and gathered in cyclone separators (Rosenthal, 1991).

Drying is the process of removing the water from a liquid substance, such as milk, to transform it into a solid. Milk powder has between 2 and 5% water, and with such a low water concentration, no germs can develop. Milk can be dried to increase its shelf life while also decreasing its weight and volume, which lowers the cost of transporting and storing the commodity (Bylund, 1995).

Commercial drying techniques use heat to evaporatively remove water from the product, leaving behind milk powder as the byproduct. In the dairy business, roller drying and spray drying are indeed the two main processes used to dry milk.

2.6.1 Rolling – drying technique

Onto heated rollers, the milk is spread. A thin coating of milk is left on the roller when the water evaporates, and this film is scraped off. This method of drying milk is no longer very useful because it cannot be easily reconstituted with water like spray dried milk can (Tull, 1996).

When a product is dried using a roller, the heat will have a substantial impact on the product. If the milk particles come into touch with the hot heat transfer surface during the drying process, the powder may then have charred particles that reduce the powder's quality (Bylund, 1995).

2.6.2 Spray – drying Technique

According to Rosenthal (1991), there are two stages to the spray-drying process. The pre-treated milk was concentrated in the first stage by evaporation to a dry solids content of 45 to 55%. In the second stage, the concentrate was fed into a drying tower for final drying. This process involves three steps: first, the concentrate is dispersed into extremely fine droplets; second, it is mixed with a stream of hot air that quickly evaporates the water; and third, the dry milk particles are separated from the drying air. He stated that when using spray drying, the air's temperature at its inlet was roughly 180 to 200°C and its output temperature was 80 to 90°C. The temperature of the milk never rises over 75°C because as milk droplets evaporate, the latent heat of evaporation continuously evaporates the surface of the droplets.

Spray dryers, according to Ozmen and Languish (2003), need to be cleaned periodically since powders tend to accumulate on the walls. Additionally, they stated that it is undesirable to have a buildup of powder deposits in a spray dryer since they can cause the final product to lose quality if they fall off and mix with it. This is because they will oxidize, brown, or scorch.

2.7 Types of milk powders:

2.7.1 Whole Milk Powder

No other drying ingredient comes close to the composition of fresh milk as closely as whole milk powder, which is a soluble powder formed by spray drying fresh whole milk (Thompson, 1996). Typically, it was made by skimming the water off of pasteurized, homogenized whole milk (USDEC, 2006). The following quality criteria apply to whole milk powder: Contains 24.0% fat, 4.0% moisture, and has a solubility index of 1.0 (Alfa-Laval Dairy Handbook).

2.7.2 Skim milk powder

According to Thompson (1996), entire milk was initially used to create the milk powder. Regular and quick varieties are both prepared from milk using a spray process.

White Whole Milk No other drying ingredient comes close to the composition of fresh milk as closely as whole milk powder, which is a soluble powder formed by spray drying fresh whole milk (Thompson, 1996). Typically, it was made by skimming the water off of pasteurized, homogenized whole milk (USDEC, 2006). The following quality criteria apply to whole milk powder: fat content 24.0%, moisture 4.0%, and solubility index 1.0. (Alfa-Laval Dairy Handbook).

2.7.3 Partially skimmed milk powder

It is a milk powder product that was once made by skimming, concentrating, and drying milk (Thompson, 1996).

2.8 The Chemical Content of Milk Powder:

According to a regulation issued by the U.S. Department of Agriculture, dried milk prepared from whole milk must have a minimum of 26% milk fat and a maximum moisture level of 5%. Therefore, the dry milk producer takes all necessary measures to guarantee a product that will adhere to American requirements.

Depending on the composition of the milk used to make it, dried milk has a different composition. The other milk ingredients are raised in roughly the same production since the drying process eliminates the majority of the water (Eckles and Macy, 2004).

2.8.1 Protein content

According to Jenness (1988), milk proteins have a significant impact on human nutrition and affect the behavior and characteristics of dairy products. According to Eckles and Macy (2004), proteins are among the most complicated organic compounds because they contain carbon, hydrogen, oxygen, sulfur, and phosphorus sometimes. The range of the protein content was 2.80 to 4.00% on average. According to Johanson (1980), the protein content is 3.50%. Casein makes up the majority of milk protein in general, with just a little amount of other protein fractions such lacto-globulin and lactoalbulin. It is a great source of proteins and has every vital amino acid needed by people (Payne, 1990). According to Philip (1984), fluid milk contains about 3.5% protein, of which 80% is casein and the remaining 20% is whey protein (globulin and albumin). Whey and casein proteins work well together to provide milk its great

biological value. According to the ADMI (1962) average protein content of dry whole milk is 26.4 g/1OOg.

2.8.2 Fat content

According to Ecklas and Macy (2004), milk's fat content is its most valuable component and plays a significant role in terms of the milk's nutritional value. They discovered that the fat content ranged from 3.5 to 5.8%, with an average of 3.8%. (Jensen, 1995). According to BSTI and ADMI (1971), dried whole milk has an average fat content of at least 26%.

2.8.3 Moisture level

According to Johanson (1980), 87.20% of cow's milk is made up of water. But according to Clarence et al. (1951), any modification in the proportion of other elements similarly affected the water content. The maximum moisture content of a powder is frequently governed by (legal) product standards. This is based on the possibility that products with excessive moisture content may have a shorter shelf life, form lumps, or experience microbial issues (Van Mil and Jans, 1991). Whole milk powder has a 4% moisture percentage, according to the BSTI.

2.8.4 Titratable Acidity

The rise in the range of acidity was caused by the breakdown of lactose to lactic acid and other acids (Gould, 1945). BSTI and ADMI (1971) suggested (0.15%) for the acidity concentration of dry whole powdered milk.

2.8.5 Ash level

Milk ash is reported to have relatively high levels of potassium, sodium, calcium, magnesium, chloride, phosphorus, and sulfur; the percentage of minerals was determined to be around 0.7%. (Ecklas and Macy, 2004). Earlier studies by Watt and Merrile and Johanson (1980) revealed findings that were similar (1963).

2.9 Issues with milk powder

2.9.1 Lactose crystallization

According to Thomas *et al.,* (2004), the functional qualities of milk protein have led to the increased acceptance of milk powder as a food ingredient. Physical and chemical changes occur during milk powder storage that are primarily caused by the lactose glass transition. According to Thomas *et al*., (2004), lactose crystallization, sticking and caking issues, and biochemical reactions, particularly the Maillard reaction, are the primary physiochemical and biochemical damages experienced during storage.

2.9.2 Crystallization of lactose

According to Thomas *et al.,* (2004), lactose is the most prevalent substance in fresh whole milk (4.9%), and lactose is amorphous when it is sprayed dried into milk powder. They claimed that during the storage of milk powder, lactose crystallization enhances the migration of internal fat onto the particle surface and creates a network of capillaries in the entire particle. Lactose crystallization is one of the main phenomena responsible for the modification of the surface chemical composition of milk powder particles. According to Thomas *et al.,* (2004), oil droplets are strained within the particles and are compelled to spread out onto the particle surface. The solubility of milk powders is harmed by lactose crystallization, which modifies the protein structures and is associated with a decline in the flowability of milk powder.

