CHAPTER 1: INTRODUCTION

Common people's eating habits are evolving. They favor tastier, healthier dishes made with fresh raw milk. Because milk products are so delicious and have high nutritional content, they make up a greater share of the everyday diets of modern, developed nations. Fresh milk and dairy products consumed by each individual are one of the most / vital indicators of the global standard of living. A dairy product called sweetened condensed milk is made by adding sugar to skim or whole milk and letting some of the water evaporate. This product, which can be eaten as a dessert, is made by heating it and adding sweeteners such as sucrose (Silva *et al*., 2020). Sweetened condensed milk, a densely sweet product, holds significant importance as a staple food item for humans due to its essential nutrient content crucial for growth and proper nourishment. This dairy product, condensed milk, has been steadily gaining popularity in Bangladesh. However, unlike the rapid growth seen in the ice- cream industry, the condensed milk industry has not experienced a similar level of development (Asaduzzaman *et al.,* 2007). Condensed milk that has been sweetened is a milk product that has had some of the milk's natural components extracted, either by the incorporation of a sweetener or via a different procedure that maintains the milk product with the same qualities. To fulfill the component requirements of this typical, milk's fat and possibly the level of protein may be considered altered, but only if milk components were added or removed without changing the modified milk's whey proteins-to-casein ratio. Condensed milk products have a long shelf life and they can be kept for several months with refrigeration even at tropical temperature (Karaman *et al.,* 2014).

Generally, milk is condensed by evaporation of some of the water in whole milk, skim milk, sugared skim milk, and sugar-free skim milk. The evaporator works by heating the milk slightly over the boiling point, to a temperature equal to the condensed substance from the water vapors, and creating a space in the evaporator chamber. The vapors in the evaporator are also condensed in a condenser. Whole cream-flavored condensed milk is typically referred to as condensed milk, but whole cream without sugar condensed milk is typically referred to as evaporated milk.

Sweetened condensed milk is a dairy item created by partially evaporating the

combination of milk and sugar. It may be found in many different products, including baked products, desserts, and beverages. Sweetened condensed milk is cheap to store and transport and it has a good nutritional content and long shelf life. Changes in flavor and color occurs when Sweetened condensed milk is heated and stored (Sing, 2004). Sweetened condensed milk differs from other dairy products in that it contains high amounts of two reducing carbohydrates lactose and sucrose. The usage of sugar substitutes in various food products has been the topic of several research articles in recent years to answer the requirement for low-calorie meals. Condensed milk sweetened with brown sugar that contains at least 8.0% milk fat and 28% total milk solids, water, and a nutritious sweetener made of sucrose, high ash, and fresh brown sugar solids syrup (Williams, 1982). The country took a while to recognize the significance of milk and milk products for both individual health benefits and economic growth. The nation's cows provided enough milk for consumers the year before. But in the last several years, the number has significantly changed. The administration is committed to bringing in sizable amounts of milk products from outside, yet there is uncertainty regarding whether these products might be adulterated by unscrupulous manufacturers. Sweetened condensed milk is a value-added milk product with extended shelf life, high levels of nutrition and economic advantages of reduced space required for storage and transportation (Fang *et al.*, 2023).

However, the preservation of this product against deterioration caused by microorganisms is well understood. The pressure of water is to a point where most bacteria are inhibited. Furthermore, the additional carbohydrates absorb water, which is akin to drying out and makes it unavailable for the process of metabolism. The higher milk solids content also successfully raises the pressure of osmosis and restricts the amount of water that is readily available (Rogers *et al.*, 1920). Despite being a widely consumed nutritious food in our country, there is a significant dearth of information available to keep an eye on the sanitary standards and nutritious content of condensed milk that has been sweetened and is made by different enterprises inside our boundaries (Asaduzzaman *et al.*, 2007).

In contrast, a lot of research has been done in nations that are both industrialized and developing to evaluate product quality under diverse environmental conditions. Therefore, further study in this area is still necessary to determine the standard of condensed milk with added sugar in our country and establish guidelines for

manufacturing high-quality products. In Bangladesh, very few or no studies were done on this topic, considering its importance to the health of the public. Thus, the current study aimed to evaluate the quality of Bangladeshi sweetened condensed milk and focused on the following objectives-

1. To assess the chemical and microbial characteristics of condensed milk produced and sold in Bangladesh.
2. To compare the quality of different brands of condensed milk with BSTI standards.

CHAPTER 2: REVIEW OF LITERATURE

To evaluate the chemical and microbial characteristics of condensed milk and compare them with BSTI standards the following literature review were done. This chapter contains definition of condensed milk, types and manufacture process of condensed milk. This chapter also contains the quality of condensed milk in terms of sensory, chemical and microbial evaluation. In this chapter the preserving properties of sugar in condensed milk is also described.

2.1 Introduction

As defined by the FAO Code of Principles, The process of heat-treating and concentrating whole or skim milk to partially remove the water yields condensed milk. While condensed milk is available unsweetened or sweetened, most are sweetened. A dairy product called sweetened condensed milk is made through the addition of sweetener to skimmed or whole cow milk and letting part of the water evaporate. This product, which may be eaten as a dessert, is made by heating and cooling it and adding sweets such sucrose (Silva *et al.,* 2020). In terms of nutrition, Sweetened condensed milk contains significant quantities of nutrients, sugars, and proteins. It also has consistency, an unusual taste, and an even brown color (Silva *et al*., 2020). Changes in flavor and color occurs when Sweetened condensed milk is heated and stored (Sing, 2004). Viscosity is another physicochemical characteristic of condensed milk that develops when sweetener is added. Sweetened condensed milk was made by pasteurization of milk at 95-96°C, with addition of 75% sugar syrup (Ilyukhina, 1970). An important occurrence in the process of producing Sweetened condensed milk is the crystallization of lactose. Aqueous alpha lactose has the ability to crystallize under the temperature and circumstances that are most favorable for the production of condensed milk. Viscosity in the Sweetened condensed milk and the presence of colloidal compounds affect how quickly lactose crystals form (Jouki *et al.,* 2021).

