STUDY ON QUALITY EVALUATION OF CHEESES MANUFACTURED IN BANGLADESH

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Roll No.: 0120/03

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Session: January-June, 2020

Semester: January- June, 2022

A thesis submitted in the partial fulfillment of the requirements for the degree of Master of Science in Dairy Science

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Chattogram-4225, Bangladesh

June, 2022

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Sujoy Barua

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ACKNOWLEDGEMENT

The author is ever grateful and indebted to the God without whose grace he would have never been able to pursue his higher studies in this field of science and to complete his research work successfully for the degree of Master of Science (M.S.) in Dairy Science.

Firstly, the author sincerely desires to express his heart felt respect, deepest sense of gratitude, sincere appreciation, and profound indebtedness to his respected teacher and research supervisor, ***Professor Dr. Goutam Kumar Debnath***, Head, Department of Dairy and Poultry Science, Chattogram Veterinary and Animal Sciences University, Chattogram for his constant supervision, scholastic guidance, uncompromising principles, helpful advice, constant inspiration affectionate feeling, radical investigation, and constructive criticism in all phases of this study and preparing the manuscript.

The author wishes to express his cordial respect and profound gratitude to his teachers ***Professor Dr. A. K. M. Humayun Kober***, ***Dr. Md Saiful Bari*** and ***Dr. Nasima Akter*** Department of Dairy and Poultry Science, Chattogram Veterinary and Animal Sciences University, Chattogram for their valuable advice and constant inspiration through the entire period of study.

The author expresses his cordial appreciation to all of the office staffs and lab assistant of Department of Dairy and Poultry Science, Chattogram Veterinary and Animal Sciences University, Chattogram.

The author humbly expressing his thanks to ***Md. Khorshed Alam*** Senior Technical Officer of Department of Animal Science and Nutrition and ***Md Shawkat Ullah*** Senior Lab Attendant of Poultry Research and Training Centre (PRTC) laboratory for their cordial and continuous support during this work. Without their assistance in this research work would be quite impossible to finish.

The author would like to express his deep sense of gratitude and thanks to **Committee for Advanced studies and Research (CASR) of CVASU** for their support. The author privileges to acknowledge the supports of **Ministry of Science and Technology of Peoples Republic of Bangladesh** for National Science and Technology Fellowship regarding research funds and other resources for this research work.

The author expresses heartfelt gratitude and gratefulness to his beloved parents, friends and well-wishers for their blessing, inspiration, sacrifice and moral support which opened the gate and paved the way to his higher studies and also provided him with the best of everything in his life.

The Author

June 2022

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LIST OF ABBREVIATIONS

Abbreviation	Meaning
%	Percent
°C	Degree Celsius
Gm	Gram
Kg	Kilogram
ADSA	American Dairy Science Association
ANOVA	Analysis of Variance
AOAC	Association of Official Analytical Collaboration
BSTI	Bangladesh Standards and Testing Institution
Cfu	Colony Forming Unit
CLA	Conjugated Fatty Acid
CMA	Chattogram Metropolitan Area
CP	Crude Protein
CVASU	Chattogram Veterinary and Animal Sciences University
DM	Dry Matter
EFSA	European Food Safety Authority
EMB	Eosin Methylene Blue
FAO	Food and Agriculture Organization
IDF	International Dairy Federation
IS	Indiam Standard
ISO	International Organization for Standardization
MFGM	Milk Fat Globule Membrane
OECD	Organisation for Economic Co-operation and Development
PDB	Primary Dilution Blank
PRTC	Poultry Research and Training Centre
r.p.m.	Revolutions Per Minute
SD	Standard Deviation
SPC	Standard Plate Count
TCC	Total Coliform Count
TVBC	Total Viable Bacterial Count
VRB	Violet Red Bile

Abbreviation	Meaning
WHO	World Health Organization

Study on quality evaluation of cheeses manufactured in Bangladesh

ABSTRACT

Cheese is a popular fermented dairy product that is produced globally either for direct consumption or as an ingredient of other food items. Nowadays, Cheese production has converted into a large industry from a cottage industry. In 2020, the global cheese market worth stands at 100 billion dollars and cheese production has increased by about 25% between 2005 and 2015. Mainly two varieties of cheeses marketed in Bangladesh. Apart from traditional Dhaka cheese, globally familiar Mozzarella cheese is sold by a leading agro-based company. Our study evaluates the quality and sanitary condition of cheeses marketed in Bangladesh. Samples were collected from three different districts of Bangladesh. The quality of the cheese was mainly evaluated based on sensory, chemical, and microbiological parameters.

Dhaka cheese is a traditional cheese variety under the semi-hard class which is mainly produced in the Haor area of Kishoreganj district. Mozzarella cheese is a member of a pasta-filata family of Italian origin. According to the study, no significant difference was found in sensory attributes among the brands of Dhaka cheese, whereas a significant difference was observed in the finish and texture score among the brands of Mozzarella cheese. The study determined Moisture (%), Fat (%), Protein (%), Titratable Acidity (%), and Salt (%) of Dhaka cheese samples were 47.36 ± 5.52, 27.03 ± 3.35, 20.64 ± 1.50, 1.39 ± 0.56, 2.57 ± 0.43 respectively. In contrast, Moisture (%), Fat (%), Protein (%), Titratable Acidity (%), and Salt (%) of Mozzarella cheese samples were 51.03 ± 4.55, 19.27 ± 4.32, 24.73 ± 2.68, 1.12 ± 0.51, 0.78 ± 0.47 respectively. A relatively higher coliform count was reported in Dhaka cheese samples than in Mozzarella cheese samples. Four out of five brands of Dhaka cheese were reported to have coliform colony growth while only one of the five brands was containing coliform colony growth. The total coliform count of Dhaka cheese samples ranges from 1.5×10^2 cfu/g to 200×10^2 cfu/g. On the other hand, the total coliform count of Mozzarella cheese samples lies between 0.9×10^2 cfu/g to 1×10^2 cfu/g.

Keywords: Dhaka cheese, Mozzarella cheese, Chemical Analysis of cheese, Microbiological Analysis, Cheeses of Bangladesh.

CHAPTER 1: INTRODUCTION

Cheese is a product made from the curd obtained from milk by coagulating the casein with the assistance of rennet or similar enzymes in the presence of lactic acid produced by added or adventitious microorganisms, from which part of the moisture has been removed by cutting, heating, and/or pressing, which has been molded in a mould, and then ripened by keeping it for some time at proper temperature and humidity (De, 2010). Generally, a coagulating agent that is used in cheese making is rennet. Rennet can be obtained from the fourth chamber of the stomach of ruminants like calves, kids, lambs, or fawn.

Some general steps need to be followed during cheese making (McMahon and Brym, 2016). The process starts with the pasteurization of raw milk and standardization to the required casein-to-fat ratio. Then, a bacterial culture is added to the milk depending on the sort of cheese that will be manufactured. After that, Rennet is added to the milk and due to the action of the enzyme of rennet, milk is coagulated and forms a solid gel. To start the whey's expulsion from the coagulum, the coagulum is cut into pieces of `small size. The remaining steps in the manufacture of curd include mechanically churning the curd grains and heating the curd following a predetermined protocol while bacteria multiply and produce lactic acid. These three acts work together to produce syneresis (expulsion of whey from the curd). Salting can be done either before or after the cheese is pressed. The finished curd is put into cheese molds or hoops, which define the shape of the finished cheese. Occasionally, external pressure is given to the cheese. Finally, cheese is allowed to ripen if required.

More than 1000 varieties of cheese persist globally (Sandine and Elliker, 1970). Walter and Hargrove (1972) made a description of 400 varieties of cheese and prepared a list containing further 400 varieties of cheese. According to Burkhalter (1981) report 510 varieties of cheese available globally. Cheese can be classified on different criteria like- moisture content, agent, or technique used for milk coagulation and ripening indices (McSweeney *et al*., 2004). The most common method for classifying cheese is based on texture or moisture content and this classification is mainly divided into four major groups- Very hard, hard, semi-hard, and soft (Davies, 1965; Walter and Hargrove, 1972). According to Scott *et al*., (1998) cheese can be classified based on moisture into three major groups namely - hard, semi-hard, and soft. Fox (1993) classified cheese

based on the coagulating agent used: Rennet coagulated cheese, acid-coagulated cheese, both heat and acid-coagulated cheese, and cheese obtained by crystallization/concentration.

Various types of fermented milk products have been documented to exist across the world (Stanley, 1998; Tamime and Marshal, 1997). The traditional food products, which have persisted for centuries and are frequently seen as a reflection of a nation's cultural heritage, have frequently developed from small-scale home production using artisanal techniques to large-scale industrial production using specialized starter cultures and modern machinery (Oberman and Libudzisz, 1998).

Dhaka cheese is one of the traditional fermented dairy products of Bangladesh. According to Berg (1988), Dhaka cheese is a Semi-hard cheese. Around a century ago, the cheese originated in a small locality called Austagram of Bangladesh. Raw milk of indigenous cows is the main ingredient for making the cheese while the abomasum of cattle is used as a source of rennet, another important ingredient of cheese making (Parvin *et al.,* 2008). For the fermentation of Dhaka, cheese artisans depend on both natural milk microbiota and the environment, like other fermented traditional dairy products. In addition, the back-slopping process is used where a par of previously fermented milk or sour cream from an earlier batch is utilized as an inoculum for the new batch (Parvin *et al.*, 2008).

The origin of Dhaka Cheese is Austagram, a small area that is approximately located 250 kilometers northeast of Dhaka. Dhaka cheese is now produced in various parts of Bangladesh since many cheese-maker families have relocated to different regions of Bangladesh, particularly where milk production is higher and milk price is lower (Parvin *et al.,* 2008). But consumers always prefer to have cheese that is produced in Austagram.

Apart from traditional Dhaka Cheese, globally popular Mozzarella cheese is also manufactured by Bangladeshi companies. With the growth of consumption of pizza or similar kinds of foods, both the production and consumption of mozzarella cheese have increased in significant amounts throughout the world (Kindstedt and Fox, 1993, Kindstedt, 2002). The scenario is not too much different in Bangladesh. In Bangladesh, the cheese is marketed not only by giant companies but also the local small-scale producers. Mozzarella is the most popular member of pasta-filata variety of cheese

which originated in the southern part of Italy (McSweeney *et al.,* 2004). Originally, Mozzarella cheese was made from water buffalo milk in Italy (Kindstedt and Fox, 1993).