2.9.3 Caking and sticking

According to Foster *et al.,* (2005), sticking and caking issues have been reported while preparing and storing high fat powders like cream powder and cheese powder. High fat powders produce smearing, which is the buildup of powder on the inside of driers, cyclones, and fluidized beds, during processing. Bulks of powder that are challenging to break up form during storage. Several studies have identified milk fat as the source of caking. In powders containing fat, viscous liquid bridges may make them difficult to flow. Some fat may melt during storage if the temperature rises, creating liquid bridges of fatty composition.

A powder collapse, according to Thomas *et al.,* (2004), happens when it is not stiff enough to support its own weight. Collapse, or the reduction of powder particle size, causes significant structural alterations in powders. Since crushing reduces particle volume, collapse is associated with a reduction in porosity and an increase in density.

Furthermore, they pointed out that particle agglomeration is a concern because caked milk powders are viewed negatively by customers and hinder flowability. They claimed that agglomeration, which corresponds to the first stage of collapse, is characterized by particle shrinkage and an increase in the amount of inter-particle content before the particles begin to adhere (stick), before becoming strongly stuck. They also claimed that caking and sticking are inter-linked and occur simultaneously during the storage of milk powder, making it difficult to study each phenomenon separately (caking).

The stickiness of milk particles is strongly influenced by both fat and lactose. Due to a rapid change in surface fat coverage from 0 to 35%, the stickiness of these particles is especially sensitive to modest changes in the fat content between 0 and 5%. (Nijdam and Longrish, 2006).

2.9.4 Maillard response

According to Thomas *et al*., (2004), the Maillard reaction occurs when reducing sugars and proteins in food interact biochemically. It starts with lactose condensation on the same amino acid residues (lactosylation) in milk products and involves a variety of chemical processes. They discovered that the nutritional quality is also worsened because the essential amino acid residues of milk protein are less available when linked to lactose and because the digestibility of milk proteins decreased as a result of this reaction, which is primarily caused by heat treatment and generally harms milk powder quality. This reaction also suggests issues with food safety (Thomas *et al.,* 2004).

2.9.5 Proteolysis and lipolysis

In whole milk powders, lipase activity and the amount of free fatty acids were investigated by Chen *et al.,* in 2003. It was discovered that lipase activity remained constant after eight months of storage. Lipases continuously released free fatty acids from triglycerides, which accumulated during powder storage. Even though lipases were active at 3°C, higher storage temperatures of 25°C led to higher values of free fatty acids. Additionally, they stated that as free fatty acids easily oxidize and produce off flavors, this has a negative impact on the quality of milk powder.

According to Chen *et al.*, (2003), proteolysis in milk powder has been assessed by keeping track of changes in nitrogen levels, such as the rise in non- protein nitrogen or decline in casein nitrogen (NPN). According to Thomas *et al.,* (2004), the proteolytic activity of whole milk powders was not considerably impacted by storage.

2.10 Microflora in powdered milk

In addition to physical and chemical properties, which primarily concern the content of moisture, fat, total protein, and non-protein nitrogen, lactose, titratable acidity, ash, and other nutrients like calcium, the important quality parameters for milk powder are microbiological quality and sensory characteristics (Laszlo, 2007). The amount and type of bacteria present in raw milk or milk byproducts, preheating temperatures, operating parameters of the evaporator, dryer, and planthygiene are just a few of the variables that affect the microflora of dried milk powder. High levels of microorganisms in raw milk may result in high levels of milk powder, and the removal of water from the powder balances out the fall in numbers that results from heat exposure (Ron *et al.,* 2006). Because microbes are ubiquitous, it is very likely and simple for organisms to contaminate our food (Pal and Mahendra, 2015). Due to microbial contamination, improper dairy equipment cleaning and sanitation will result in food poisoning (Pal and Mahendra, 2015). Dried milk powder can get spoiled by a variety of germs, including bacteria (Alkaligenese, Bacillus, Escherichia coli, Enterococcus, Hafnia, Micrococcus, and Streptococcus) and fungi (Aspergillus, Mucor, and Penicillium) (Paland Jadhav, 2013).

Milk is a very nutrient-dense food that is a great environment for a variety of microorganisms to develop. Since milk has a high nutritional value for both microbes and humans as well as newborn mammals, the microorganisms in the liquid milk have a significant impact on the quality of milk powder (Soomro *et al.,* 2002). Milk has the ability to spread a number of diseases. Infected cows with tuberculosis, brucellosis, or mastitis, the exterior of the teats and udder, milk handlers, and storage equipment can all contaminate milk (Szita *et al.,* 2008). The Codex Alimentarius Commission advises pasteurizing all milk and liquid products first, then concentration, and then drying. Therefore, insufficient pasteurization may make it easier for germs to survive in dried milk. The measurement of microbial load is a critical hurdle for public health in our nation. There are numerous proven approaches for identifying microorganisms. According to the American Public Health Association's advice, the current study's Total Viable Bacterial Count (TVC), Coliform Count, and Staphylococcal spp. tests were carried out (APHA, 1960). Staphylococcal spp. was done in accordance with the American Public Health Association's advice (APHA, 1960).

2.10.1 Total viable bacterial count (TVC)

Total bacterial count is an approximate indicator of milk quality, herd health, farm sanitation effectiveness, milk handling and storage, and temperature during shipping. Total viable count (TVC) provides a numerical estimation of the quantity of microorganisms, such as bacteria, yeast, or mold spores, in a sample. A high TVC count implies a high number of microbes, which could suggest a lack of quality in the water or food being consumed (Biyani *et al.*, 2018). Milk powder should have a maximum bacterial count of 50,000 cfu/gm in accordance with Codex Stan (1999) requirements.

2.10.2 *Escherichia coli*

Milk is a good culture medium for the development and multiplication of many different types of microorganisms due to its complex metabolic makeup and high water activity. Escherichia coli is one of the most common microbes to contaminate water, food, milk, and other dairy products. It is also a good predictor of fecal pollution in general in unsanitary environments (Diliello,1982). Since both of the affected individuals drank raw milk, Martin *et al*., (1986)'s report of two instances of hemolytic uremic syndrome offers evidence that raw milk may be a source of *E. coli* O157:H7 transmission. The existence of enteropathogenic and/or toxigenic microorganisms, which could pose a threat to the public's health, is suggested by the recovery of E. coli from food. In newborns and young children, enteropathogenic *E. coli* (EEC) can cause severe diarrhoea and vomiting (Kumar *et al.*, 2011). Humans can contract a number of diseases from *Escherichia coli* O157:H7, from mild diarrhea to severe hemorrhagic colitis and hemolytic uremic syndrome (Kiranmayi., 2010). At a storage temperature of 5 °C, *Escherichia coli* O157:H7 viable cells can endure in infant formula powder for up to a year (Koseki., 2015). *Escherichia coli* O157:H7 exhibits a high level of virulence and can infect humans at low doses of 5 to 50 cells (Farrokh., 2013).

Given that these microorganisms are prevalent in the environment and feces, the presence of Coliforms in food with an animal origin denotes fecal and environmental contamination (Shojaei and Yadollahi, 2008). *E. coli* is a significant mastitis pathogen that is widely present in agricultural settings (Hogan and Smith, 2003). *Escherichia coli* organisms are the most prevalent coliform pollutants in both raw and pasteurized milk (Quinn *et al.*, 2002). It serves as an accurate indicator of fecal contamination of food and drink, including milk and dairy products (Todar, 2008).