Sweetened condensed milk thickens deserts and sweets and gives them an ice-cream texture. It is also used to flavor baked goods, custards, pastries, pies, and beverages. The food and beverage sector has reacted to consumer demand for inexpensive in calories products by making considerable strides in recent years in researching the

effects of sucrose replacements on a variety of food items (Mariotti & Alamprese, 2012).

Sweetened condensed milks are dairy goods derived from milk which have had a portion of its water content removed and sugar added, but they can be made in any way that yields a product with identical components and attributes. The addition as well as removal of milk components in a way that does not change the milk's whey protein to casein ratio is the sole option to modify the fat as well as protein content of the milk in order to meet the compositional criteria in Section 3 of this Standard. In Bangladesh, very few or no studies were done on this topic, despite its importance for public health.

2.2 Types of Condensed milk

Condensed milk can be categorized as PFA (1976), the various milk concentrates are as follows:

2.2.1 Unsweetened Condensed Milk

It is an item that comes from normal milk that has had some of the water eliminated or from a combination of cow or buffalo milk. Up to 0.3% of the final product's weight may consist of calcium chloride, sodium citrate, sodium salts of orthophosphoric acid, and polyphosphoric acid. These alterations do not have to be listed on the label. A minimum percentage of 26% milk solids and 8.0 percent milk fat is required for unsweetened condensed milk.

2.2.2 Sweetened Condensed milk

It is an item produced by partially eliminating the liquid and adding cane sugar into either cow or buffalo milk, or their mixture, or typical milk. Up to 0.3% of the end result's weight may be comprised of pure lactose, calcium chloride, citric acid sodium citrate, sodium salts of orthophosphoric acid, and polyphosphoric acid. These additions do not have to be listed on the label. At least 9.0 percent milk fat, at least 31% milk solids, and at least 40.0 percent cane sugar must be included in sweetened milk concentrate.

2.2.3 Unsweetened condensed skim milk

It is an item produced by partially eliminating liquid milk from cows, buffaloes, or a mixture of them. Up to 0.3% of the finished product's weight can be composed of calcium chloride, sodium citrate, sodium salts of orthophosphoric acid, and polyphosphoric acid. The alterations do not have to be listed on the label. There must

be a minimum percentage of 20.0 percent of unsweetened condensed milk in the whole amount. The maximum amount of fat permitted per weight is 0.5%.

2.2.4 Sweetened condensed skim milk

It is an item produced by partially eliminating the liquid from cow or buffalo milk and mixing sugar from cane to the mixture. Up to 0.3% of the finished item's weight may be composed of pure lactose, calcium chloride, citric acid, sodium citrate, sodium salts of orthophosphoric acid, and polyphosphoric acid. These additions do not have to be listed on the label. The sugar content of cane must comprise at least 40% and the solids composition of sweetened nonfat condensed milk has to be at least 26.0%. The maximum quantity of lipid allowed per weight is 0.5%.

2.3 Process of Manufacture Condensed milk

After thermal processing, sugar is included in the milk at a temperature between 265°F and 310°F. The temperature is not so hot that the product browns, but hot enough that the characteristic of excess viscosity is removed. The sugar is then added in large quantities without any order. The sugar solution is very close to the saturation point, and the milk is vacuum evaporated to concentrate it. The process of making Sweetened Condensed Milk brings the total Solids (including Sugar) of the final product from about 70% to about 75%. The process is shown below

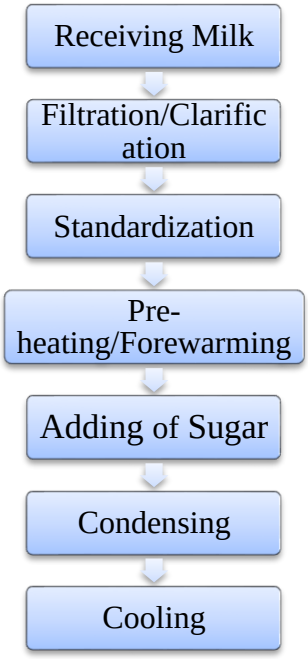

Figure 1 Manufacture of condensed milk.

2.4 Quality Evaluation of Condensed milk

It is widely accepted that milk and its derivatives are regarded as the most important nutrients for humans. Because they provide an exceptionally balanced combination of easily absorbed fats, carbohydrates, proteins, minerals, fluids, and vitamins, all of which are essential for human growth and proper nutrition. As stated by the United States Department of Agriculture, sweetened condensed milk is obtained by mixing sucrose or glucose to milk and permitting a significant amount of the water evaporate. This product comprises no fewer than of 8.5 per cent fat from milk and 28% milk solids (MS) (Lampert, 1970).