Despite conducting cheese-making practice for more than a thousand years, it was confined to the cottage industry. As industrialization advanced towards the end of the 19th century, the production of cheese migrated to factories (Bennett and Johnston, 2004). Since then cheese production was going to increase drastically. Now, the global cheese market is worth 100 billion dollars (OECD/FAO, 2020). By the report of FAO global cheese production in 2005 was approximately 18.43 million tons (Banks, 2007). In 2015, global cheese production grew by approximately 23 million tons (IDF, 2016). FAO (2020) expects to increase cheese consumption by 13.8% between 2019 and 2029. It is a clear indication that the global cheese market is expanding and production has increased by about 25% between 2005 and 2015.

Cheese is an excellent source of essential nutrients like proteins, lipids, minerals, and vitamins. Certain peptides and free fatty acid is liberated after the digestion of cheese which plays an important role in anti-carcinogenic, anti-thrombotic, and anti-microbial activity in the body (Kwak *et al.,* 2011). According to Kwak *et al.,* (2011), Cheese contains some health-beneficial biologically active conjugated fatty acid (CLA) and milk fat globule membrane (MFGM). During the production of cheese, water-insoluble nutrients of milk like- coagulated casein, colloidal minerals, fat, and fat-soluble vitamins are retained in the cheese curd (O'Brien and O'Connor, 2007). But, the watery part of the milk termed whey is drained out during processing cheese which contains a significant amount of lactose, minerals, water-soluble vitamins, lactalbumin, and traces of fat (Bylund, 2003).

Food safety is one of the major concerning issues in food industry. Some authors regarded cheese as one of the safest food products (Little et al., 2008). But according to some scientific reports, several food poisoning outbreaks were associated with various types of cheese consumption. In 2006, the consumption of contaminated cheese was responsible for 0.4% of total food-borne outbreaks in Europe (EFSA, 2008). So, the quality of cheese must be checked before marketing. The final measurement of the moisture, fat, and salt in produced cheese should be checked before storing because it helps to determine whether produced cheese meet government standard (McMahon and

Brym, 2016). Standards for different major varieties of cheese has published by FAO/WHO in multiple editions of the Code of Quality Standards for Cheese which is included in the Joint FAO/WHO Codex Alimentarius (McSweeney *et al.*, 2004).

The size of the cheese market is expanding globally. Bangladesh is no exception. Different leading agro companies are commercially producing cheese since the consumption of cheese is increasing day by day. So, it has become a matter of concern to judge the cheese quality in terms of composition as well as hygienic conditions for the welfare of consumers. Despite having public health significance, very few or no study was conducted regarding this aspect in Bangladesh. Our study has been conducted to cover the knowledge gap and to attain the following objectives.

Objectives:

- To evaluate the chemical composition and the sanitary standard (coliform load) of cheeses manufactured and marketed in Bangladesh
- To compare the quality of different brands of cheeses marketed in Bangladesh with the Codex Standard of FAO and the standard of BSTI

CHAPTER 2: LITERATURE REVIEW

2.1 Introduction:

According to Code of Principles FAO definition, Cheese is the fresh or matured product obtained by draining after coagulation of milk, cream, skimmed or partly skimmed milk, buttermilk or a combination of some or all of these products (Berg, 1988). Cheese is nutritious dairy product which contains high quality milk proteins, calcium, phosphorus and energy, cheddar cheese gives about 400 calories/100g (De, 2010). Cheese composition depends upon variety. All cheese contains the same constituents of milk but in varying proportions. Fat and water are the most variable constituents of cheese (Eckles *et al.,* 1951). It is a common belief that cheese originated some 8000 years ago in Iraq, between the Tigris and Euphrates rivers as a method of preserving the nutritive value of milk (Fox, 1993; McSweeney, 2007). Owing to a continual development of the range of products available and an increase in the consumption of a western style diet, global cheese production has seen elevation over the century and this trend is likely to continue in next days too (OECD/FAO, 2011). Since 1970, global cheese production has gradually expanded at a rate of about 3% per year, and this trend is likely to continue in next years (Banks, 2017). Apart from this, cheese is now introduced as a functional food since it contains high concentration of essential nutrients (Walther *et al.,* 2008). Since the demand for the product and scale of production have increased, cheese industry has transformed itself to large scale industry from cottage industry (Legg *et al.,* 2017). According to International Dairy Federation estimation global cheese production in 2015 totaled approximately 23 million tons (IDF, 2016). Production of Cheese spread across the six continents the world. Cheese is produced mainly from cow milk which count is about 20.7 million tons while remainder portion of produced cheese comes from other species like- buffalo, goat and sheep as well as home-made and farmstead cheeses which contribution do not appear in national statistics.

2.2 Types of Cheese

Cheeses can be categorized based on- which substance is used to coagulate milk during making cheese, hardness of cheese, manufacturing technique is used, eye development in cheese, whether rind is allowed to from or not, which type of microbial growth is occurred during ripening (McMahon, 2015). Berg (1988) referring Cheese standard,

1973 by FAO classified according to fat percentage (%) in dry matter. Mainly classification of cheese is done on the basis of moisture percentage, e.g. hard cheese, semi hard cheese, soft cheese etc. Commonly, cheese is classified based on moisture content and under this classification -cheese mainly divided into four major groups: very hard, hard, semi hard, soft (Davis, 1965; Walter and Hargrove, 1972).

McSweeney (2007) classified cheeses into about 12 major families, based on method of coagulation of the milk and various technological parameters:

- Acid-coagulated varieties (e.g. Cottage cheese, Quarg, Cream cheese).
- Varieties coagulated by a combination of both heat and acid (e.g. Ricotta).
- Rennet-coagulated cheeses (most varieties), which can be subdivided based largely on the technology of their manufacture and ripening into:
    1) extra-hard (Grana-type) cheeses (e.g. Grana Padano)
    2) hard cheeses (e.g. Cheddar)
    3) semi-hard cheeses (e.g. Monterey Jack)
    4) Swiss-type cheeses (e.g. Emmental)
    5) Dutch-type cheeses (e.g. Gouda)
    6) varieties ripened under brine (e.g. Feta)
    7) Pasta-filata varieties (e.g. Mozzarella)
    8) surface (white) mould-ripened cheeses (e.g. Camembert)
    9) Blue cheese (e.g. Roquefort)
    10) surface (smear)-ripened cheeses (e.g. Tilsit)

Ottogalli (1998, 2000) classified cheese in three major groups namely 'Lacticinia' (milk-like), 'Formatica' (shaped), 'Miscellanea' (miscellaneous). The group Lacticinia covers the products that are obtained from milk, cream, whey or buttermilk by acid coagulation (lactic or citric) with or without a heating step. The second group Formatica includes the cheeses that are obtained by rennet induced coagulation, this group includes highest number of cheese varieties. Third group Miscellanea is a diverse range of cheese variations that comprises processed, smoked, grated, and pickled cheeses, cheeses incorporated with non-dairy elements (fruit, vegetables, and spices), cheese analogues, and ultrafiltration cheeses. Each group is subdivided into different classes and families based on different criteria.

2.3 Marketed cheese in Bangladesh

Unlike other dairy products cheese was not too popular in Bangladesh in last few decades. Market of cheese was fully depended on imported cheese or cheese produced by local producers of specific localities. With the changes of time cheese consumption has increased in Bangladesh (Proma, 2016). Consequently, demand of cheese has elevated than previous years. To cope with increasing demand of cheese some dairy processing companies have started to manufacture cheese in large scale (Proma, 2016). Mainly two types of cheese are manufactured in Bangladesh, they are traditional Austogram cheese or Dhaka cheese and mozzarella cheese (Das, 2019).

2.3.1 Dhaka cheese

Dhaka cheese is a white, semi-hard artisanal cheese which has been manufactured traditionally in Bangladesh (Habib, 2015). The origin of Dhaka Cheese is Austagram, a small area which is approximately located 250 kilometers north-east of Dhaka (Parvin *et al.,* 2008). Davis (1976) reported that previously this variety of cheese mainly produced in Haor area of Mymensingh, Bangladesh. Now, this variety is manufactured all over the county, specially the places where milk price is lower and milk production is abundant (Parvin *et al.,* 2008). Usually this type of cheese is made from cow milk, sometimes mixture of cow and buffalo milk is used. This type of cheese contains a characteristic hard, crumbly texture and a strong salty flavor (Habib, 2015). Common salt (Sodium chloride) is an important constituent of Dhaka cheese for its role in preservation and organoleptic quality (Habib, 2015).

2.3.2 Manufacturing Process of Dhaka cheese

Parvin *et al.,* (2008) described detail manufacturing procedure of Dhaka Cheese. According to their description- Special type of coagulating solution is required for manufacturing Dhaka Cheese which is locally known as MAWA or medicine water. To prepare MAWA, cow's abomasum is collected from local slaughter house. Collected abomasum is washed and dried in sun using normal salt. After that, the dried abomasum is added to mixture of water and milk and kept for 12 hours in ambient temperature. The prepared solution is used to coagulate milk. Coagulation ability of the solution need to be checked and filtration is done before adding to the milk. The solution is added gently to the raw milk collected from local market and is allowed to coagulate for 30 minutes. Coagulation condition is checked by probing bamboo knife into the curd. The

curd is then sliced into squares with the help of a bamboo knife and put aside for another 15-20 minutes. After manual milling by hand, the curd is retained for another 30 minutes to drain away the whey. In next step, curd is bundled by using hand and the container is tilted to separate the card from whey. The curd is then chopped with a knife and put in a bamboo-made basket with pressure applied by hand to drain the whey. The curd is then sliced into pieces with a knife and stuffed into a unique bamboo-made basket with pressure applied by hand to drain the whey. For optimum shape and size, inversion is performed every 4/5 h for a total of 16 hours. After the curd has hardened sufficiently, the salting procedure with table salt begins. At 24 hour intervals, three 7.5 cm diameter holes are drilled in the middle of the upper and lower surfaces of the cheese, and salt is inserted into these holes. The exterior surface of the cheese is additionally scraped with salt every 24 hours for 72 hours. The cheese is now ready to be sold. However, the surface of the cheese is sprinkled with salt every day until it is sold. It should also be mentioned that cheese is cleaned with water before consumption.

2.3.3 Mozzarella cheese

Mozzarella is an elastic, smooth cheese with a long stranded fibrous protein structure that is parallel-oriented and devoid of curd particles (Joint FAO/WHO, 2006). No ripening process is involved while manufacturing mozzarella cheese that's why it is classified as unripened cheese. Mozzarella is a rindless cheese and can be found in different forms. When mozzarella has a high moisture content, is classified as soft cheese and the upper layers may develop pockets that contain liquid that has a milky appearance. It may be packaged either with or without the liquid. The color of cheese is almost white. Low moisture mozzarella is a firm/semi-hard cheese that is homogeneous and free of holes, this characteristic make it ideal for shredding. "Pasta filata" technique is used to make mozzarella cheese which involves heating curd with a correct pH level, kneading, and stretching the curd until it is smooth and lump-free (McMahon and Brym, 2016).