2.10.3 Staphylococcus aureus

Staphylococcus aureus infections in humans are thought to be mostly caused by milk and its derivatives (Zecconi and Piccinini, 1998). In Europe, 5% of staphylococcal epidemics are attributed to milk and other dairy products (Bianchi *et al.*, 2014). Both humans and animals can contract a number of diseases from S. aureus, and the infections can be quite severe. Mild skin infections to severe pneumonia and septicemia are possible (Lowy *et al.,* 1998). S. aureus can enter milk either directly through the excretion of udders with clinical or subclinical staphylococcal mastitis or indirectly by environmental contamination during the handling of raw milk (Scherrer *et al.*, 2004; Jorgensen *et al.,* 2005). Staphylococcus aureus must not be present in 25gm of the product in accordance with Codex stan criteria from.

2.10.4 Screening methods for microorganisms in milk microscopy:

2.10.4.1 Direct microscopy

Initially, traditional microscopy is often used to quickly analyze milk samples. In this method, *Escherichia coli* appears as rod-shaped, Gram-negative bacilli, and Staphylococcus aureus as spherical, Gram-positive cocci with clusters that resemble grapes (Iorio *et al.*, 2007).

2.10.4.2 Cultural resources

Blood agar

E. coli appears as non-hemolytic, grey whitish, wet, glistening, opaque, round, convex colonies with complete edge on bovine or ovine blood agar (Soomro *et al.*, 2002). Unlike S. aureus, which has colonies that are shiny, smooth, complete, elevated, translucent, and frequently have a golden tint. After 24 hours of incubation, the colonies are 2-3 mm in diameter, and the majority of strains exhibit ß- haemolytic colonies (Bottone *et al.,* 1984).

MacConkey agar

For the purpose of preferentially isolating Gram-negative and enteric (often found in the digestive tract) bacilli and differentiating them based on lactose fermentation, MacConkey agar is an indicator, selective and differential growth medium for bacteria. By preventing the growth of Gram-positive organisms, crystal violet and bile salts enable the selection and isolation of gram-negative bacteria. Using the carbohydrate lactose and the pH indicator neutral red, enteric bacteria that can ferment lactose can be

found. Smooth, round pink colonies with spreading growth are produced by Escherichia coli (Anderson *et al.,* 2013).

Mannitol Salt Agar

To isolate S. aureus, mannitol salt agar, or MSA, is frequently employed as a selective growing medium. While preventing the growth of other bacteria, it promotes the growth of S. aureus. Since most other bacteria are inhibited by this medium's high concentration of salt (approximately 7.5–10%), staphylococci are the only bacterium that can grow there.

Additionally, it is a selective medium for mannitol-fermenting staphylococci, containing the carbohydrate mannitol and the indicator phenol red, a pH indicator for detecting acid produced by mannitol-fermenting staphylococci like Staphylococcus aureus, which produces yellow colonies with yellow zones, while other staphylococci produce small pink or red colonies with no color change to the medium (Anderson *et al.,* 2013).

2.10.4.3 Biochemical properties

2.10.4.3.1 Tests for Escherichia coli

An indole test

The indole test is a biochemical procedure used to assess a bacterial species' capacity to transform tryptophan into indole. A mechanism commonly referred to as "tryptophanase"—a chain of several intracellular enzymes—carries out this division.

Results of an indole test are revealed by a change in color following a reaction with an additional reagent, similar to many other biochemical tests on bacteria. The presence of a red or red-violet hue in the broth's surface alcohol layer indicates a successful reaction. An unfavorable outcome is colored yellow. An orange color may appear as a result of a varied result. This is owing to the presence of skatole, another potential byproduct of tryptophan decomposition, also known as methyl indole or methylated indole. E. coli demonstrated successful indole cleavage (MacFaddin, 1980).

Test for triple sugar iron (TSI)

The Triple Sugar Iron (TSI) test is a microbiological procedure that evaluates a microorganism's capacity to create hydrogen sulfide and ferment sugars. Any of the three sugars in the medium that are fermented by bacteria will result in byproducts. These byproducts, which are frequently acids, will cause the pH-sensitive red dye (phenol red) to turn yellow. The location of the color shift distinguishes between the acidic byproducts of lactose or sucrose fermentation and the acid generation associated with glucose fermentation. Enterobacteria make up a large portion of bacteria that can ferment carbohydrates in the anaerobic portion of the tube. Because it ferments lactose and produces acid and H_2S. E. coli serves as a representative of the TSI test (Tille, 2014).

2.10.4.3.2 Tests for Staphylococcus aureus

Catalase test

The abundance of catalase produced by S. aureus can combine with hydrogen peroxide to create oxygen. The test separates cocci that produce catalase from those that don't (like staphylococci) (e.g., streptococci). Blood agar cannot be used for a catalase test since blood contains catalase (Davis and Hoyling, 1973).

Coagulase test

When fibrinogen is converted to insoluble fibrin in the presence of plasma by coagulase, which is generated by some Gram-positive cocci, including S. aureus, either in bound form (attached to the bacterial cell wall) or as a free enzyme, clotting occurs. S. aureus can be distinguished from coagulase-negative Staphylococci (CoNS) by the presence of coagulase (Brown *et al.*, 2005).

2.11 Hygiene aspects of dried milk powder

By following the instructions below, dried milk powder's hygienic quality can be preserved (Pal, 2012).

1. It is crucial to note that raw milk with excellent microbiological purity is required for the production of dried milk powder.
2. Roller drying is successful because it eliminates the majority of germs.
3. Because they could be a source of microbial contamination, evaporators should be thoroughly cleaned and sanitized.

4. To prevent the contamination of the powder, the drying chamber should be kept in extremely sanitary conditions.
5. Filtered air needs to be supplied for the processes of drying, conveying, cooling, and air sweeping. Filter pads must therefore be cleaned periodically to remove collected dust.
6. The packaging room, storage container, concentration tank, pipelines, and vacuum pans must all be completely cleaned and sanitized.

Chapter III - Materials and method

3.1 Test location

The research was carried out at the Department of Applied Food Science and Nutrition, the Department of Dairy and Poultry Science, the Department of Animal Science and Nutrition Chattogram Veterinary and Animal Sciences University (CVASU) to analyze the chemical and microbiological properties of different brands of powdered milk available in Bangladesh. Analytical process or assay was performed at the Poultry Research and Training Centre (PRTC) and Department of Dairy and Poultry Science laboratories of CVASU from April to September 2021.

3.2 Collection of samples

Five well-known commercial brands of powdered milk (A, B, C, D, E) were selected. A total of 15 poly packets of powdered milk from five brands (3 packs for each brand) were purchased from neighborhood shops and super shops in Khulshi town (Basket, Shopno, Agora, and Khulshi mart).

3.3 Chemical Analysis

Different chemical qualities of milk have been measured, such as fat% (Gerber method), and moisture% using a PMB 202 moisture analyzer, by the FAO's recommended standards (1984). Following the IDF20-1 approach, the protein% was calculated (2014). Ash% was calculated by AOAC procedures 2016. By titrating with 0.1N sodium hydroxide solution according to Aggarwala and Sharma's method, titrable acidity (TA) was determined (1961).