2.4.1 Sensory Evaluation

Defined attributes for condensed milk sensory evaluation are scent, color, brightness, consistency, structure, flour texture, sugar content, and cooked taste (Renhe *et al.*, 2017). Physical score includes flavor, body and texture, color, and taste (Asaduzzaman *et al.,* 2008). According to (Rehne *et al*., 2017Inadequate lactose crystallization, contamination from bacteria, heating intensity, and milk quality are all connected with sensory problems in Sweetened condensed milk. Two distinctions between proper plants operations can lead to rancidity in Sweetened condensed milk, as stated by Rice (1926): 1) Porous connections which enable a tiny quantity of icy milk to drip into the heater while it's in operation; 2) negligent high heating, that prohibits every drop of milk from reaching the appropriate temperature necessary to destroy the lipase. Sensory evaluations for flavor in skimmed milk, skimmed milk condensed milk, and whole milk yielded scores of 18.23±0.33, 16.13±0.28, and 27.83±0.13 out of a total score of 30, respectively. Condensed milk (with a score of 23.27±0.26) exhibited superior body/structure compared to whole milk (14.07±0.29) and skimmed milk (11.97±0.13) out of a possible 25 points. Regarding appearance, non-fat condensed milk (scoring 8.83±0.14) demonstrated better results compared to whole milk (6.13±0.19) and skim milk (4.84±0.14) out of a total of 10 points. The process of skimming significantly affected both the chemical and sensory attributes of the milk (Hassan *et al.,* 2015).

In order to provide standards and ensure consistent, high-quality products, the American Dairy Science Association (ADSA) suggested scorecards for condensed milk and evaporated milk.

Table 1 Scorecard for condensed milk.

Items	Full Marks
Flavor and odor	30
Body and texture	25
Color	05
Fat content	10
Solids	10
Bacteria	10
Sugar	10

Source American Dairy Science Association (ADSA)

2.4.2 Quality in terms of chemical compositions

The moisture content in skimmed milk stood notably higher (88.90±0.15%) compared to skim Full-fat milk (84.18±0.34%) and condensed milk (75.80±0.55%). The variations in the water content is attributed to the skimming method, effectively segregated the milk's fat content and raised the moisture content as a result. Conversely, the condensation process led to a reduction in moisture content. The medium fat dry matter content was initially recorded at 41.40±0.69%, which decreased substantially to 2.14±0.12% following the condensation procedure. Although this process enhanced the average fat content of skimmed milk to 2.42±0.19%, the statistical differences were insignificant ($P > 0.05$). Analyzing the ash content, buffalo whole milk exhibited an average ash content of 5.09±0.09% on a dry matter basis (DMB), rising to 7.45±0.09% DMB when the cream was dispersed. In the case of condensed milk, there was a significant raise in ash content from 7.45±0.09% to 8.69±0.21%. The titratable acidity of lactic acid was relatively higher in condensed milk (0.21±0.003%) compared to skimmed milk (0.19±0.002%) and whole milk (0.16±0.003%), albeit within normal range. Moreover, specific gravity measurements revealed that whole milk had an average of 1.031±0.003. Upon segmentation of the cream from whole milk, the specific gravity increased to 1.035±0.0002 in skim milk. Skimmed milk condensing significantly elevated the

specific gravity is greater in the finished product in contrast to whole and skim milk (Hassan *et al.,* 2015).

The Sweetened condensed milk sample's total moisture, protein, fat, and dry content were 27.6%, 8.43, 72.4%, and 8.43 percent, correspondingly. Other investigations on the proportion of chemical substances in Sweetened condensed milk have revealed identical findings (Renhe *et al*., 2018). As reported by Ahmed *et al* (2019), the samples of sweetened condensed milk had contents of 19.9, 8, 7.95, 80.1%, and fat, protein, and moisture. The titratable acidity of sweetened condensed milk was 0.146 to 0.155% (Asaduzzaman *et al*., 2008). In the sweetened condensed milk samples under examination, the average measurements for total solids, fat, protein, sucrose contents, and sugar/water ratio were 29±0.31, 8.5±1.3, 7.9±1.1, 43±0.32, and 61.91±0.35, accordingly (Ghazaly *et al.,* 2016).

The Codex Alimentarius standards, widely applied in global commerce, stipulate a minimum of 25% total milk solids (TMS) and 7.5% milk fat in milk that has been evaporated (EM). Different countries have enacted legislation defining EM composition requirements. As per the conventional British norm, EM should contain 9% or lower of milk fat and 31% or higher of TMS. In contrast, 7.9% milk fat and 25.9% TMS are the lowest levels stated in the United States standard. Consequently, the range of EM concentrations is 2.0 to 2.5. The Codex permits adjustments to the protein percentage within the non-fat solids (NFS), requiring a minimum of 34%, by incorporating lactose, milk penetrates. But modifications that alter the ratio of the casein to protein from whey are not permitted, and some municipal laws still forbid protein standardized procedures. For every 100 grams of sweetened condensed milk, the composition comprises 26.5g of water, 73.5g of total solids, 8.1g of fat, 8.1g of protein, 55.7g of carbohydrates, and 1.6g of ash (Webb and Jones, 1965). Danish, No.1, Carnation, and Goalini Plus sweetened condensed milk that had fat values of 8.10±0.34, 8.00±0.32, 8.00±0.32, and 8.10±0.34, respectively. Major variations were seen in the protein levels in all the brands; Danish and Goalini Plus condensed milk had the greatest protein content (7.50±0.13), while the No. 1 brand had the lowest (6.50±0.45%) (Siddique *et al*., 2017).

The PFA (Prevention of Food Adulteration) Act 2000 standards are listed beneath, and the following table lists the Bureau of Indian Standards for various kinds of condensed milk.