2.3.4 Manufacturing Process of Mozzarella cheese

General steps that are followed in manufacturing process of mozzarella cheese is demonstrated in **Figure 1.**

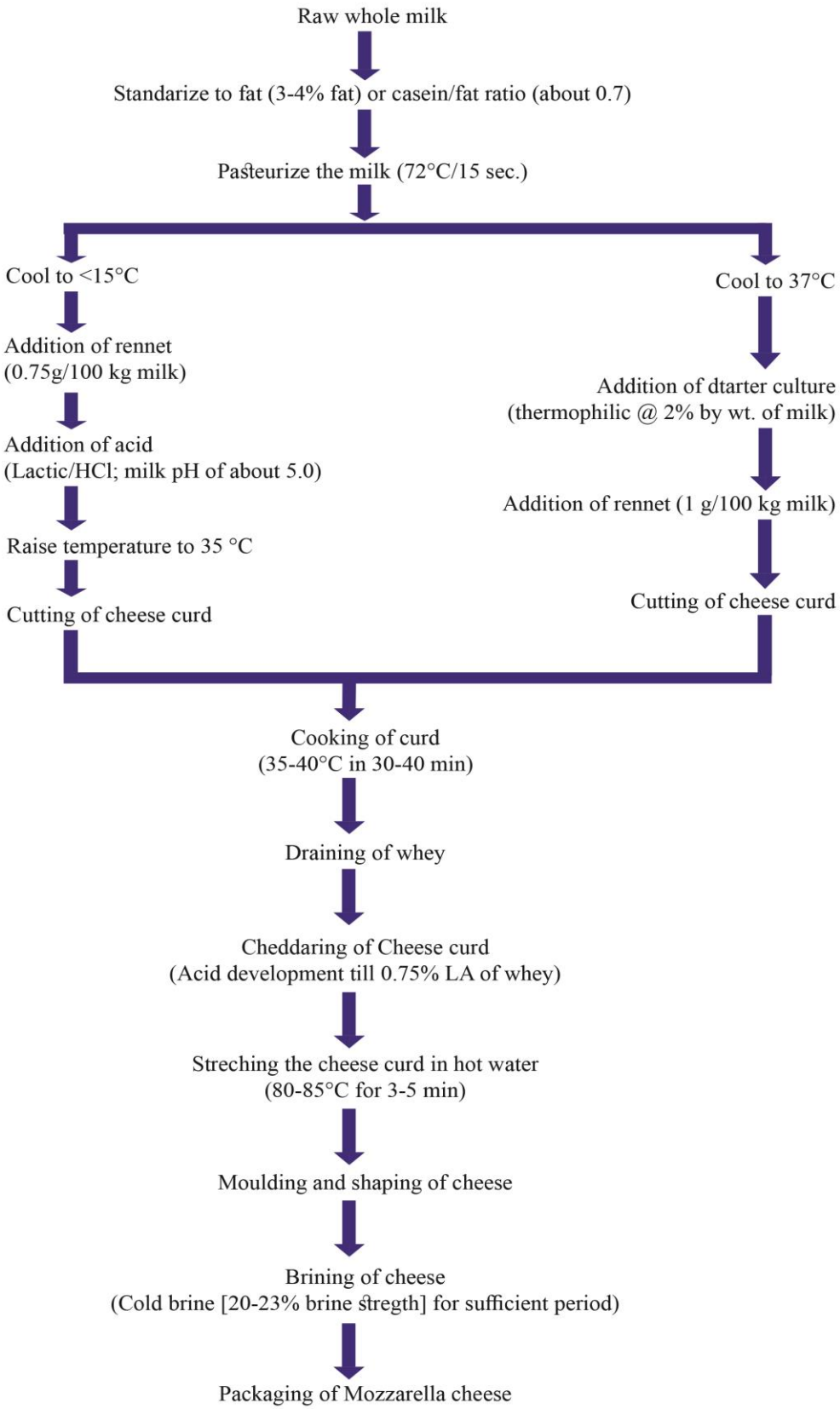


Figure 1: Flow chart for preparation of mozzarella cheese

Source: Ghosh and Singh (1996); Jana and Tagalpallewar, (2017)

2.4 Quality evaluation of cheese

Gunasekaran (2016) suggests that quality attributes of cheeses can be grouped into different categories like- microbial, chemical, physical etc. Evaluation of quality of cheese mainly includes - sensory evaluation, chemical analysis and microbiological analysis. Kumar *et al.,* (2014), conducted sensory and chemical analysis in Paneer (cheese) quality evaluation while Mizan *et al.,* (2010), conducted sensory and chemical analysis only in mozzarella cheese evaluation. Khatun *et al.,* (2019) implemented organoleptic, chemical and microbiological analysis in Qualitative study on the cottage cheese.

2.4.1 Sensory Evaluation:

Both Kumar *et al.,* (2014) and Mizan *et al.,* (2010), evaluated cheese physically by using a score card. Score card used for judging was provided by American Dairy Science Association (ADSA) for Cheese (De, 2015). Sensory analysis of three traditional Ethiopian cheese was conducted by based on taste, color, aroma, texture, appearance and overall acceptability by using questionnaire (Eshetu and Asresie, 2019). Kumar *et al.,* (2014) conducted sensory evaluation of Paneer on 4 atributes- Flavour, body and texture, color and texture and packaging. They also mentioned that Paneer could be divided into four grades based on total score obtained during sensory evaluation; they categorized in following way- Paneer having total score of 90 or more = Excellent/A grade, 80–89 = Good/B grade, 60–79 = Fair/C grade, 59 or less = Poor/D grade. Sensory evaluation of Mozzarella and Cheddar cheeses were evaluated by two experienced judges based on flavor, body, and texture using the American Dairy Science Association score card for Cheddar cheese as a guide (Covacevich and Kosikowski, 1978). Zedan *et al.,* (2014) used three attributes- appearance, body & texture and flavor as a part of sensory evaluation of mozzarella cheese. Land and Shepherd (1988) used hedonic scale containing nine points for sensory evaluation of cheese where they used four attributes flavor, taste, texture and overall acceptability. Sameen *et al.,* 2008 stated that flavor of cheese is most important than any other sensory attributes to determine consumer's response. The principal objective of all the studies that were reviewed were to evaluate whether there was any significant differences in flavor and taste, body and texture, finish and colors. When grading cheese, a plug is drawn from a central location in a block of cheese. This provides a representative

sample to be assessed. The cheese plug and the block as a whole are then examined visually for finish and appearance characteristics in addition to color characteristics. The body and texture of the plug are observed by manipulating a small portion of the plug using the fingers and then examining the characteristics. The flavor characteristics are then observed by smelling and tasting the cheese sample. Some body and texture characteristics can also be detected by testing the sample.

2.4.2 Quality in terms of chemical compositions:

Mizan *et al.,* (2010) analyzed all mozzarella cheese samples for moisture (g/kg), total solids (g/kg), fat (g/kg), protein (g/kg), ash (g/kg), lactose (g/kg), acidity (%) and pH.

In their study Mizan *et al.,* (2010) found that moisture (%) 47.4 ± 0.3, total solids content (%) 52.9 ± 0.2, fat content (%) 24.2 ± 0.4, protein content (%) 20.5 ± 0.6, ash content (%) 2.7 ± 0.1, acidity content (%) 0.7 ± 0.1, pH content 5.0 ± 0.1 of mozzarella cheese made from cow milk. Johnson and Olson (1985) used Mohr method to determine amount of Sodium Chloride present in cheese. They also recommended this method in unripened cheese when speed is necessary.

No study was found regarding chemical analysis of marketed Dhaka cheese as par our best knowledge. In their study Parvin *et al.,* 2008 registered the value of total Nitrogen, ash, moisture, acidity of collected Dhaka cheese samples and physiochemical changes during ripening. Habib *et al.,* (2012) researched on effect of pressing on Dhaka cheese quality where he mentioned Dry matter (%), fat (%), protein (%) and pH of Dhaka cheese in different pressure while manufacturing.

2.4.3 Quality in terms of microbial analysis

Dairy products acts as an excellent growth medium for a wide range of microorganisms. So, it is necessary to perform microbial analysis of dairy products to check the presence of harmful bacteria; particularly total viable count and *Escherichia coli*, since they are indicators of the hygienic condition of these products (Losito *et al.,* 2014).

Kumar *et al.,* (2014) used Standard Plate Count (SPC) and Coliform count to evaluate microbial quality of cheese. Kumar *et al.,* (2014) mentioned Standard Plate Count (SPC) in cheese samples less than 10,000 count/g is excellent quality and coliform count less than 10 cfu/g is satisfactory quality. Nur *et al.,* (2021) conducted total viable bacterial count (TVBC) and total coliform count (TCC) by pour plate method for

microbiological quality assessment of milk and milk products including cheese. Kunová *et al.,* (2015) used Violet red bile agar for determining Coliform bacteria in cheese samples. In addition they used dilutions of 10^{-3} and 10^{-4} to determine of Coliform Bacteria.

2.5 Conclusions

Cheese industry is growing in Bangladesh. But no study has not been conducted to determine the chemical compositions and microbial loads in marketed cheeses in Bangladesh. This study is designed to minimize the existing knowledge gap about cheeses manufactured in Bangladesh and figuring out current status of cheese that are available in Bangladesh. In addition to this study will help to figure out whether Bangladeshi cheese manufacturers are following Bangladesh Standards and Testing Institution (BSTI) standards or not.

CHAPTER 3: MATERIALS AND METHODS

To conduct the experiment, technical assistance was required from three different laboratories. Chemical analysis of cheese was performed in Dairy Science laboratory and Animal Nutrition laboratory of Chattogram Veterinary and Animal Sciences University (CVASU). In contrast, microbiological analysis was performed in the Bacteriological Laboratory of Poultry Research and Training Centre (PRTC).