3.4 Reconstitution of Powdered milk

To get liquid milk with a similar chemical composition to whole milk, whole milk powder is dissolved in water. 100 ml of 45°C water was combined with 13g of milk powder for reconstitution. 1000 ml (1 liter) of distilled water was first heated to 100°C. We took 5 clean, dry, 300 ml beakers and labeled them with the names of the powdered milk brands: Dano, Marks, Aarong, Diploma, and Pran. The label instructed us to add 13g of powdered milk from each packet to the beaker. Each beaker received 100 ml of 45°C water, mixed for 90 seconds with a clean stirrer to remove any lumps.

3.5 Procedure for the Chemical analysis of powdered milk

3.5.1 Determination of acidity percentage

First, 10 ml of reconstituted milk and 10 ml of deionized water were poured into a beaker and stirred together using a pipette. There was added 1 ml of phenolphthalein to it. The initial reading was then taken after the addition of 0.1 N NaOH in the burette. Then titration was carefully carried out till a bright pink color developed. It establishes the titration's termination point. The burette's final reading was recorded (Fahmid et al., 2016). The same process was carried out three times using reconstituted milk from all brands, and the mean value was noted.

Calculation:

$$\% \text{ of lactic acid} = \frac{volume\ of\ alkali\ used \times (N) of\ NaOH \times 0.09}{volume\ of\ milk\ sample} \times 100$$

Figure 2: The appearance of faint pink color indicating the end point of titration

3.5.2 Determination of fat percentage by Gerber Method

The butyrometers were first placed in the test-tube rack after being labeled with the powdered milk brand name. After that, 10 ml of sulphuric acid was added to each butyrometer. According to the labeling, 11ml of reconstituted milk from the designated beaker was introduced to the butyrometers. Each butyrometer then had 1ml of isoamyl alcohol poured into it. As the milk was being digested in the acids and heat was being produced by the reactions, the butyrometers were gently shaken after the caps were closed until the curd was dissolved. After that, the butyrometers were attached, and the centrifuge was run for 5 minutes at 1100 rpm. These butyrometers underwent

centrifugation and then spent 3 minutes in a water bath heated to 65°C. Then, by removing the golden coating from there, the fat% was seen (Kleyn *et al.*, 2001).

Figure 3: Reading of fat recorded from butyrometer at vertical position

3.5.3 Determination of moisture percentage

Each sample contained 5gm of milk powder, which was weighed and distributed on the analyzer disk. After that, the disks were sequentially placed in the analyzer with the timer set to 2 minutes and 15 seconds and the temperature to 117°C. The findings were written down on a notepad.

(A) Moisture analyzer machine, (B) After burning of the powdered milk in the analyzer

Figure 4: Steps of moisture measurement

3.5.4 Determination of Crude Protein

A technique outlined in IDF20-1 was used to evaluate the crude protein content of a powder milk sample (2014)

Digestion

A sample of two grams was weighed and transferred to a Kjeldahl digestion tube. Twenty milliliters of a digestion mixture that serves as a catalyzer and contains 98% sulphuric acid are introduced to the digestion tube. For three hours, a digestion tube was inserted into Kjeldahl's digestion unit. The substance that had been digested was allowed to cool to room temperature.

Distillation

50 ml of distilled water was added to the digested mixture to dilute it. In a conical flask, 10 ml of 4% boric acid was introduced along with two drops of methyl indicator. In the distillation unit were a digestion tube and a conical flask holding boric acid. Automatic distillation from a linked flask carrying NaOH solution supplied 40% NaOH solution to the diluted mixture. Conical flasks were used to collect liberated ammonia that was produced during chemical reactions and distillation.

Titration

A reading was recorded after titrating the distilled solution against a solution of 0.1N HCl.

Figure 5 : Steps of protein measurement

3.5.5 Determination of Ash:

AOAC procedures were used to determine the ash content (2016). The inorganic residue left over after organic stuff is destroyed is known as ash content. A pre-weighed, dried 5 gm sample was added to the crucible. Then it was turned into charcoal. The charcoal was then placed in a muffle furnace and heated for 4 hours at a temperature of about 600°C to remove all of the charcoal. Then the crucible was removed from the furnace. It was properly cooled in a desiccator before being weighed.

Procedure

The empty crucible was taken out, allowed to sit in a desiccator for an hour, dried at 105°C, and then weighed to a constant weight. The weighted empty crucible was filled with approximately 1g of sample. The specimen in the crucible was burnt on a low flame after 1 drop of nitric acid was applied. The crucible was then placed in a muffle furnace with the temperature adjusted to climb to 650°C and maintained there for three hours. After that, it was pulled out, cooled, and held in a desiccator while the weight of the crucible with the ash was measured.

$$\%Ash = \frac{\text{Weight of Crucible and Ash } - \text{Weight of Crucible}}{\text{Weight of sample}} \times 100$$

Figure 6 : Placed sample in a muffle furnace

3.6 Bacteriological analysis

To assess the microbiological quality of samples obtained from various super shops, total viable bacterial count (TVC), and the isolation of *Escherichia coli* and *Staphylococcus aureus* were performed. Different bacteriological media, including mannitol salt agar (*S. aureus*), plate count agar (TVC), and MacConkey agar (TCC), were employed (APHA, 1992).

3.6.1 Preparation of agar medium

Every medium was produced by the manufacturer's instructions. The medium was divided up into various screw-top bottles and autoclaved at 121°C for 15 minutes to sterilize it. Once sterilized, the bottle was kept in a water bath at 45°C until it was needed. It is advised that a hot water bath not exceed a temperature of 50°C.

3.6.2 Enumeration of Total viable bacterial count (TVC)

3.6.2.1 Spread Plate Technique

First, a group of test tubes (5) was taken, each holding 9 ml of diluents. A 9 ml mixture of diluents and a 1gm of milk sample were homogenized to create a suspension in a beaker. 1 cc of the original sample was transferred to test tube number 1 and properly mixed. 1 ml from the first test tube to the second was transferred, and so on until the last one, and 1 ml from the last test tube was discarded. Three petri plates containing PCA medium were removed from each tube. Next, 0.5 ml of the mixture was moved from one test tube to the next. For each tube, only one pipette should be used. Gently touch the medium with the test tube's tips. Using a glass spreader or sterilized swab

stick, diluted samples were applied to the media's surface. To encourage the growth of live bacteria, the petri dishes were labeled (with the sample number, date, etc.) and placed in an incubator inverted for 24 to 72 hours at 37°C. The colonies were visible after 1 day and up to 3 days of incubation. Which 30-300 plate colonies should be included, and the rest should be thrown away. Three plates of colonies from each sample were counted, and an average was calculated. The final count was represented as colony forming units per milliliter (CFU/ml) after counting.

Figure 7 : Counting of bacteria in a colony counter

Calculation:

Total no. of bacteria = No. of colonies × Dilution factor

3.6.3 Isolation of *E coli*.

The confirmation test for E. coli was conducted using Eosin methylene blue (EMB) agar, which was isolated from *E. coli*. A confirmed positive brilliant green bile broth culture streaked a loop of suspension onto the plates. For 18 to 24 hours, inoculated plates were incubated at 35°C. Discrete dark-centered nucleated colonies with metallic sheen were seen after incubation, indicating a successful outcome for each (EMB) agar plate. For the morphological analysis of gram-negative *E. coli*, two or more colonies were selected and cultivated on nutrient agar slants (Cheesbrough, 1985).