Table 2 PFA Standard for condensed milk

Characteristics	**Requirement**
Total solids (% wt) min	31.0
Fat (% wt)	Not less than 9
Sucrose (% wt) min	40
Acidity (% lactic) max	0.35
Bacterial count (per g) max	500
Coliform count (per g)	Negative
Yeast and Mold count (per g) max	10

2.4.3 Quality in terms of microbial analysis

Raw milk and milk products consumption continue to be identified as risks factor for foodborne illness (Das *et al.*, 2015). In case of condensed milk chance of microbial growth is lower due to its high sugar contents and manufacture process. According to (Ghazaly *et al*., 2016) It has been discovered that sweetened condensed milk and evaporated milk contain mold and yeast. Typically, *Aspergillus* and *Penicillium* are separate genera. Other fungal genera *Aspergillus* spp were isolated with sweetened condensed milk ratios of (32%) and (44%), respectively, In the studied samples containing (87.5%) and (27.5%) condensed milk and evaporated one flavus, respectively, was the most prevalent species. In contaminating sweetened condensed milk specimens, the majority of prevalent genus among the identified yeasts was Saccharomyces (40%) followed by Rhodotorula (12%) and Candida (8%). Rhodotorula Candida and Saccharomyces were the yeasts that were identified from evaporated milk, with rates of12%, 8%, and4%, accordingly. According to Das *et al.* (2015) The Mean viable count of condensed milk was 4.3×103 cfu/g. The total Coliform and fungus count was absent in condensed milk and was acceptable in quality according to FDA standards. The total viable count ranged between

13.60±2.51 and 16.60±2.41 (per 10^2/g), and no coliform bacteria were detected in Danish, Fresh milk, Starship, and Goalini condensed milk in Bangladesh (Asaduzzaman *et al.,* 2007).

As per Siddique *et al.* (2017), no coliform bacteria were detected in any of the sweetened condensed milk brands. BSTI (2000) and Yankov (1967) also reported a zero count of coliforms in sweetened condensed milk. The presence of coliform contamination in processed foods is often linked to unhygienic production practices, encompassing inadequate factory cleanliness and post-processing exposure (Arum *et al.,* 1979). Notably, there have been no reported instances of foodborne illness outbreaks involving individuals consuming sweetened condensed milk, as indicated by ICMSF (1998) and a total of 4,444 cases. These products generally exhibit resistance to microbial growth and boast shelf stability. However, variations in bacterial presence, including coliforms, yeasts, and molds, may occur contingent upon potential contamination during concentration, cooling, crystallization, and packaging processes. Developments of molds require oxygen for survival, much like yeasts do, therefore oxygen deprivation can inhibit their growth; low storage temperatures can also have this impact. However, occasionally, visible mold colonies (diameter 1 cm and larger) will grow because milk coagulates in these areas, forming so-called "buttons." Because of the action of enzymes, these "buttons" keep growing even after the molds have stopped growing due to a shortage of oxygen. They are typically dark brown in color and have an unpleasant flavor. The genus *Aspergillus* contains the most prevalent molds (Vandenberg, 1962).

2.5 Preserving properties of sugar in Condensed milk

Theoretically, dextrose has a preserving potency that is almost twice the potency of sucrose (assuming the preserving potency is proportional to the Osmotic Pressure). However, in numerous experiments, dextrose demonstrated to be only slightly more potent than sucrose in concentrated solutions. The typical data presented in Tables 3 indicate that sucrose may have a higher preserving potency at high concentrations (RJ Ramsey *et al*., 1933)

(Table 3) the difference in bacteriological activity between dextrose & sucrose in simple broth solutions Inoculated wish large spore-forming rod separated from sweetened condensed milk.

Table 3 Preserving properties of sugar in condensed milk

Percentage of sugar	PH of Media	Inoculation	Count at the end of 14 days
40% Dextrose	6.3	10,000	Spoiled
50% Dextrose	6.2	10,000	3,00,000
65% Dextrose	6.0	10,000	1,800
40% Sucrose	6.8	10,000	Spoiled
50% Sucrose	6.7	10,000	2,00,000
65% Sucrose	6.6	10,000	42,000

Source (Ramsey *et al*., 1933)

Conclusions

Bangladesh's business for condensed milk is expanding. However, very little research has been done to find out the microbiological loads and chemical contents of condensed milk which is marketed in Bangladesh. The aim of this study is to close the present information gap about condensed milk produced in Bangladesh and to determine the condensed milk market's current state in Bangladesh. Furthermore, this research will assist in determining whether or not condensed milk manufacturers in Bangladesh follow the Bangladesh Standards and Testing Institution (BSTI) standards.

CHAPTER: 3 MATERIALS AND METHODS

Technical support from three separate laboratories was needed to experiment. The condensed milk underwent chemical analysis at the Dairy Science laboratory of Chattogram Veterinary and Animal Sciences University (CVASU) and the laboratory of Bangladesh Council of Scientific and Industrial Research (BCSIR), Chattogram. Additionally, its microbiological analysis was carried out at the Bacteriological Laboratory of Poultry Research and Training Centre (PRTC) during May to August, 2023.

3.1 Sample collection

Four commercial organizations that provide full cream sweetened condensed milk were selected to conduct this investigation- Danish, Starship, Goalini plus, and No1 condensed milk. The samples of different batches of production were collected at random from the super shops of Chattogram district. Three samples from each brand were collected at different times of the year. Total 12 samples were collected. Samples were labeled as D1, D2, and D3 for Danish condensed milk; S1, S2, and S3 for Starship condensed milk; G1, G2, and G3 for Goalini plus condensed milk and N1, N2, and N3 for No 1 condensed milk.

3.2 Chemical Analysis of Condensed Milk

3.2.1 Preparation of Sample

After 15 minutes of heating at 45 degrees Celsius in a water bath, the can of sweetene d condensed milk was shaken. After being cleaned with warm water, the cans outside were wiped and dried. After that, the can was dried and cleaned with disinfectant. The surface was perforated after being swabbed with ethanol.