3.1 Study Design and Selection of the Study Area

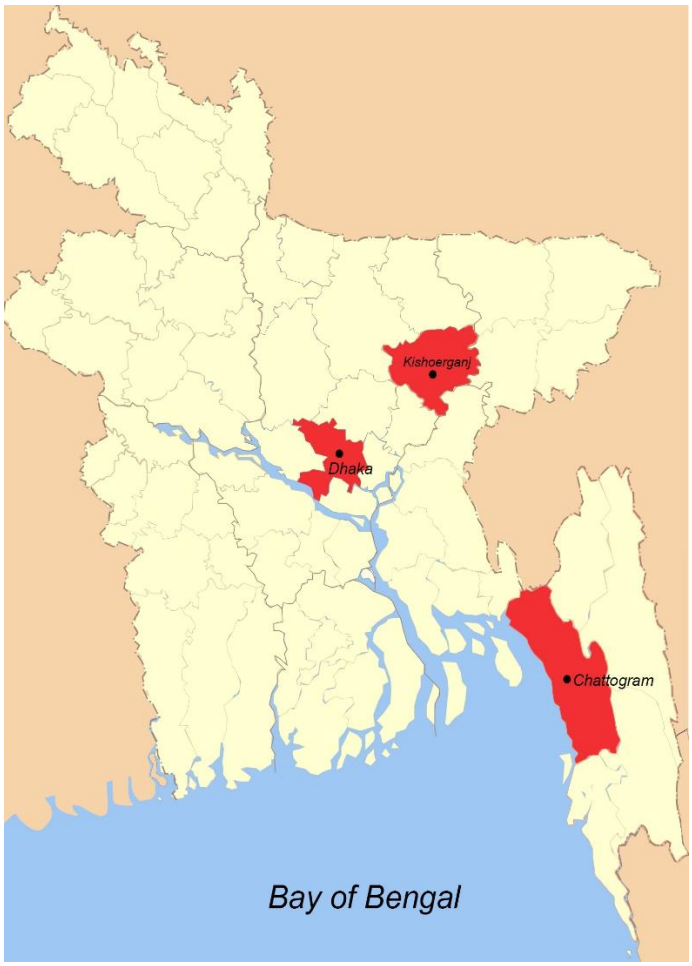


Figure 2: Map of Bangladesh indicating the area from where sample were collected

Areas from where samples were collected are demonstrated in Figure 2. Most of the samples were obtained from superstores in the Chattogram Metropolitan area (CMA). All of the mozzarella cheese samples used in the study came from reputable dairy producers in Bangladesh. However, when it comes to Dhaka cheese, three of the samples were produced by three of the country's top dairy producers, while the remaining two samples were procured from regional producers in the Dhaka

Metropolitan area of Dhaka district and Austagram Upazila of the Kishoreganj district who were engaged in the cheese-making industry as a part of their inherited culture. For improved results, three (3) batches of each sample were run.

3.2 Labelling of samples

Brands of Dhaka Cheese that were considered for our study were marked as DC_1, DC_2, DC_3, DC_4 and DC_5. Among the brands DC_4 and DC_5 were selected as well as collected from local cheese makers from Dhaka Metropolitan Area and Austagram upazila respectively while other three brands from leading dairy manufacturing companies of Bangladesh. In contrast, all mozzarella cheese brands that were taken into the study marked as MC_1, MC_2, MC_3, MC_4 and MC_5 accordingly and derived from five different cheese manufacturing companies of Bangladesh.

3.3 Sensory Evaluation of Cheese

Three judges evaluated cheese sensory quality impartially and independently. All samples were placed on plates before being given to the judges, and all manufacturer labels were taken off (Figure 3). After that, all samples were noted under a unique identification code so that the judges could not see any indication of the brand or company. Marks were assigned based on the cheese's finish and appearance, body and texture, color, and flavor and aroma. The judges marked the samples in a supplied organized scorecard. Score Card for sensory evaluation of cheese:

Items	**Full Marks**
Color	10
Finish and appearance	15
Body	15
Texture	15
Flavor and aroma	45

Source: De, 2010

Figure 3: Prepared sample for sensory evaluation of cheese

3.4 Chemical Analysis of Cheese

3.4.1 Preparation of Sample

Samples were prepared according to AOAC method No. 955.30 (Bradley, 2023). First of all, the rind, smear, or moldy surface layer of the cheese sample was removed. A portion of cheese was taken from a representative sample of cheese with great caution and proper procedure to prevent contamination. Before taking the sampling portion, it was thoroughly mixed by intensive stirring and kneading. The test sample was transferred to an airtight container and stored at 10–12°C up to further examination.

3.4.2 Determination of Dry Matter

Determination of Dry Matter in cheese samples were carried out following AOAC method No. 926.08 (Bradley, 2023). Clean and properly dried petri dishes were weighed and marked properly. Ten (10) grams of cheese samples from each selected brand were taken in each petri dish (figure 4A). Afterward, the petri dishes were transferred to a hot air oven to remove all the moisture from the cheese sample (Figure 4B). The samples were kept to dry in a hot air oven at 105°C for 24 hours. After oven drying for 24 hours the plates were transferred to a desiccator for cooling down and the weights of the plates were recorded (Figure 4C). The process was repeated every 6 hours till constant weight was obtained. Finally, the weight of the petri dish containing the dried sample was recorded.

Dry matter content was calculated according to formula given below:

$$Dry\ Matter(\%) = \frac{(W_2 - W_1)}{W_S} \times 100$$

W_s= Weight of sample

W_1= Weight of empty petri dish

W_2= Weight of petri dish containing dried sample

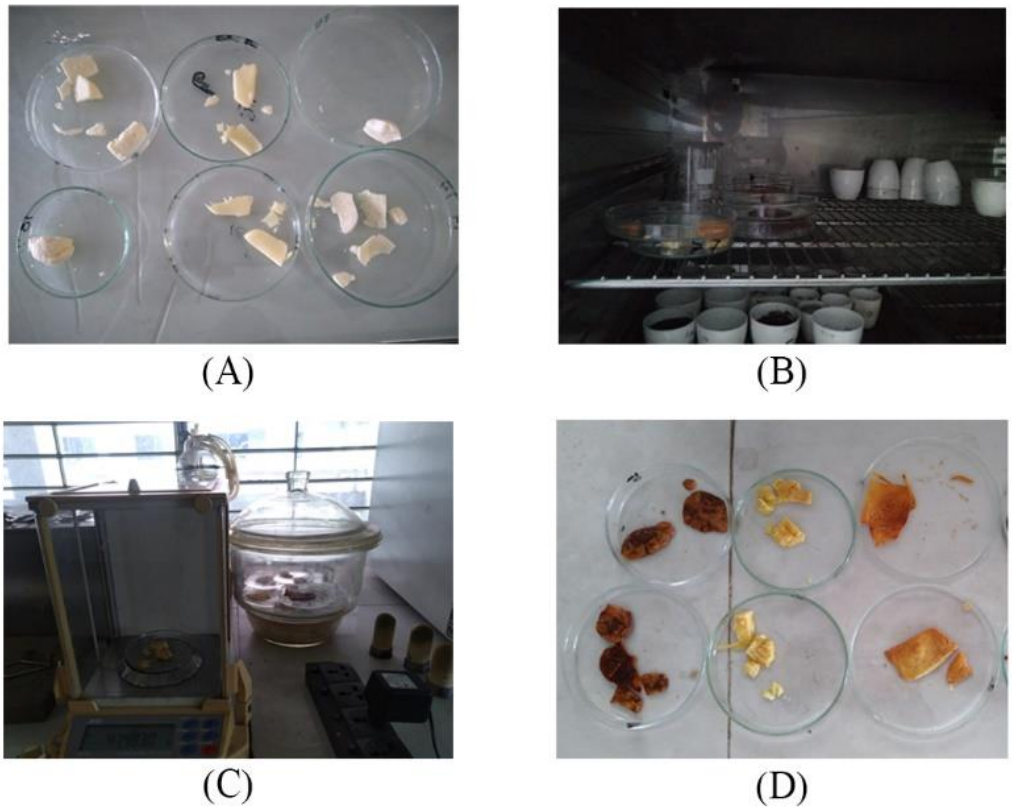


Figure 4: Moisture Estimation of cheese

A: Fresh weighted sample; B: Sample placed in hot air oven for drying; C: Weighing oven dried sample after cooling in desiccator; D: Dried sample

3.4.3 Determination of Moisture

Determination of Moisture in cheese samples were carried out following AOAC method No. 926.08 (Bradley, 2023). Clean and properly dried petri dishes were weighted and marked properly. Ten (10) grams of cheese sample from each selected brands were taken in each petri dish **(Figure 4A)**. Afterwards, the petridishes were transferred to hot air oven to remove all the moisture of cheese sample. The samples were kept to dry in hot air oven at 105°C for 24 hours **(Figure 4B)**. After oven drying

for 24 hours the plates were transferred to desiccator for cooling down and weights of plates were recorded **(Figure 4C)**. The process was repeated every 6 hours till constant weight were obtained. Finally the weight of petri dish containing dried sample was recorded.

Moisture content was calculated according to formula given below:

$$Moisture\ (\%)\ of\ cheese = \left\{1 - \frac{(W2 - W1)}{Ws}\right\} \times 100$$

W_s= Weight of sample

W_1= Weight of empty petri dish

W_2= Weight of petri dish containing dried sample

3.4.4 Determination of Fat

Fat determination of cheese is done by Gerber's method as described in the Madras Veterinary College Manual. One gram of cheese sample was taken in a clean and dry Butyrometer. In the next steps, 10.75 ml of hot water (60°C to 70 °C) and 10 ml of Sulphuric acid (specific gravity 1.82) were added to the Butyrometer (Figure 5 A). Finally, 1 ml of Amyl alcohol was added to the mixture. Butyrometer was closed properly by the stopper and inverted several times. The butyrometer was placed in a water bath at 70°C for 10 minutes with periodical removal and inverting (Figure 5 C). Butyrometer was placed in the centrifuge machine and centrifuged at 100 r.p.m. for 5 minutes. Reading of the fat column was taken by adjusting it with the scale of a butyrometer.

$$Fat\ (\%)\ of\ cheese = \frac{Observed\ reading \times 11.25}{Weight\ of cheese}$$

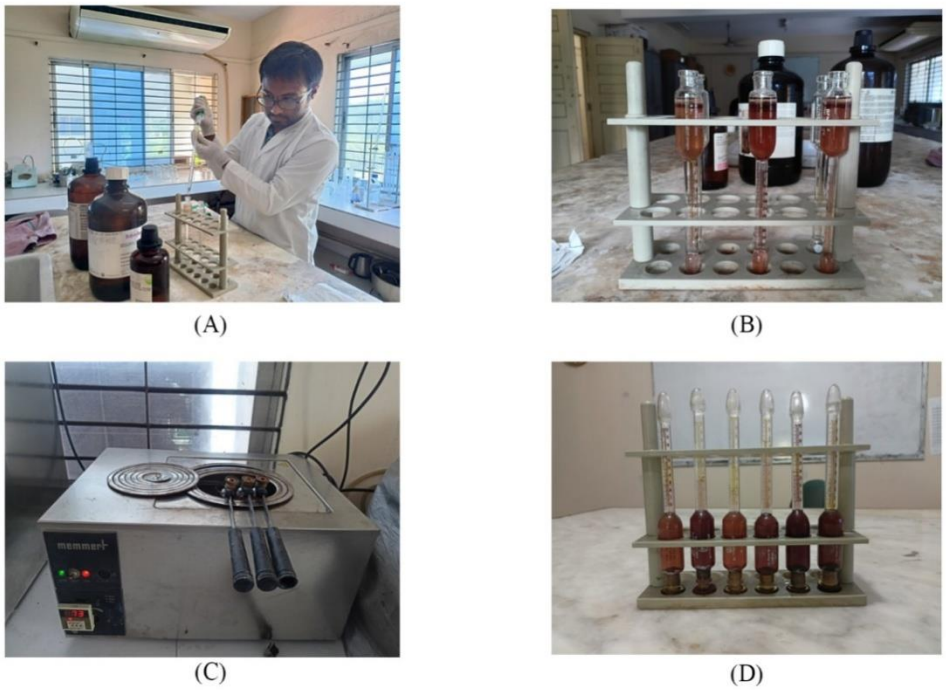


Figure 5: Fat Estimation of cheese

A: Addition of acid in Butyrometer; B: All ingredients in Butyrometer; C: Placing Butyrometer in hot water bath; D: Butyrometer reading after centrifugation

3.4.5 Determination of Crude Protein

Crude protein content of Cheese sample was determined by following the process described in IDF20-1 (2014).