3.6.3.1 Identification of *Escherichia coli*

3.6.3..3.1 Morphological characterization of organisms by Gram's staining method

For gram's staining, a glass slide was initially taken. A sterile, dry, clean, grease-free glass slide was centered with a loop filled with sterile distilled water. A single colony with comparable characteristics was picked up on the slide using an inoculating loop, and it was combined with distilled water. On the slide, the colony was shaped like a thin smear. The smears were removed by letting them air dry. The smear was then exposed to 0.5% crystal violet solution for 1 minute. Gram's iodine solution was then added and allowed to work as a mordant for 1 minute. The next step was decolorizing for 1-2 seconds with acetone alcohol. After that, water was used to rinse the slide. A counter stain of safranin solution (2%) was applied, and it was let to stand for one minute. After that, water was used to rinse the slide. The slide was then blotted with blotting paper and left to dry naturally. The slide was looked at using immersion oil under a light microscope with a high power objective (100X) (Merchant and Packer, 1969).

3.6.3.3.2 Biochemical characterization

Following biochemical analyses, all the positive samples were verified, and the following is described:

An indole test

5 ml of pure bacterial culture was inoculated with 2 ml of sterile tryptophan or peptone broth before the test, and the mixture was then incubated at 37°C for 24-48 hours. After incubation, the culture broth was well mixed with 0.5 ml of Kovac's reagent (isoamyl alcohol, para-Dimethyl- amino-benzaldehyde, concentrated hydrochloric acid). The tube was then let to stand for some time. The appearance of a crimson or red-violet tint in the broth's surface alcohol layer indicated that the test for E. coli had been successful. A bad outcome is shown in yellow (Cheesbrough, 1985).

Testing with Triple Sugar Iron Agar (TSI) procedure

The colony was scooped up using a sterilized platinum loop. This colony splattered on the surface of the agar slant after being inoculated into TSI agar by first poking through

the middle of the medium to the bottom of the tube. The tube's cap was loosened, and it was incubated for 18 to 24 hours at 35°C in free air. Lactose (or sucrose) fermentation results in the production of a significant amount of acid, which causes the phenol red indicator to turn yellow both in the butt and the slant. E. coli was reported to be present (Carter, 1986).

Test for methyl red (MR)

A pure colony of the test organism was inoculated in 5 ml of sterile glucose phosphate peptone broth before being subjected to the Methyl Red (MR) test. 5 drops of the methyl red indicator were added following a 48-hour incubation period at 37°C. Indicating the presence of E. coli and a pH of 4.5 or less due to the fermentation of glucose, a red coloring appeared positive (Cheesbrough, 1984) fermentation of glucose (Cheesbrough, 1984).

3.6.4 *S. aureus* isolation

The following media were employed to check for S. aureus in the milk samples collected:

Selective media: Mannitol Salt Agar (HiMedia®, India),

Enriched media: Blood agar (Oxoid Ltd.), Brain Heart Infusion Broth (Oxoid Ltd.),

Milk samples were smeared and streaked onto Mannitol Salt agar (HiMedia®, India) in loops, and they were then incubated aerobically for 24-48 hours at 37°C. The bacterial growth was seen after incubation. To get pure culture, the S. aureus colonies were repeatedly sub-cultured and then cultured onto mannitol salt agar (MSA). The isolates also fermented mannitol, as evidenced by the MSA (Mannitol Salt Agar) changing color and the growth of tiny yellow colonies. *S. aureus* was suspected of being present in any colonies that produced a golden yellow color (Anderson *et al.,* 2013). These colonies were subsequently subcultured onto blood agar. Initially, Gram's staining and a battery of advised biochemical assays were used to confirm that S. aureus was indeed growing in any smooth colonies on blood agar causing beta-hemolysis (Bottone *et al.,* 1984).

3.6.4.1 Identification of S. aureus

3.6.4.1.1 Gram's staining

Gram’s staining was performed according to the conventional procedures. A loop full of sterile distilled water was placed in the center of a sterile, clean, dry, grease free glass slide. On the slide, a single colony with similar characters was picked up with an inoculating loop and was mixed with distilled water. Briefly, a portion of a suspected colony on a blood agar was thin-smeared over a slide, heat fixed, stained with crystal violet treated with Gram’s iodine, decolorized with acetone-alcohol and finally counter stained with safranin. The slide was then microscopically examined. Gram-positive cocci with cluster like cellular arrangements were suspected for the presence of *S. aureus* (Iorio et al., 2007).

3.7.4.1.2 Biochemical tests

Catalase Test

For the catalase test, a 3% hydrogen peroxide (H_2O_2) solution was employed. Suspected colony was placed on a glass slide that was clean, dry, and free of oil. Loop full of water was poured, and then one drop of 3% H_2O_2 was added. On the basis of the creation of active bubble formation, a favorable outcome was suggested. This test might also be performed directly on a suspected colony on agar media, however it risks producing false-positive results if blood agar is used (Davis and Hoyling, 1973).

Coagulase Test

Coagulase, a virulence factor that can coagulate plasma into gel in a tube or agglutinate cocci on a slide, is known to be produced by pathogenic *Staphylococcus aureus*. The majority of pathogenic strains of *S. aureus* produce both free and bound coagulases. Bound coagulase is a protein connected to the cell wall, whereas free coagulase is an extracellularly released enzyme. In the tube coagulase test, free coagulase is found, while the slide coagulase test finds bound coagulase. Tube coagulase test may be used for confirmation and a slide coagulase test may be used to screen isolates of *S. aureus* (Brown *et al.*, 2005).

Slide coagulase test

On two opposite ends of a clean glass slide, dense suspensions of *Staphylococci* grown in culture were prepared. One end was designated as the "test," while the other was designated as the "control." The use of the control suspension allowed the researchers to rule out the possibility of a false positive caused by auto agglutination. The test

suspension was given a single drop of citrated plasma, which was then thoroughly mixed in. Agglutination, or the coming together of the cocci in clusters, within 5-10 seconds was considered positive (Brown *et al.,* 2005).

Tube coagulase test

Three test tubes were labeled with the words "test," "negative control," and "positive control." Each tube received 0.5 ml of 1:10 diluted horse plasma. 0.1 ml of overnight broth culture of a test isolate was added to the tube labeled test. 0.1 ml of overnight broth culture of known *S. aureus* was added to the tube labeled positive control, and 0.1 ml of sterile broth was added to the tube labeled negative control. All of the tubes were incubated at 37°C for 4 to 24 hours. Gelling of the plasma indicated a positive result, as it remained in place even after inverting the test tube (Ryan and Ray, 2004).

3.8 Statistical Analysis

The data were recorded and sorted in Microsoft Excel 2007. The mean of different variables were compared by using STATA 2014

Chapter IV: Results

The results of nutritional and microbiological analysis of different brands of powdered milk in Bangladesh, obtained through the investigation are presented below through tables.