3.2.2 Determination of Fat

Fat was determined by Gerber’s Method. The Madras Veterinary College Manual's Gerber's technique is used to determine the fat content in condensed milk. 25 grams of homogenous sample was taken into a clean beaker about 50 ml of distilled water was added and mixed thoroughly by stirring. The reconstituted milk had been shifted in a 100 ml volumetric flask. Then distilled water was mixed by washing the beaker several times to make it 100ml. 10 ml of Gerber’s sulphuric acid was taken into the butytometer then 11 ml of well-mixed reconstituted milk was added. And at last, 1 ml of Amylalcohol was added. The butyrometer was stopped by a stopper and it was

inverted 2-3 times to mix the content. After that, the butyrometer remained ten minutes in a water bath at 65°C. After that, it was centrifuged for five minutes at 1200 rpm. A reading from a butyrometer was obtained.

Calculation

$$\text{Fat}\ (\%)\ = \text{Observed reading} \times 100/\text{W}$$

Here,

W= Weight of condensed milk taken for analysis.

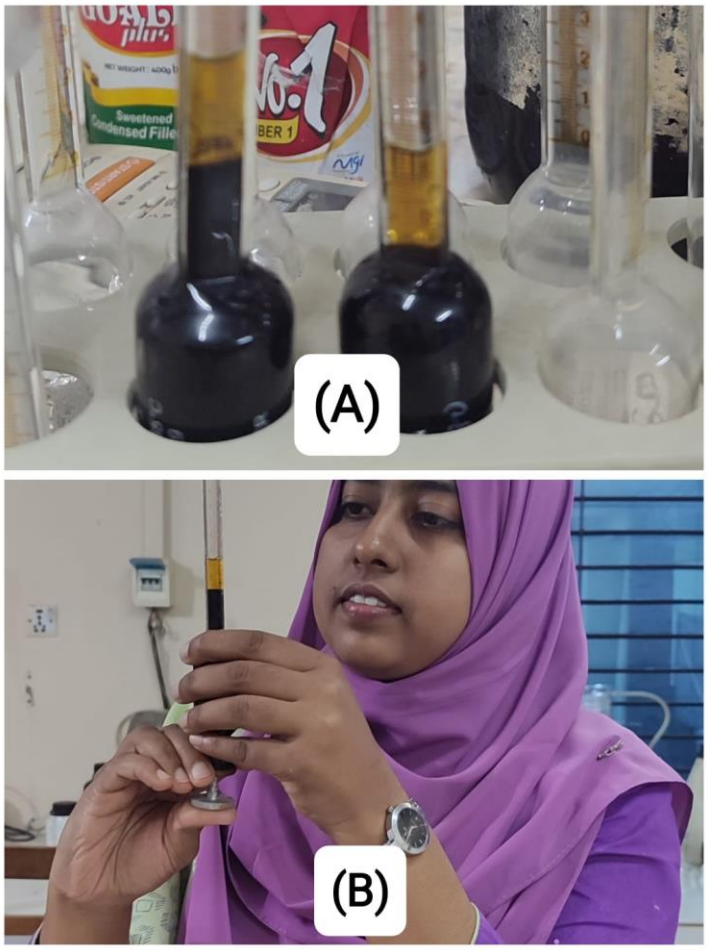

Figure 2 Determination of Fat

A: Separation of fat B: Taking a reading of fat from a butyrometer.

3.2.3 Determination of Sucrose

Sucrose is the ordinary table sugar that we eat every day. Sucrose is used in sweetened condensed milk for sweetness and also for preservation. It is a disaccharide, which on hydrolysis with dilute acids yields an equimolar mixture of units of D-glucose and D-fructose. Fehling's solution may be used for calculating the amount of reducing sugar in a solution (such as glucose, lactose, and fructose). It consists of complex cupric ions and is prepared by adding Fehling A solution which contains blue copper (II) sulfate, and Fehling B solution which contains sodium hydroxide and sodium potassium tartrate. The cupric ions are reduced to cuprous ions which cause the sugar's aldehyde group to precipitate as red copper (I) oxide, oxidizing the aldehyde to the carboxylic acid group. Depending on how many copper (II) ions are present, the final solution's color may vary from green to brick red.

Accurately about 1.30 grams of the sample of condensed milk was weighted. The sample was dissolved in 20 ml of deionized water within a tiny conical vial. 10 mL of 0.5 M HCl was introduced and then subjected to heating in a boiling water bath for duration of 20 minutes. Following this step, the solution was allowed to cool down and neutralize by adding 10 mL of 0.5 M NaOH solution and diluting it to 250 mL with boiled-out distilled water in a volumetric flask. Titration was done with 20.0 mL of the standardized Fehling's solution with the inverted sugar solution, using the same procedure as employed for the standardization of Fehling's solution.

3.2.4 Determination of Acidity

Method 920.124 of the AOAC (1995) was used to determine acidity. A sample of 10 grams of condensed milk was obtained and placed in a dry, clean porcelain dish. The porcelain dish was filled with 30 cc of distilled water (60°C to 70°C) and well stirred. To the porcelain dish, 1 milliliter of 0.5% phenolphthalein indicator was put. The mixture was titrated up against a solution of 0.1 N NaOH until a little pink color remained **(Figure 3).** The amount of NaOH needed to finish the titration was noted. The acidity percentage was determined using the following formula:

Calculation

$$Acidity\ of\ condensed\ milk\ \% = 9\ AN/M$$

Here,

A = the amount of standard sodium hydroxide in ml needed for the titration.

N = Normality of standard sodium hydroxide solution.

M = The material's weight, measured in grams, for the test.

Figure 3 Determination of Acidity

A: Titration of condensed milk; B: Faint pink color after titration.