3.4.5.1 Digestion

Two grams of sample was weighted and taken to Kjeldahl digestion tube. Twenty milliliters of 98% Sulphuric acid and digestion mixture which act as catalyzer added to digestion tube. Digestion tube was placed in Kjeldahl's digestion unit for digestion for 3 hours (Figure 6 A). The digested material was allowed to cool at room temperature.

3.4.5.2 Distillation

The digested mixture was diluted by adding 50 ml of distilled water. 10 ml of 4% boric acid was taken in a conical flask and two drops methyl indicator was added. Digestion tube and conical flask containing boric acid was placed in distillation unit (Figure 6 B). 40% NaOH solution was added automatically by distillation unit from connected flask

containing NaOH solution to diluted mixture. Liberated ammonia derived from chemical reaction and distillation was collected in conical flask.

3.4.5.3 Titration

Distilled solution was titrated against 0.1 N HCl solutions and reading was taken (Figure 6 C).

Protein content was calculated according to formula given below:

To determine protein (%) of sample, it is necessary to determine Nitrogen (%) of sample before. Total Nitrogen (%) can be determined by formula given below-

$$Total\ Nitrogen(\%) = \frac{14.007 \times (T - B) \times N \times 100}{W}$$

Where,

T: Volume in mL of standard Hydrochloric acid used in titration for test sample

B: Volume in mL of standard Hydrochloric acid used in titration for blank determination

N: Normality of standard Hydrochloric acid

W: Weight of Sample

$$Crude\ Protein(\%) = Total\ Nitrogen\ (\%) \times 6.38$$

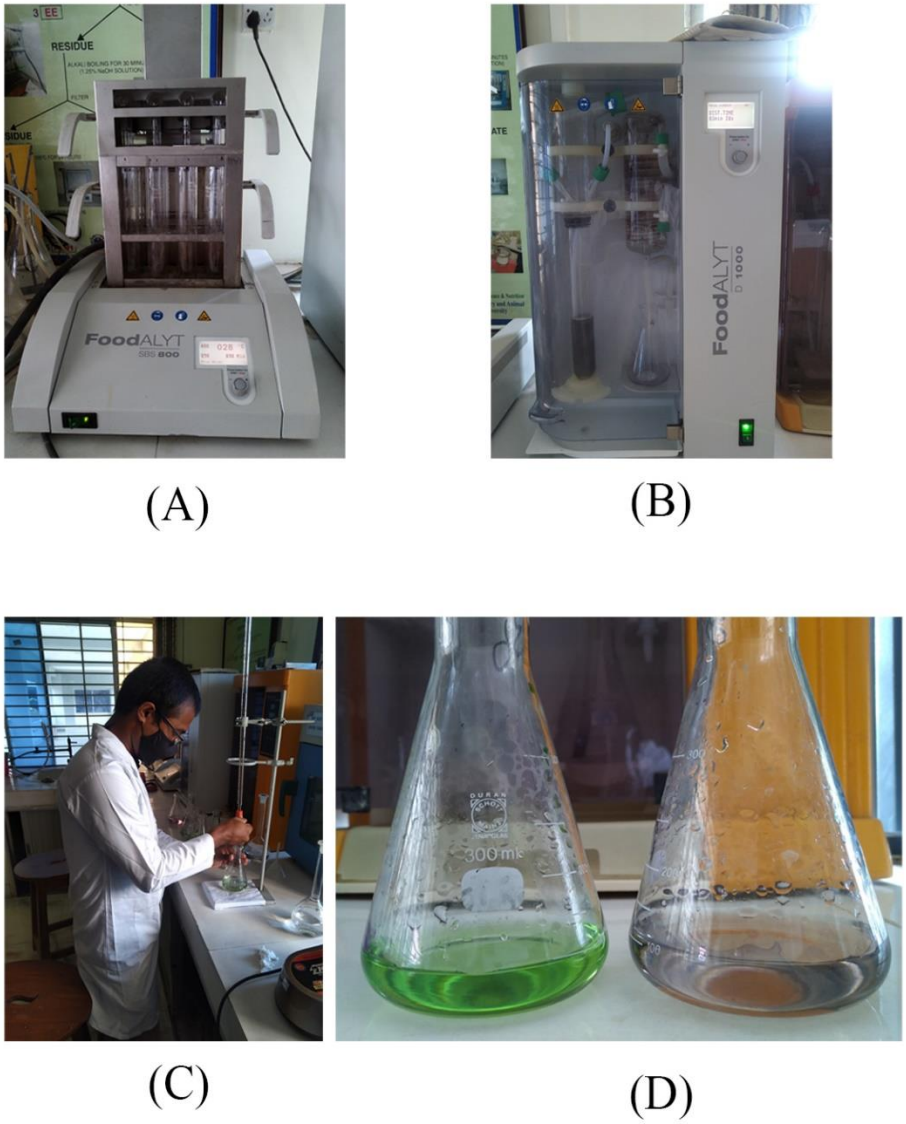


Figure 6: Crude Protein Estimation of cheese

A: Digestion of sample and digestion mixture; B: Distillation;
C: Titration; D: Change of color after titration

3.4.6 Determination of Ash:

Determination of Ash from cheese sample was determined by AOAC (1995) method No. 935.42. Weight of clean and properly dried crucible was recorded. Three (3) gram of cheese sample was taken in each crucible (Figure 7A). The crucibles were heated in a heater at 100°C until fume was removed (Figure 7B). Then the crucibles were transferred to muffle furnace and pattern of placed crucibles was drawn in a notebook (Figure 7C). The samples were heated in muffle furnace at 550°C for 5 hours (Figure 7D). Finally the weight of crucibles containing dried sample was recorded.

Ash content was calculated according to formula given below:

$$\boldsymbol{Ash}(\%) = \frac{(\boldsymbol{W_2} - \boldsymbol{W_1}) \times \mathbf{100}}{\boldsymbol{W_s}}$$

W_s= Weight of sample

W_1= Weight of empty crucible

W_2= Weight of crucible containing dried sample

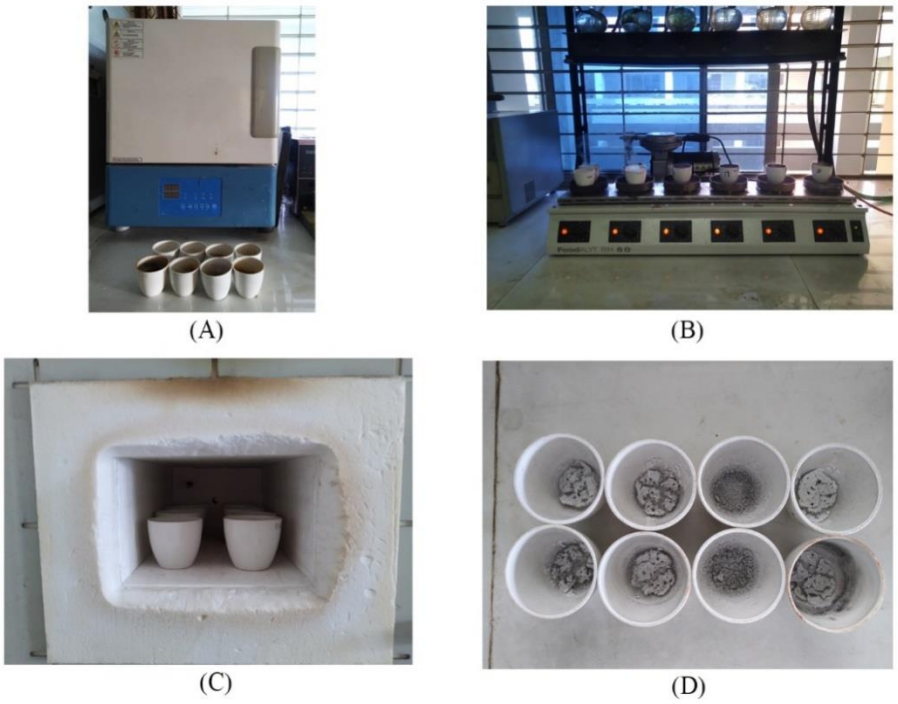


Figure 7: Ash estimation of cheese

A: Fresh weighted sample; B: Burning sample for making it fume free; C: Placing crucibles in Muffle Furnace; D: Sample after burning

3.4.7 Determination of Acidity

As per AOAC (1995) method 920.124 Acidity were determined. Two grams of Cheese sample was taken in a clean and dry conical flask. 20 ml of distilled water (temperature 60°C -70 °C) was added to conical flask and fine paste was made with a glass rod. 2 drops of 0.5% phenolphthalein indicator was added to conical flask. Titration of the mixture was performed against 0.1 N NaOH Solution till faint pink color persisted (Figure 8 B). The volume of NaOH required for completing titration was recorded.

Acidity % was calculated according to formula given below:

$$Acidity(\%) = \frac{0.009 \times V \times 100}{Ws}$$

W_s = Weight of sample

V = Volume of 0.1N NaOH required for titration

(A) (B)

Figure 8: Acidity estimation of cheese

A: Titration of sample; B: Change of color after titration

3.4.8 Salt (NaCl) Determination

Mohr's method (IS:3507-1966) was used to determine salt (%) in cheese sample. 3-5 g of sample was taken in clean and dry conical flask. 50 ml of distilled water was added to conical flask. Flask was continuously swirled for 30 minutes. 1 ml Potassium chromate which acts as indicator was added to the mixture. Titration was performed using 0.1N Silver Nitrate solution, end point of titration was determined by orange coloration of mixture which lasted for at least 30 seconds (Figure 9). Volume of Silver Nitrate used for titration was recorded.

$$\boldsymbol{NaCl}(\%) = \frac{\mathbf{5.844} \times \mathbf{C} \times (\boldsymbol{V_S} - \boldsymbol{V_0})}{\boldsymbol{W_S}}$$

V_S: Volume in mL of Silver Nitrate solution used in titration for test sample

V_o: Volume in mL of Silver Nitrate solution used in titration for blank determination

C: Concentration of Silver Nitrate solution (in mol/L)

W_s: Weight of Sample

Figure 9: Salt (Sodium chloride) Estimation of cheese

Right Conical flash: cheese sample with distilled water;
Middle flash: color after addition of indicator
Left Conical flash: Change of color after titration

3.5 Microbiological Analysis of Cheese

As a part of microbial analysis, coliform count in cheese samples were conducted. Whole analysis procedure was performed by following ISO 4832:2006. The coliform count was done in a VRB agar (Himedia, India) plate. An incubation temperature of 37°C was used for 24 hours in the Incubator (J. P. SELECTA, Barcelona, Spain).