Table 1 shows that the commercial powdered milks are found in the nearby super shops and local markets-

Table 1. Types of powdered milk

No	Commercial powdered milk
1	A
2	B
3	C
4	D
5	E

[A= Dano, B= Marks, C= Diploma, D= Aarong , E= Pran]

4.1 Chemical properties of milk

4.1.1 Fat content

Table 2. Fat content of powdered milk

Treatment	Brands					P value
	1	2	3	4	5	
1	33.33±1.52	32.66±1.52	33.5±2.5	33.5±1.5	31.5±.86	0.79
2	32.33±1.52	33.66±3.05	33±1	33.33±2.51	32.83±1.60	0.18
3	35.67±3.05	33.4±2.62	33±2	34.56±2.38	35.33±3.51	0.54

Fat of milk powder obtained from Brands 1, 2, 3, and 4 for Treatment 1 was 33.33 ± 1.52, 32.66 ± 1.52, 33.5 ± 2.5, 33.5 ± 1.5, and 31.5 ± 0.86 g/100g, respectively, and the difference was not statistically significant. For Treatment 2, the fat content of milk powder from Brands 1, 2, 3, 4 and 5 was 32.33 ± 1.5, 33.66 ± 3.05, 33±1, 33.33 ± 2.51 and 32.83 ± 1.60, respectively; however, the difference was not statistically significant. For Treatment 3, the fat content of milk powder from Brands 1, 2, 3, 4 and 5 was 35.67

± 3.05, 33.4 ± 2.62, 33 ± 2, 34.56 ± 2.38, and 35.33 ± 3.51 g/100g, respectively, with no statistically significant differences.

4.1.2 Protein content

Table 3. Protein content of powdered milk

Treatment	Brands					P value
	1	2	3	4	5	
1	22.13±0.23	22.14±0.23	22.25±0.36	22.56±0.68	21.91±0.36	1.02
2	22.11±0.22	22.14±0.26	22.12±0.22	22.55±0.21	22.31±0.26	1.88
3	22.33±0.15	22.31±0.15	22.00±0.58	22.24±0.76	22.22±0.30	0.24

Protein content for Treatment 1 was 22.13 ± 0.23, 22.14 ± 0.23, 22.25 ± 0.36, 22.56 ± 0.68, 21.91 ± 0.36 g/100g, respectively, with no statistically significant variation across the brands. For Treatment 2, the protein content of milk powder from Brands 1, 2, 3, 4, and 5 was 22.11 ± 0.22, 22.14 ± 0.26, 22.12 ± 0.22, 22.55 ± 0.21, and 22.31 ± 0.26 g/100 g, respectively. However, the difference was not statistically significant. Protein content of milk powder from Brands 1, 2, 3, 4, and 5 for Treatment 3 was 22.33 ± 0.15, 22.31 ± 0.15, 22.00 ± 0.58, 22.24 ± 0.76, 22.22 ± 0.30 g/100 g, respectively; nevertheless, the difference was not statistically significant.

4.1.3 Ash content

Table 4. Ash content of powdered milk

Treatment	Brands					P value
	1	2	3	4	5	
1	5.49±0.09	5.52±0.04	5.52±0.04	5.45±0.08	5.26±0.07	6.87
2	5.46±0.11	5.22±0.31	5.40±0.04	5.46±0.12	5.46±0.12	1.06
3	5.46±0.60	5.44±0.10	5.39±0.03	5.46±0.07	5.26±0.07	4.04

For Treatment 1, the ash of milk powder from Brands 1, 2, 3, 4, and 5 was 5.49 ± 0.09, 5.52 ±0.04, 5.52 ± 0.08, 5.45 ± 0.08, and 5.26 ± 0.07 g/100g, respectively, with no statistically significant difference between the values. Ash of milk powder from Brands 1, 2, 3, 4, and 5 was obtained for Treatment 2 at 5.46 ± 0.11 g/100 g, 5.22 ± 0.31 g/100 g, 5.40 ± 0.04 g/100 g, 5.46 ± 0.12 g/100 g and 5.46 ± 0.12 g/100 g, respectively; the differences were not statistically significant. For treatment 3, the ash of milk powder from Brands 1, 2, 3, 4, and 5 was 5.46 ± 0.60, 5.44 ± 0.10, 5.39 ± 0.03, and 5.46 ± 0.07, 5.26 ± 0.07 g/100 g, respectively; the differences between these values were not statistically significant.

4.1.4 Moisture level

Table 5. Moisture content of powdered milk

Treatment	Brands					P value
	1	2	3	4	5	
1	3.3±0.26	3.21±0.38	3.54±0.24	4.14±0.06	3.41±0.35	4.99
2	3.16±0.21	3.2±0.4	3.52±0.22	4.08±0.30	3.62±0.16	5.59
3	3.33±0.25	3.25±0.38	3.25±0.15	3.87±0.46	3.01±0.37	2.56

The moisture of milk powder from Brands 1, 2, 3, 4, and 5 for Treatment 1 was 3.3 ± 0.26, 3.21 ± 0.38, 3.54 ± 0.24, 4.14 ± 0.06, and 3.41 ± 0.35 g/100g, respectively. However, the difference was not statistically significant. For Treatment 2, the moisture of milk powder from Brands 1, 2, 3, 4, and 5 was 3.16 ± 0.21, 3.2 ± 0.4, 3.52 ± 0.22, 4.08 ± 0.30, and 3.62 ± 0.16 g/100g respectively. However, the difference was not statistically significant. The moisture of milk powder from Brands 1, 2, 3, 4, and 5 for Treatment 3 was 3.33 ± 0.25, 3.25 ± 0.38, 3.25 ± 0.15, 3.87 ± 0.46, and 3.01 ± 0.37 g/100g, respectively. The difference was not statistically significant.

4.1.5 Acidity percentage

Table 6. Acidity of powdered milk

Treatment	Brands					P value
	1	2	3	4	5	
1	0.16±0.05	0.13±0.02	0.15±0.02	0.15±0.02	0.14±0.02	0.62
2	0.15±0.02	0.13±0.02	0.14±0.03	0.15±0.03	0.14±0.03	0.02
3	0.14±0.04	0.14±0.04	0.14±0.04	0.13±0.03	0.14±0.01	0.02

There was no statistically significant difference in the values for the acidity of milk powder from Brands 1, 2, 3, 4, and 5 for Treatment 1. The values were 0.16 ± 0.05, 0.13 ± 0.02, 0.15 ± 0.02, 0.15 ± 0.02 and 0.14 ± 0.02 respectively. Acidity of milk powder produced from Brands 1, 2, 3, 4, and 5 for Treatment 2 was 0.15 ± 0.02, 0.13 ± 0.02, 0.14 ± 0.03, 0.15 ± 0.03, and 0.14 ± 0.03 correspondingly, with a statistically significant difference between the two. For Treatment 3, the acidity of milk powder from Brands 1, 2, 3, 4 and 5 was 0.14 ± 0.04, 0.14 ± 0.04, 0.14 ± 0.04, 0.13 ± 0.03, 0.14 ± 0.01 g/100 g respectively, with a statistically significant difference.

4.2 Microbiological analysis

4.2.1 Total Viable Bacterial Count (TVC)

Table 7 reveals that, the highest TVC was 5.8×10^4 CFU/ml in P2 in case of powder milk sample and the lowest was 3.3×10^3 CFU/ml found in case of Da 1 in the powdered milk samples.