3.2.5 Protein Determination (%)

The procedure outlined in IDF 20-1(2014) was used to determine the protein content of the condensed milk sample.

3.2.5.1 Digestion

After weighing two grams of the sample, it was transferred to the Kjeldahl digestion tube. A digestion mixture that serves as a catalyst is introduced to the digestion tube along with twenty millimeters of 98% sulfuric acid. For three hours, the digestion tube was kept in the Kjeldahl digestion unit. The substance that had been digested was let to cool at room temperature.

3.2.5.2 Distillation

50 ml of distilled water was added to dilute the digested mixture. In a conical flask, 10 ml of 4% boric acid and two drops of methyl indicator were added. Within the distillation unit a conical flask holding boric acid and a digesting tube. The distillation device automatically added 40% of the NaOH solution from the attached flask containing the solution to the diluted mixture. A conical flask was used to hold the ammonia that was liberated during the chemical reaction and distillation process.

3.2.5.3 Titration

A reading was obtained when the distilled solution was titrated against 0.1 N hydrochloride solutions. Determining the sample's nitrogen (%) is necessary before determining the protein content. The protein content was computed using the following formula:

$$\text{Protein } \% = \frac{\text{Titration value} \times \text{Normality of HCl } (0.1) \times 0.014 \times 6.25}{\text{Sample weight}(0.5\text{g})} \times 100$$

3.3 Microbiological Analysis of Condensed milk

As a part of microbial analysis, coliform count and total viable count. Yeast and Mold counts in condensed milk samples were conducted. Sweetened condensed milk is viscous due to the presence of sucrose or other sweetening agents. Before withdrawing the sample, the jar of condensed milk was placed inside a water bath set at 45°C for around fifteen minutes. After making the sample homogenous by mixing in the can 11gm of the sample was transferred aseptically in a 99 ml dilution blank the content was mixed thoroughly to make the sample 1:10 dilution.

3.3.1 Total Viable Count

The standard Plate Count (SPC) strategy suggested for dairy items (APHA 1960) was followed for quantitative investigation of microscopic organisms: enumeration of total viable count- A nutrient agar medium was utilized for the count of all reasonable microbes. pH of the medium was changed to 6.8 before sterilization. The diluted condensed milk sample was inoculated into the agar plates. The sample was then uniformly distributed over the medium by repeatedly rotating the plates in both clockwise and counterclockwise directions. Every petri plate was meticulously labeled, with duplicates taken for every plate. After being incubated for 24-48 hours at 37°C in an inverted posture, the colonies were counted (Benson, 2002). Following incubation, plates with evenly spaced colonies were chosen for counting.

3.3.2 Coliform count

The members of the coliform group of bacteria may be found in dairy products produced when handled under insanitary conditions. In this study coliform count of condensed milk was done by following process by using MacConkey's agar. Serial dilution of the sample was prepared. A sterile petriplate was inoculated with a 1 ml amount of the necessary dilutions (in duplicate). Next, each plate was provided 10-15 milliliters of previously melted MacConkey's agar that had cooled to 45°C. Everything was well combined. It was decided to let the agar harden. Over the top of the hardened medium, a second layer of three to four milliliters of medium was poured. The dishes were turned upside down and then kept for 24 hours at 37°C in an incubation chamber. After incubation for 24 hours dark red colonies measuring at least 0.5mm indicate a positive test.

3.3.3 Yeast and Mold

The presence of Yeast and Mold was detected in Potato dextrose Agar Medium. 5ml of 1:10 was transferred to duplicate petridish for plating in 1:2 dilution. pH of the potato dextrose agar was adjusted to 3.5 by adding a calculated amount of sterile tartaric acid solution at the time of pouring plate. The melted cooled agar was added then it was allowed to cool and set. The plates that underwent incubation were flipped upside down and left to incubate at a temperature of 25°C for 5 days. The colony was examined.

3.4 Statistical Analysis

One-way ANOVA (STATA version 18) was used for statistical analysis of all data stored in a Microsoft Excel 2010 spreadsheet. Significant difference at $P \leq 0.05$ was used to assess the significance of the mean difference.

CHAPTER 4: RESULTS

In this research, a comprehensive analysis was conducted on 12 samples, consisting of three distinct samples from each brand. The assessment encompassed both chemical and microbiological analysis conducted according to established Standard methods.

4.1 Chemical Analysis

Table 4 displays the chemical compounds of the condensed milk samples. The sucrose contents in sweetened condensed milk from Danish, Goalini Plus, No.1, and Starship were 40.70±0.46, 41.90±0.31, 41.70±0.26, and 41.70±0.44, respectively. Significantly differing sucrose content ($p=0.002$) was seen among the different kinds of condensed milk that had been sweetened and bought from nearby marketplaces. Significant differences were found Danish condensed milk with Goalini plus, No1 and Starship condensed milk. But no significant difference was found within Goalini plus, No1 and Starship condensed milk ($p>0.05$). The sucrose contents of all brands are lower than the BSTI standard of sucrose.

Table 4 Chemical Analysis of Condensed milk

Para-meter	Brands				P Value	BSTI Standard	Level of Sign.
	Danish	Goalini plus	No1	Starship			
Sucrose	40.20±0.46	41.83±0.31	41.70±0.26	41.70±0.44	0.002	42-47	**
Protein	7.47±0.23	7.14±0.16	6.60±0.28	7.21±0.16	0.006	No standard	**
Fat	7.73±0.61	8.53±0.23	8.00±0.40	7.73±0.23	0.12	8.0 (Min.)	NS
Acidity	0.15±0.014	0.16±0.018	0.17±0.015	0.14±0.018	0.19	0.32 (Max.)	NS

NS= Not statistically significant; **statistically significant at $p \leq 0.05$

The protein contents acquired from Danish, Goalini Plus, No. 1, and Starship, sweetened condensed milk were 7.47±0.23, 7.14±0.16, 6.60±0.26, and 7.21±0.16, respectively. Significant variations in protein content ($p=0.006$) were observed across different brands of sweetened condensed milk gathered from nearby markets. Specifically, notable differences were observed between No.1 with Danish and Starship condensed milk. However, no significant differences ($p>0.05$) were found between Danish with Goalini Plus and Starship brands, as well as between Goalini Plus with No.1 and Starship.