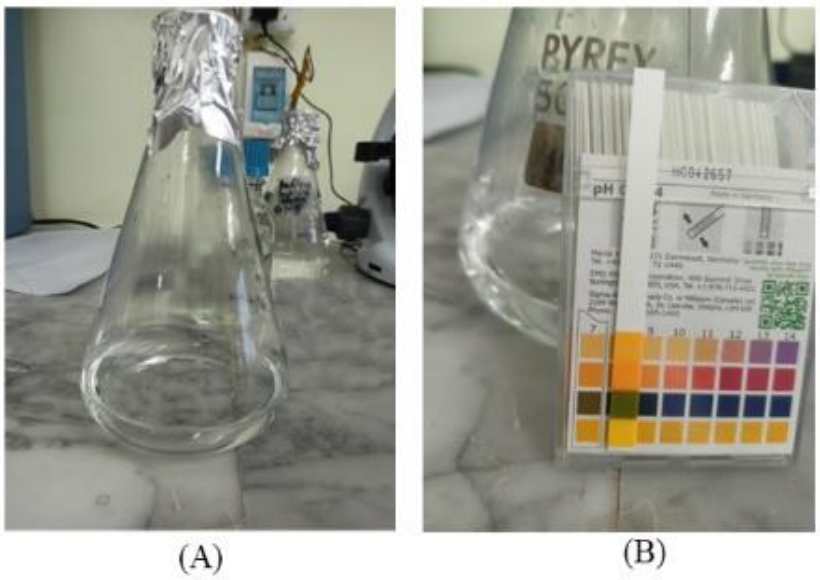
3.5.1 Preparation of Butterfield's Phosphate Diluent:

Preparation of Stock solution:

34 g of potassium dihydrogen phosphate (KH_2PO_4) was dissolved in 500 mL of purified water. Freshly prepared 175 ml of 1N NaOH solution added to KH_2PO_4 solution and more distilled water was added to make the solution total 1000 ml(figure 10 A). Optimum pH (7.2) of the solution was ensured by using pH paper (figure 10 B). Stock solution was stored under refrigeration.

Preparation of working solution:

1.25 mL of stock solution added to 1 L volume with purified water. Dilution blanks were prepared by using the solution.



(A) (B)

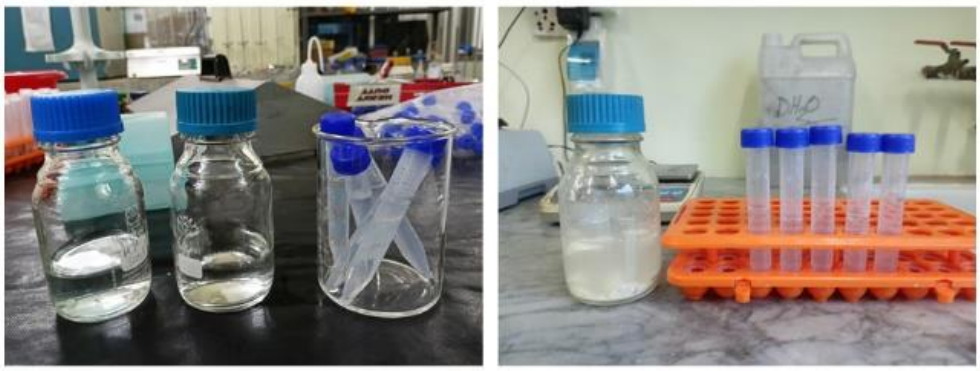
Figure 10: Preparation of Butterfield's Phosphate Diluent;

A: Prepared Butterfield's Phosphate solution;

B: pH test for prepared diluent

3.5.2 Preparation of dilution blank:

Clean and dry dilution blanks and falcon tubes were taken; each dilution blank and falcon tubes were filled with 90 ml and 9 ml diluents, respectively (Figure 11A). Caps of all dilution blanks and falcon tubes those were supposed to be sterilized were loosely tightened up. A portion of the diluent was lost during sterilization, so the sterilized blanks were brought to the proper volume with the sterile diluents after sterilization.



(A) (B)

Figure 11: Preparation of dilution blank A: Sterilized dilution blanks with diluent; B: Preparation of dilution blanks for serial dilution

3.5.3 Preparation of Media:

41.53g Violet Red Bile (VRB) agar was suspended in 1000ml sterilized distilled water. The media was placed on a heater and heated until boiling to dissolve completely (Figure 12). The media was transferred to hot water bath at 50°C prior to pour on petri dishes.

Figure 12: Preparation of VRB media

3.5.4 Sample dilution:

Ten grams (10 g) of the sample was weighted aseptically into a sterile 90 mL water blank and homogenized. This is considered primary 1:10 dilution blank (PDB). One (1) mL of the PDB was aseptically transferred to another sterile 9 mL falcon tubes and the sample is diluted by a factor of 10, 100, 1000, etc. (Figure 13). One mL of each dilution was pipetted to appropriately mark duplicate Petri dishes.

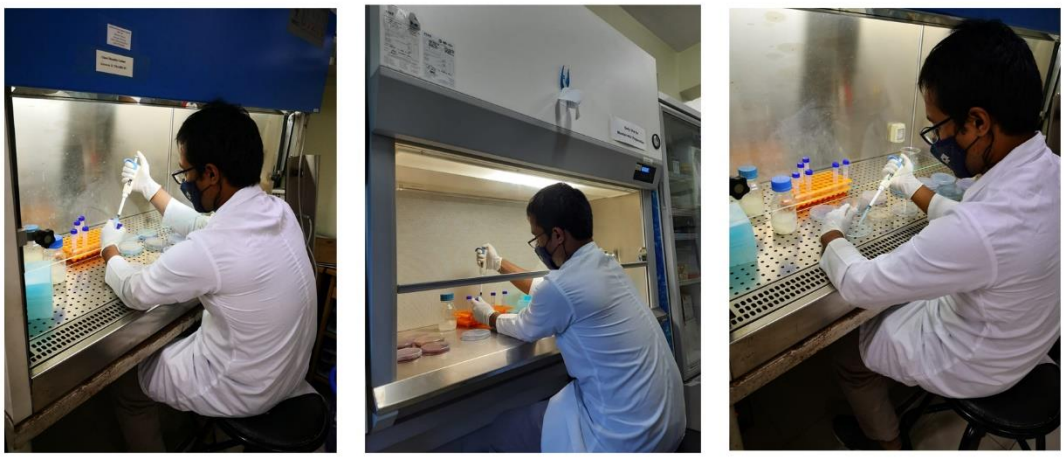


Figure 13: Dilution of sample and transferring sample to petri dishes

3.5.5 Pouring Media

15-20 mL of VRB agar was poured into each dish, which had been cooled to 45°C (Figure 14). The plates were swirled for proper mixing and kept them to solidify. Additional 3-4 ml of VRB was poured into plates.

(A) (B)

Figure 14: Process of pouring media

A: Pouring media in petri dishes: B: Plates kept for solidification

3.5.6 Incubation and counting:

The plates were inverted and incubated at 37°C for 24 hours. Dark-red colonies with reddish zone of precipitated bile having an estimated diameter of 0.5 mm or more were considered as coliform colonies and the results were recorded (Figure 15). Plates with no colony growth are shown in (Figure 16) Number of colonies in per plate were calculated using colony counter (Figure 17). The number of coliforms per gram of sample was calculated.

Number of coliforms per gram = Average number of coliform colony × Dilution factor