4.2.2 Escherichia coli

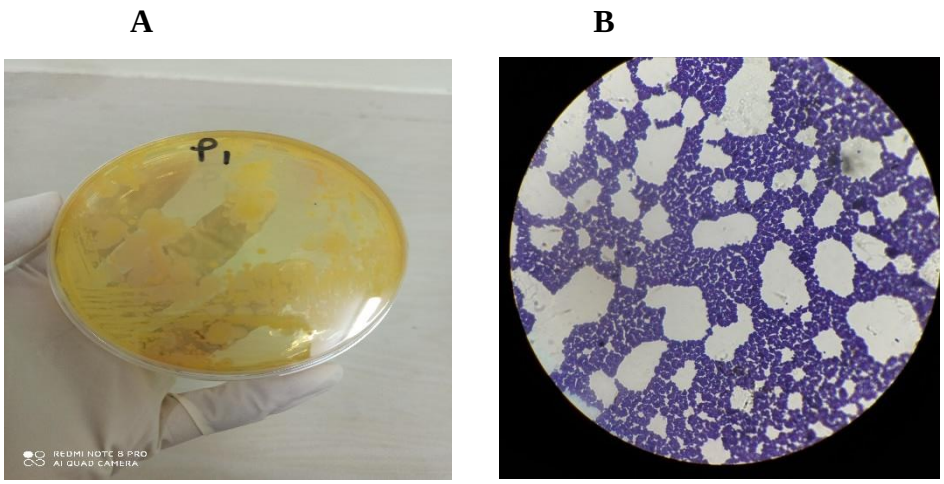
None of the collected milk samples have produced characteristic colony with greenish metallic sheen on EMB agar. **Table 7** shows that all the collected milk samples were found negative for *E. coli*.

Table 7. Microbial analysis of powdered milk sample

Sample	TVC	*E. coli*	*Staphylococcus aureus*
A1	5.4×10^3	Nil	Positive
A2	4.3×10^3	Nil	Negative
A3	3.8×10^4	Nil	Positive
P1	8.2×10^3	Nil	Positive
P2	7.8×10^4	Nil	Negative
P3	5.8×10^4	Nil	Positive
Da 1	3.3×10^3	Nil	Negative
Da 2	4.2×10^3	Nil	Negative
Da 3	6.3×10^3	Nil	Negative
Di 1	5.4×10^3	Nil	Negative
Di 2	7.2×10^3	Nil	Negative
Di 3	9.5×10^3	Nil	Negative
M 1	8.4×10^3	Nil	Negative
M 2	6.7×10^3	Nil	Negative
M 3	4.7×10^3	Nil	Negative

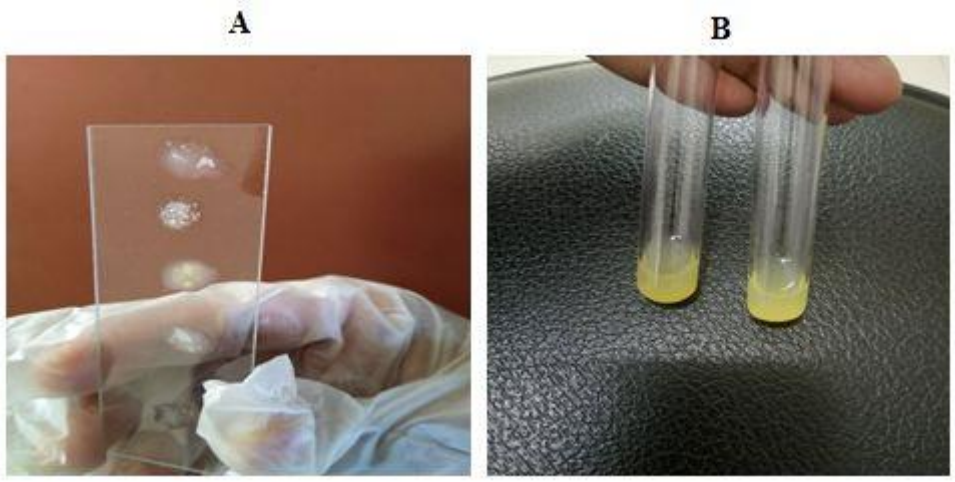
4.2.3 Staphylococcus aureus

From all of the 15 collected milk samples 4 samples were found positive for *Staphylococcus aureus*. *S. aureus* was confirmed by observing the cultural and morphological characteristics of the isolates grown on specific culture media (**Figure 8**). Among 4 positive isolates of *S. aureus*, 1 sample was found positive for coagulase test (**Figure 9**).



(A) Golden yellow colored colony of *S. aureus* on Mannitol Salt Agar, (B) *S aureus* under microscope.

Figure 8 : Isolation and identification of *S. aureus*



(A) Formation of bubble in catalase test, (B) Formation of clotting in tube coagulase.

Figure 9 : Biochemical tests for *S. aureus*

Chapter V: Discussion

5.1 Nutritional properties of powdered milk

5.1.1 Fat

It is the primary and most important flavoring agent in milk and milk powder. Full cream milk powder should have a percentage range of 26-42%, while skimmed milk powder should have a percentage range of 1.5- 2.5%. (Pugliese *et al*., 2017). It plays a significant role in reconstituted powdered milk and related hazards during powder drying.

The current study estimated fat percentage in powder milk samples ranged from (31.5 ± 0.86) to (35.67 ± 3.05), which is significantly different from the findings of Pijanowski *et a.,l* (1975), who discovered that the average fat percentage of whole milk powder was 25.4%. The average value of fat obtained from **A** (Treatment 3) (35.67 ± 3.05) was statistically non-significantly higher and from **E** ((Treatment 1) (31.5 ± 0.86) was lower than the fat of milk powder of the other three samples (**Table 2**). According to the BDS 860: 2020, the typical range for the amount of fat contained in dry whole milk powder is between 26 and 42 percent. The current investigation fell into the same ballpark as the guidelines outlined in BDS 860: 2020. (Kajal *et al.,* 2012) conducted a study in which it was observed that the fat content of **C** was 27.26 ± 0.95%. The results of our investigation showed that the fat content of **C** was 33.5 ± 2.5, 33 ± 1, and 33 ± 2 for the three treatments. Our findings differ from those of (Kajal *et al.,* 2012). Fat content in dry whole milk powder must meet or exceed the BSTI standard of 26% on average. A, B, C, D and E all listed different fat percentages on their packaging, ranging from (not indicated) to 26-28%. The variances may be the result of changes in the packaging, improper storage conditions, or different methods of calculating the fat percentage.

5.1.2 Protein

According to our findings, the protein content of the powdered milk falls between (21.91 ± 0.36) and (22.56 ± 0.68). (**Table 3**). Within the protein of several varieties of milk powder obtained from the local market, statistically non-significant differences were discovered (**Table 3**). In this study, **E** (treatment 1) has a lesser protein content of (21.91 ± 0.36), while **D** has a greater protein content of (22.56 ± 0.68). The average protein level of dried whole milk is 25.0-25.4%, according to Simova and Ruzickova

(1979). Our findings show that the protein content of powder milk is close to these levels. According to the ADMI, the average protein concentration of dry whole milk is 26.4 g/1OOg (1962). This study was nearly identical to ADMI. Dry whole milk contains 27.20% protein. (Eckles *et al*., 1951). Our findings contradict these assertions**.** The protein composition of the A, B, C, D, E packing forms was (not indicated) 22 gm, 26 gm, 24 gm, (25-27) gm, (34-36) gm. However, in our research, we discovered some departure from the previously indicated numbers (**Table 3**). For example, **B**s' protein content was listed on the packaging as 26gm/100 grams, while in our analysis it was (22.14 ± 0.23), (22.14 ± 0.26), and (22.31 ± 0.14) for treatments 1, 2, 3.