Sweetened condensed milk from Danish, Goalini Plus, No. 1, and Starship had fat percentages of 7.73±0.61, 8.53±0.23, 8.00±0.40, and 7.73±0.23, in that order. It was determined that there were no significant differences ($p>0.05$) in the fat percentages of the different brands of condensed milk available in Bangladesh. However, it is noteworthy that the fat contents of Danish and Starship condensed milk were slightly lower, while Goalini Plus condensed milk slightly exceeded the BSTI standard.

The acidity percentages of sweetened condensed milk obtained from Danish, Goalini Plus, No.1, and Starship were measured at 0.15±0.014, 0.16±0.018, 0.17±0.015, and 0.14±0.018, respectively. No significant differences ($p>0.05$) were observed among

the different brands of condensed milk. Moreover, all the brands' acidity percentages fall within the BSTI standard.

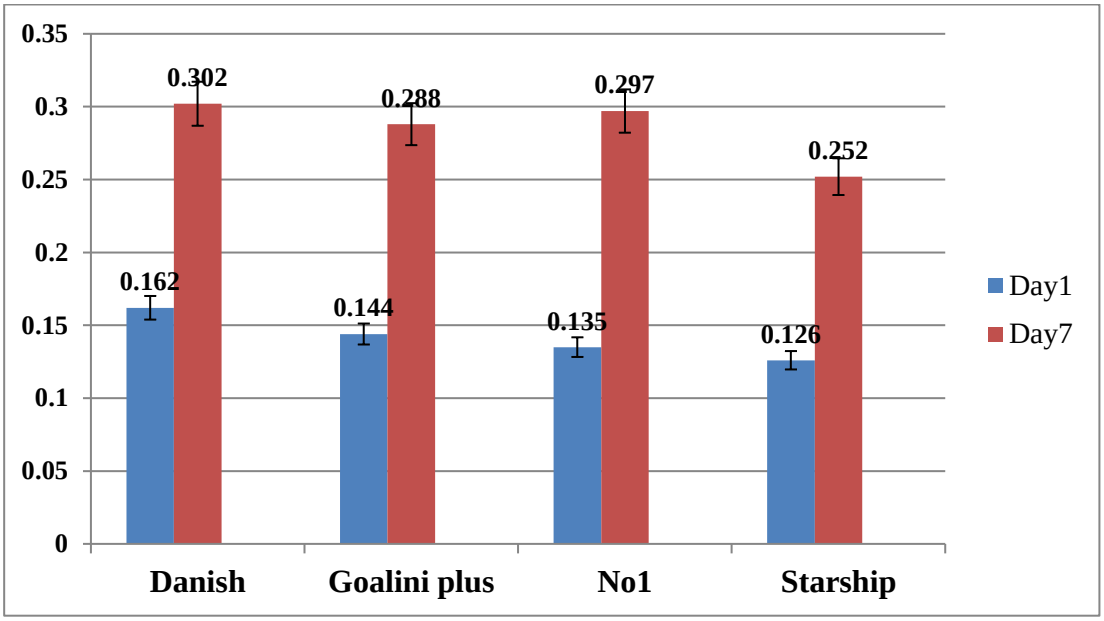

Figure 4 Acidity% on day1 and day7.

4.2 Microbial Analysis

The colony forming unit (cfu) per milliliter, or cfu/ml, was displayed as the result.

4.2.1 Total viable count

Table 5 Total viable count of condensed milk of different brands

Brands	Total Viable Count (mean±SD)	P Value	BSTI Standard	Level of Significance
Danish	5800±458			
Goalini plus	8300±557	0.002	Max. 10000	**
No1	6200±458			
Starship	6500±600			

NS= Not statistically significant; **statistically significant at p≤0.05

The total viable counts of sweetened condensed milk obtained from Danish, Goalini Plus, No.1, and Starship were recorded as 5800±458, 8300±557, 6200±458, and 6500±600, respectively. A significant variance (p=0.002) was detected among the total viable counts of many varieties of condensed milk that has been sweetened, gathered from nearby marketplaces. Specifically, the average total viable count obtained from Goalini Plus was observed to be higher than the others. Notably, there was a significant difference between Goalini Plus and the other brands, whereas no significant differences were found between the other brands individually. It's worth mentioning that the total viable counts of condensed milk from all brands fell within the BSTI standard.

4.2.2 Coliform Count of Condensed milk

No coliform bacteria were found in any of the samples. It follows the BSTI standard and also indicates the sanitary standard of condensed milk.

4.2.3 Yeast and Mold

After incubation of the samples at 25°C for a period of 5 days, no yeast and mold were found.

CHAPTER 5: DISCUSSION

In this study, a total of 12 samples from 4 brands of condensed milk were taken for analysis. Chemical (sucrose, fat, protein, acidity) and microbial (total viable count, coliform count, yeast and mold) analysis was performed based on the respective AOAC method. All the parameters were compared with BSTI standars.