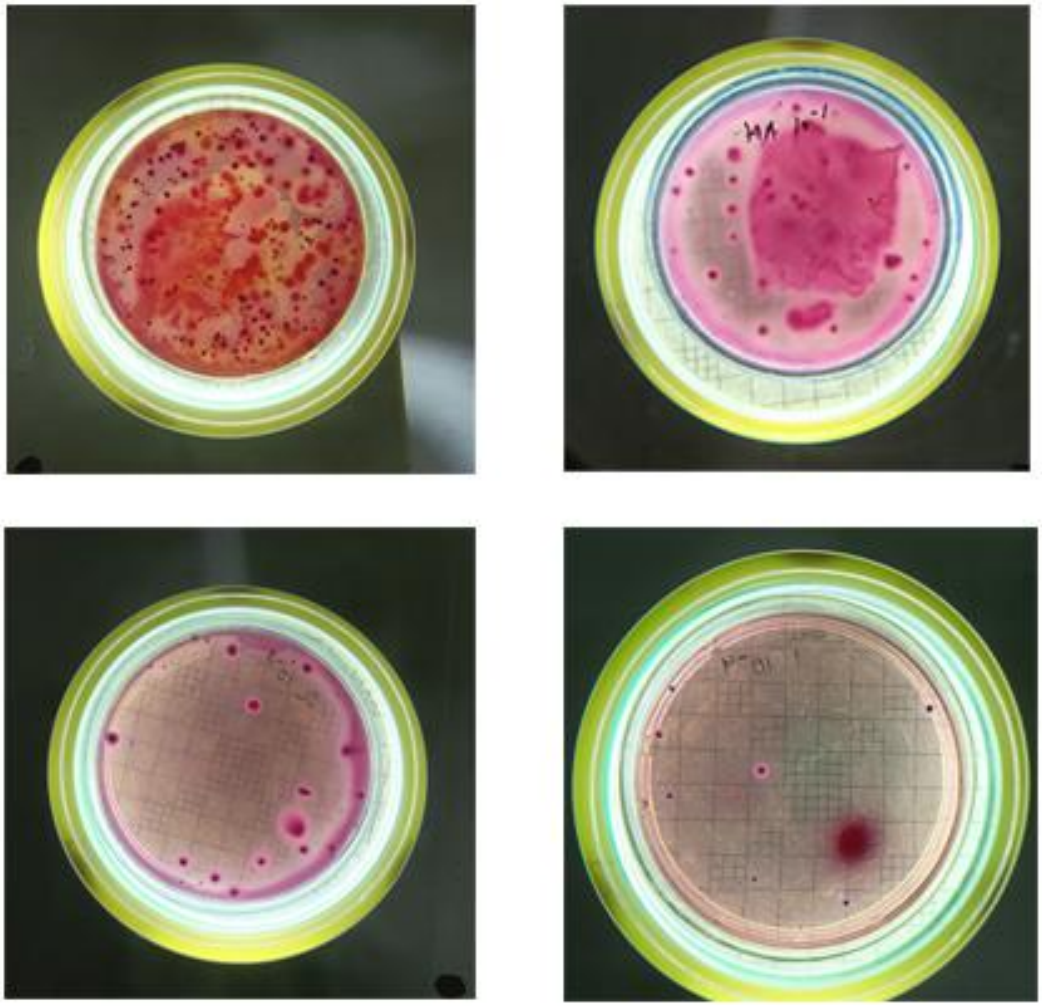


Figure 15: Evidence of bacterial colony growth in VRB media

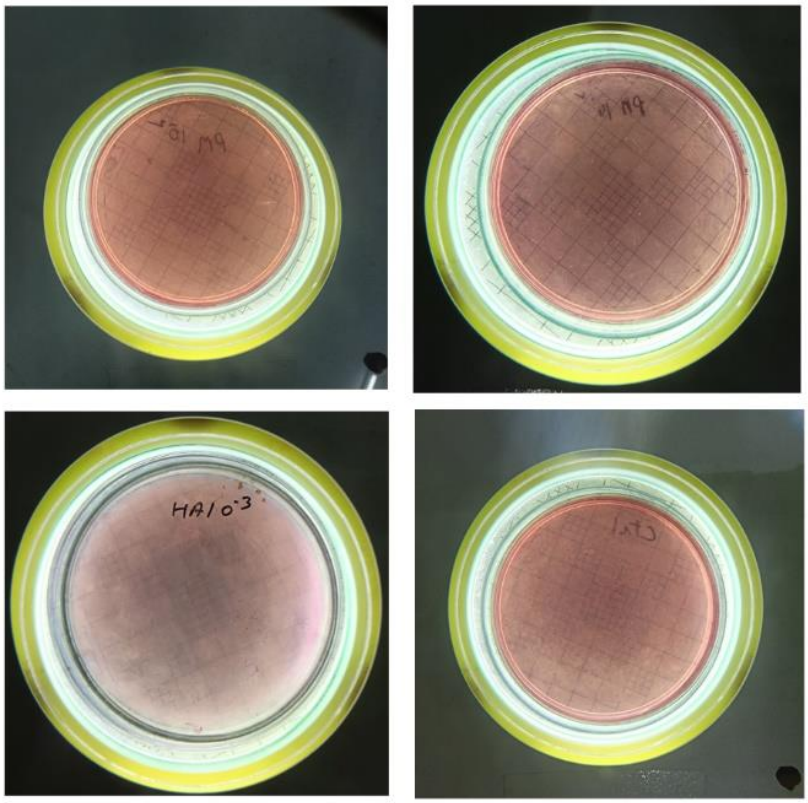
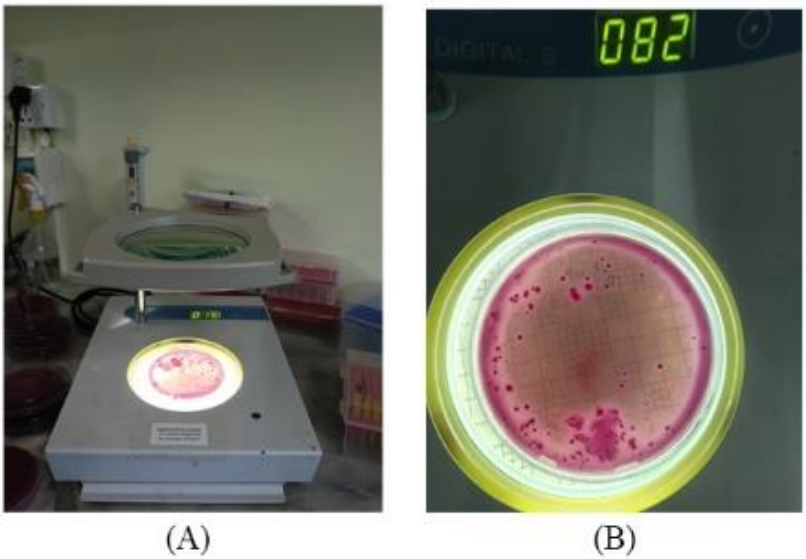


Figure 16: No bacterial colony growth in VRB media



(A) (B)

Figure 17: Procedure of counting bacterial colony; A: Colony counter; B: Petri dish with bacterial growth

3.5.7 Confirmation of presence of *E. coli* in cheese samples:

Subsequent colonies from VRBL agar are streaked on the petri dishes containing EMB agar (Figure 18 A). Plates are kept in incubator for 24 hours. Metallic sheen on bacterial colony indicates presence of *E. coli* in cheese samples (Figure 18 B).

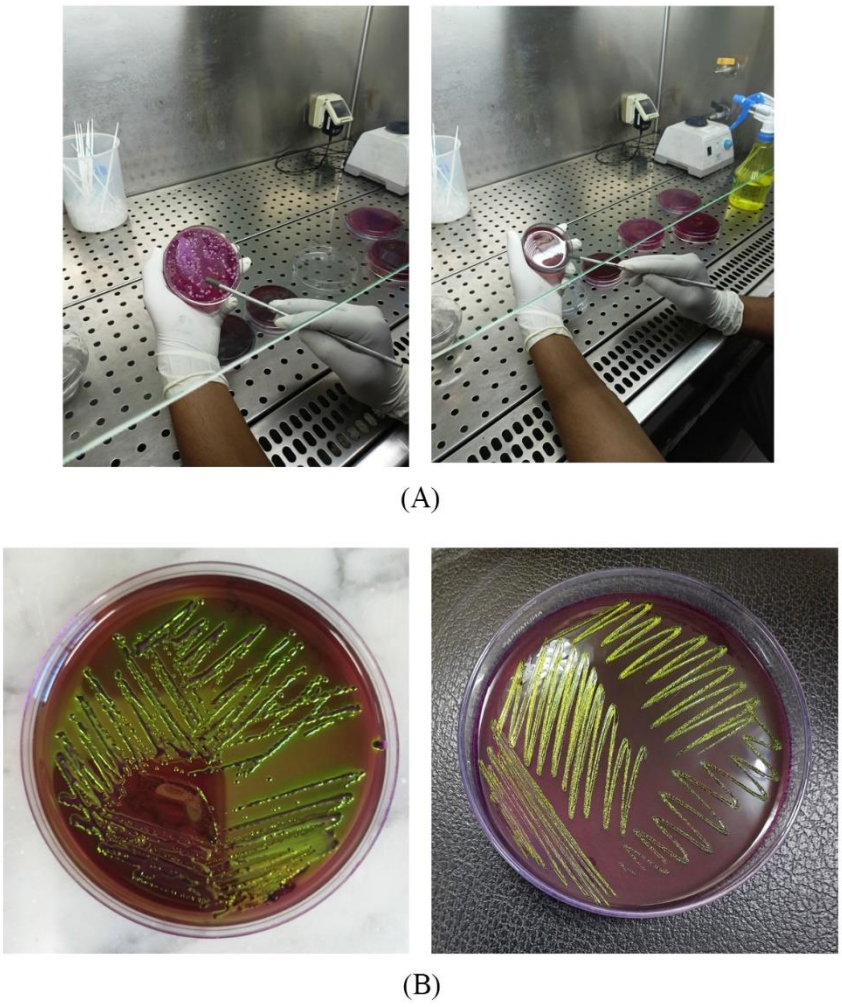


Figure 18: Confirmatory test for *E. Coli;*
A: Subculture from VRB agar to EMB Agar; B: Metallic Sheen in EMB Agar

3.6. Statistical Analysis

The 2013 version of Microsoft Excel was used for all types of data analysis. The one-way Analysis of Variance (ANOVA) test was used to determine whether there were any significant mean differences in the chemical, physical, and sensory characteristics of the cheese samples.

CHAPTER 4: RESULTS

4.1 Sensory Evaluation

A panel of judges conducted the sensory evaluation of cheese. Each judge assigned a score to each cheese sample based on its color, finish, body, texture, and flavor. The sensory evaluation scores of Dhaka Cheese samples and Mozzarella cheese samples are shown in Table 1 and Table 2, respectively.

According to the study, there was no significant variation in scores in the sensory evaluation of Dhaka Cheese. According to statistical analysis, the jury's scores on color, Finish, body, texture, and flavor were nearly identical.

In the case of Mozzarella cheese samples, judge's scores on color, body, and flavor were nearly identical. However, there was significant variation in the scores for both finish and texture. The texture score (Mean ± SD) of the MC_1 sample was 10.25 ± 0.5, which was significantly lower than the texture scores of the other samples. For both MC_1 and MC_2 significant score difference was found in finish and appearance compared to others.

Judges also mentioned some defects in their observations. e.g. Texture defects like (gas holes, mechanical holes), Flavor and aroma defects (over salted, bitter flavor).

Table 1: Sensory Evaluation of Dhaka Cheese:

Attributes	***Full Score***	***DC_1***	***DC_2***	***DC_3***	***DC_4***	***DC_5***	***P Value***	***Level of Sign.***
Color	10	7.67± 0.58	8.50 ± 1.50	8.67 ± 1.15	8.67 ± 0.58	8.67± 0.58	0.660	NS
Finish and Appearance	15	10.17 ± 0.29	12.50 ± 1.32	12.50 ± 1.50	12.33 ± 2.89	11.67 ± 1.53	0.450	NS
Body	15	10.50 ± 0.50	12.67 ± 0.58	12.50 ± 0.50	12.00 ± 1.73	12.00 ± 1.00	0.134	NS
Texture	15	12.67 ± 0.58	12.67 ± 1.15	12.50 ± 1.32	12.00±2.65	11.33 ±1.15	0.792	NS
Flavor and Aroma	45	37.17± 0.76	38.33 ± 1.53	37.67± 2.08	39.33± 1.15	38.00± 2.00	0.558	NS

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

Table 2: Sensory Evaluation of Mozzarella Cheese:

Attributes	***Full Score***	***MC_1***	***MC_2***	***MC_3***	***MC_4***	***MC_5***	***P Value***	***Level of Sign.***
Color	10	7.00± 1.41	5.57 ± 0.53	8.33 ± 0.58	7.87± 0.83	8.43± 0.40	0.197	NS
Finish and Appearance	15	8.50 ± 1.29	8.86 ± 0.69	11.83 ± 0.76	12.75 ± 1.16	11.67 ± 0.58	0.006	*
Body	15	10.75± 2.06	9.28 ± 1.38	12.6 ± 0.53	13.12 ± 0.83	12.17 ± 0.76	0.180	NS
Texture	15	10.25 ± 0.50	12.14 ± 1.21	13.17 ± 0.29	12.50 ± 0.76	12.83± 0.76	0.0096	*
Flavor and aroma	45	31.50 ± 3.79	29.57 ± 0.53	37.00 ± 2.65	37.50 ± 2.27	38.67± 1.15	0.077	NS

NS= Not statistically significant; *statistically significant at $p \leq 0.01$

Sensory evaluation score of Dhaka cheese and Mozzarella cheese against every sensory attributes are presented in Figure 19 and Figure 20 respectively.