5.1.3 Ash

This study discovered that the average Ash content in powder milk samples ranged from (5.22 ± 0.31) to (5.52 ± 0.05). In this study, the lower ash content is in **B** from treatment 2, with an ash concentration of (5.22 ± 0.31), whereas **C** has a higher ash concentration of (5.52 ± 0.05). The ash of various types of milk powder collected from a local market showed statistically insignificant variations (**Table 4**). The ash content of the powder samples was found to be lower than the Sudanese (SSMO, 1999) standard of 7.3% and the USA standard of 6.0% (**Table 4**) (FDSPM, 2003).

5.1.4 Moisture

Because increased moisture would create a humid area that is suitable for the growth of molds or some anaerobic bacteria, the moisture percentage in milk powder should not exceed 5% (Gasmalla *et al*., 2013). This is because the percentage of moisture in milk powder should not exceed 5%. Again, a low moisture content would indicate that the manufacturing process may have involved overdrying, which would have the effect of diminishing the flavor of the final product.

According to the findings of our data analysis, the moisture content of powdered milk typically falls somewhere in the range of (3.01 ± 0.37) to (4.14 ± 0.06) g/100g on average (Table 5). In this investigation, the **E** sample had a moisture content that was significantly lower than that of the **D** sample (3.01 ± 0.37 g/100g for treatment 3), while the moisture content of the **D** sample was significantly higher (4.14 ± 0.06 g/100g for treatment 4). We found the moisture content of **C** to be (3.54 ± 0.24), (3.52 ± 0.22), and (3.25 ± 0.15), all of which are in stark contrast to the findings of the study conducted

by Kajal *et al.,* 2012, in which they discovered that the average moisture content in **C** was (4.49 ± 1.04%). Our findings on the moisture content of **C** are much more accurate. It was discovered that the moisture content of the various kinds of milk powder that were purchased from the neighborhood market did not differ significantly from one another (**Table 5**). The BDS 860:2020 report indicates that the typical amount of moisture present in dried whole milk powder is 5.0. The current investigation fell into the same ballpark as the standards outlined in BDS 860: 2020. According to the ADMI (1971), the amount of moisture that whole milk powder contains falls somewhere between two and five percent. The findings of the current study were in agreement with the ADMI standard's range. On the contrary, the BSTI estimates that the amount of moisture that can be present in whole milk powder is no more than 4%. Therefore, the findings of our study support the statement (**Table 5**).

5.1.5 Acidity

Acidity means a solution contains hydrogen ions. Acidity percentage is the amount of hydrogen ions in 100 ml of solution. Normal powdered milk acidity is 0.17%; deviations indicate improper processing (Priyanka *et al.,* 2022).

Our study found an acidity average of (0.13 ± 0.02 to 0.16 ± 0.05) **(Table 6)**. It was found that there were statistically significant differences between the brands in treatment 3. In comparison to the acidity of the other five samples of powder milk, the average value obtained from **C** (0.16 ± 0.05) for treatment 1 was significantly higher, while the average value obtained from marks (0.13 ± 0.01) was significantly lower for treatments 1 and 2 **(Table 6)**. Dry whole milk powders should have an acidity level of (0.15%), as suggested by BSTI and ADMI (1971). According to the BSTI and ADMI benchmarks, the accuracy of the current study was satisfactory. It was reported by Judkins and Keener (1960) that the typical acidity of commercial milk is between 0.08 and 0.23%. The acidity of regular milk samples is typically between 0.10 and 0.18 percent, with an average of 0.16 percent. The acidity of regular milk samples, which we know to be between 0.10 and 0.18 percent, with an average of 0.16 percent (Eckles, 1951), is very close to the acidity percentage of milk powder samples collected during the experiment.

5.2 Microbiological analysis

5.2.1 Total Viable Bacterial Count (TVC)

The total viable bacterial count in a sample is the number of bacteria that can grow and form countable colonies on nutrient agar medium (i.e., Plate Count Agar) after an overnight incubation at 37°C. In this study, the bacterial load in all powdered milk samples ranged from (7.8×10^4 to $3.3\times10^{3)}$ CFU/ml **(Table 7)**. Powdered milk should contain less than 3.0×10^4 CFU per gram, according to BDS 860: 2020. The maximum bacterial count in dried whole milk is 31,000/g (American Dry Milk Institute, 1971). The bacterial count of dried whole milk is limited to 20,000/g, according to BSTI. Except for **D** for treatment 3 and **E** for treatment 2 and treatment 3, the bacterial population in all samples was below the maximum range of the BSTI standard **(Table 7)**. Arora (1989) observed that the bacteria count in spray dried skim milk ranged from 2000 to 160,000/g. Powder milk's total viable count ranged from 200,000 to 4,30,000/g (Rahman *et al.,* 1988).

5.2.3 *Escherichia coli*

Commonly, the presence of coliform bacteria, such as *E. coli*, in milk is indicative of fecal contamination. In this study, it was determined that none of the collected milk samples contained E. coli. This result is highly compatible with BDS criteria (BDS, 2000).

5.2.4 *Staphylococcus aureus*

In this study, the results indicated that 4 milk samples among 15 samples were found to be positive for *S. aureus*. Among 4 positive samples, 1 sample was found positive for coagulase test. Several studies have been conducted to assess the level of contamination of milk with S. aureus obtained from communal and commercial farms. This higher staphylococcal count in milk samples may be the result of ineffective management and improper hygienic practices carried out during farming, milking, transportation, processing, and preparation of the samples (Zakary *et al*., 2011).

Chapter VI- Conclusion

Milk is one of the advantageous foods that can be digested and absorbed into the human system with relative ease. It is made up of all of the necessary nutrients that are required for the body's healthy development and upkeep. Because of this, milk is an excellent food option for people of all ages, but especially for infants. Milk's quality could suffer as a result of ruthless business practices, poor hygiene during production, and careless handling and storage. Powdered milk samples from various supermarkets were tested in this study. The nutritional quality (fat, protein, ash, moisture, and ash) of five brands was similar. Acidity varied significantly according to statistical analysis. This result indicated that all milk powder companies studied adhered to the legal standard for powder milk composition. The majority of the powder milk samples collected from various Chattogram supermarkets have satisfactory keeping quality and microbiological quality. However, *Staphylococcus aureus* was found in couple of milk samples, which is not acceptable according to the BDS Standard. The presence of *S. aureus* in powder milk samples indicates that improper hygienic measures were used during production. To ensure milk quality, advanced methods of analysis and monitoring milk production and processing are required, as is a greater emphasis on regulatory aspects. Based on the parameters examined, this study concluded that all of the milk samples available in the selected areas were of acceptable quality. However, more research is needed on trace elements, metals, antibiotics, shelf stability, and so on.

Chapter VII- Limitations and Recommendations

Limitations:

1. Research fund was inadequate.
2. Lab facility should be more improved.
3. Specific minerals present in the ash content were not determined for insufficient fund and facility.
4. Sample size of this investigation was not representative to the population due to short period of the study

Suggestions for future research work:

From the present study, the following suggestions can be made for future work-

1. Future research should include a larger number of samples and a more detailed examination of the milk.
2. More research is needed to determine the chemical (adulteration) and microbiological quality of all types of milk sold in retail stores.
3. Prior to the distribution of raw and reconstituted milk, proper heat treatment and the use of potable water must be guaranteed.
4. Future research on this will add a new dimension to controlling food-borne diseases and reducing public health risks in Bangladesh.

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