5.1 Chemical Analysis

In our study, Danish, Goalini Plus, No.1, and Starship provided sweetened condensed milk whose sucrose percentages were analyzed and Significant variations (p=0.002) were noted in the sucrose percentage of the different varieties of sweetened condensed milk. Comparatively, according to Ghazaly *et al.* (2016), the sucrose content reported was higher (43±0.32) than the values found in all brands during our study. As per the BSTI (Bangladesh Standards and Testing Institution) standard, the acceptable range for sucrose in sweetened condensed milk is set at 42-47%. Interestingly, our study revealed that all the brands examined had lower sucrose contents than the specified range outlined by the BSTI standard. Sucrose contents may be lower due to the breakdown of sucrose into simple sugar or the storage conditions such as exposure to light heat or air can lead to chemical reactions that break down sucrose molecules. Condensed milk can be contaminated by microbes that ferment sugar which can convert sugar into other products.

The results of our investigation showed that the protein content of various kinds of sweetened condensed milk varied significantly. Danish had higher protein content, whereas No.1 condensed milk exhibited lower protein content, consistent with findings reported by Siddique *et al.* (2017). According to the BSTI standard, the acceptable protein level in milk solids, not fat, is set at 34. However, studies by Renhe *et al*. (2018) reported a protein percentage of 8.43%. Conversely, other research studies, such as Ghazaly *et al*. (2016) and Ahmed *et al.* (2008), indicated lower protein percentages of 7.9±1 and 7.95%, respectively. These variations might be attributed to the condensation process itself or the potential denaturation of protein due to heat treatment during manufacturing.

No noticeable variations were found in our investigation between the fat percentages of the various brands, aligning closely with the findings reported by Siddique *et al.* (2017). However, Danish had slightly lower fat content, whereas Goalini Plus

exhibited slightly higher fat content. In studies conducted by Renhe *et al*. (2018), Ghazaly *et al*. (2016), Amer and Mossalami (2006) and Ahmed *et al*. (2008), the fat contents of condensed milk were reported as 8%, 8.5±1.3, 8.54±0.12 and 8%, respectively. Comparatively, the fat percentage in Danish and Starship brands was found to be lower than the BSTI standard. It needed to be mentioned that a few companies label condensed milk that has vegetable fat instead of milk fat as "filled condensed milk".

Our study showed that there were no notable variations ($p>0.05$) across the various condensed milk brands. This aligns with the acidity levels reported by Asaduzzaman *et al.* (2008), ranging from 0.146% to 0.155%. According to the BSTI Standard, the maximum allowable acidity percent is 0.32, and the acidity values for the experimental samples fell within this acceptable range. These acidity percentages observed in all brands are consistent with the results reported by Asaduzzaman *et al*. (2008) and Siddique *et al*. (2017). Additionally, Mia (1995) found an average acidity percentage of 0.167 in sweetened condensed milk, which is slightly lower than the typical acidity content in this product. High acid levels in dairy products are often linked to inferior microbial quality, as supported by the studies of Khaleque (1983) and Rahman (1988).

5.2 Microbial Analysis

As per the BSTI Standards, the acceptable Coliform count in sweetened condensed milk should be nil. Microbial analysis confirmed the absence of coliform bacteria, meeting the quality standards outlined by the United States Food and Drug Administration (FDA). A study carried out by Asaduzzaman *et al*. (2007) and Siddique *et al.* (2017) also revealed the absence of coliform bacteria in Danish, Fresh milk, Starship, and Goalini condensed milk in Bangladesh. Moreover, based on Yankov (1967) and BSTI (2000), the prescribed count of coliforms in sweetened condensed milk is zero, further supporting the findings that no coliform bacteria were detected in any of the brands of sweetened condensed milk as observed in various studies and in compliance with regulatory standards.

Significant variations among the TVCs of several brands of sweetened condensed milk were found in our study, differing from the findings of Asaduzzaman *et al*. (2008) and Siddique *et al.* (2017). As per BSTI Standards, the maximum permissible

Colony count should be 10000 cfu/g, and the total viable counts for all brands examined fell within this acceptable limit. Despite the significant differences observed among the brands in our study, all brands maintained total viable counts that complied with the maximum permissible value set by BSTI standards.

In this study, no yeast and mold were found. According to the BSTI standard, the maximum allowable presence of Yeast or Mold is 10/ml. The absence of Yeast and Mold indicate the good sanitary condition and proper packaging of condensed milk. As per Vandenberg (1962) developments of molds require oxygen for survival, much like yeasts do, therefore oxygen deprivation can inhibit their growth.

CHAPTER 6: CONCLUSIONS

The study aimed to evaluate the level of quality of various brands of condensed milk available in Bangladesh, focusing on their chemical and microbiological characteristics. Specifically, four condensed milk brands were the primary focus of the evaluation. The findings revealed varying sucrose percentages among the brands, with Danish exhibiting the lowest sucrose content. Notably, all brands fell below the BSTI standard for sucrose levels. Coliforms were absent in all samples, while the total viable count met the recommended levels for all brands. Regarding acidity, fat, and protein content, all four brands were deemed acceptable. Considering the comprehensive analysis of these parameters, Danish and Starship emerged as superior to the other two brands, primarily due to their higher protein content and favorable acidity levels.

CHAPTER 7: LIMITATIONS

Throughout the study, a considerable number of limitations emerged. These could be taken into account in the future before starting a study-

- No molecular method was applied to determine the microorganisms' identities.
- The study's sample size was relatively small.
- No sensory evaluation for condensed milk was done.
- Microbial count after preserving the opened can of condensed milk was not done.
- Different tests for condensed milk were not possible in our lab. So different Test was done in different laboratories.
- No automated method or device was used to determine the titration endpoint during the study; instead, visual power was applied.

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