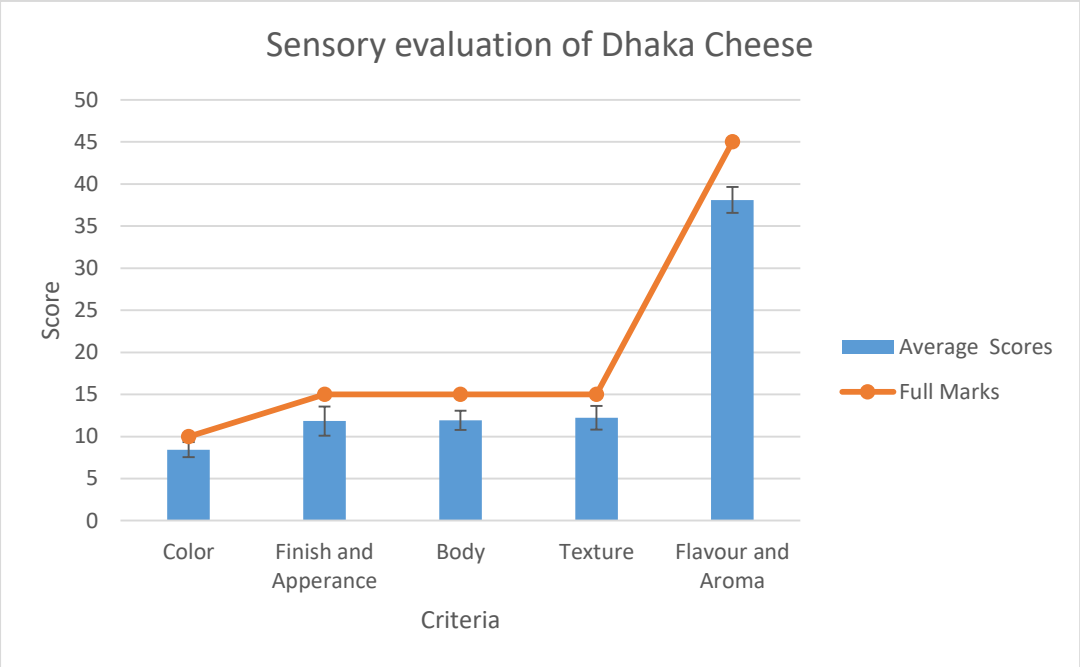


Figure 19: Sensory evaluation scores of Dhaka Cheese

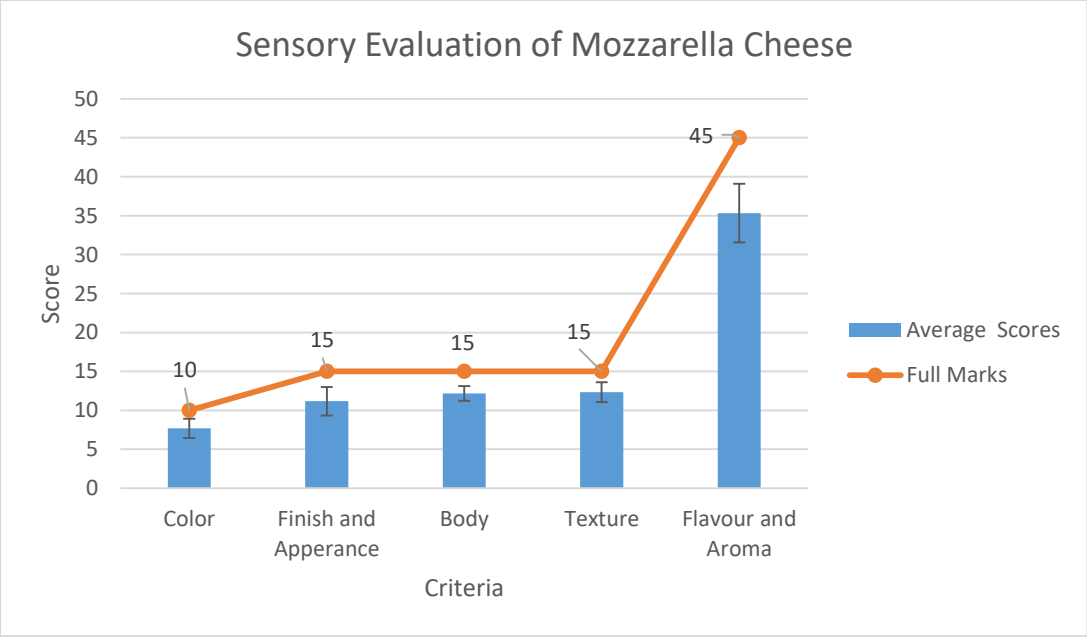


Figure 20: Sensory evaluation scores of Mozzarella Cheese

Comparative sensory scores (Mean ± SD) on different attributes of Dhaka cheese and Mozzarella cheese are demonstrated in Table 3. Sensory scores are almost identical of two cheese types.

Table 3: Comparison of Sensory test scores (Mean ± SD) of Dhaka cheese and Mozzarella Cheese:

Attributes	Dhaka cheese	Mozzarella cheese
Color	8.43 ± 0.90	7.69 ± 1.24
Finish and Appearance	11.83 ± 1.72	11.17 ± 1.83
Body	11.93 ± 1.15	12.15 ± 0.95
Texture	12.23 ± 1.40	12.33 ± 1.28
Flavor and Aroma	38.10 ± 1.54	35.33 ± 3.77

4.2 Chemical Analysis

4.2.1 Chemical Analysis of Dhaka Cheese

The chemical composition of Dhaka Cheese samples is shown in Table 4. The moisture, dry matter, and protein content of the Dhaka Cheese samples tested were nearly identical. DC_2 Cheese had the highest moisture content (50.319 ± 3.977) and DC_3 Cheese had the lowest (41.406 ± 2.570). The fat (%) of the tested samples showed significant variation. According to the study, DC_1 Cheese has the highest fat content (29.968± 4.443), while DC_2 Cheese has about (22.734 ± 1.096%). Significant differences were also observed in the acidity (%), ash (%), and salt (%) of the tested samples.

Table 4: Chemical Analysis (Mean±SD) of Dhaka Cheese:

Attributes	***DC1***	***DC2***	***DC3***	***DC4***	***DC5***	***P Value***	***Level Of Sign.***
Moisture	49.88 ± 9.12	50.32 ± 3.98	41.41 ± 2.57	48.47 ± 3.24	46.73 ± 2.96	0.130	NS
Dry Matter	50.12 ± 9.12	49.68 ± 3.98	58.59 ± 2.57	51.53 ± 3.24	53.27 ± 2.96	0.130	NS
Fat	29.97 ± 4.44	22.73 ± 1.10	29.45 ± 1.83	24.17 ± 2.19	27.93 ± 1.12	8.69×10^{-8}	*
Crude Protein	20.97 ± 1.47	21.76 ± 1.61	20.76 ± 0.83	19.16 ± 1.88	20.56 ± 0.73	0.166	NS
Acidity	1.48 ± 0.19	0.86 ± 0.26	1.28 ± 0.07	2.30 ± 0.46	1.02 ± 0.06	1.71×10^{-9}	*
Ash	3.49 ± 0.17	5.02 ± 0.58	4.79 ± 0.42	3.22 ± 0.23	3.50 ± 0.49	2.21×10^{-5}	*
Salt (NaCl)	1.88 ± 0.57	2.66 ± 0.42	2.86 ± 0.17	2.43 ± 0.05	2.85 ± 0.14	0.015	**

NS= Not Statistically Significant; **statistically significant at $p\leq0.05$; *statistically significant at $p\leq0.01$

From the data of Table 4 it is clear that there are notable differences in chemical compositions of different brands of Dhaka cheese. Moisture (%), Fat (%), Protein (%), Acidity (%), Ash (%), Salt (%) of different brands of Dhaka cheese are represented graphically in Figure 21, Figure 22, Figure 23 Figure 24, Figure 25 and Figure 26 respectively.

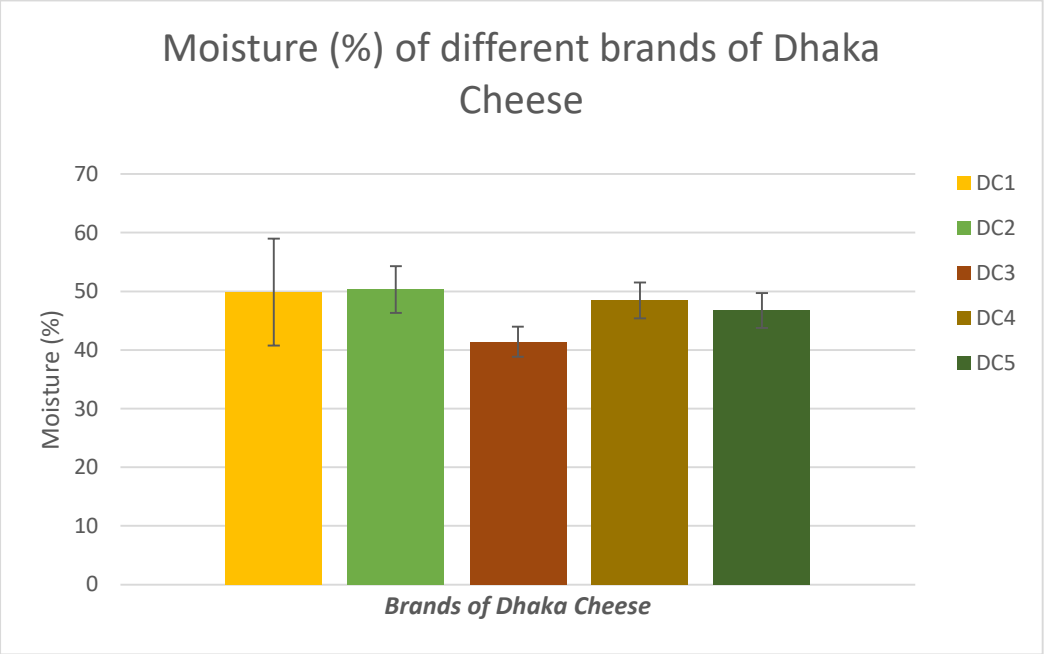


Figure 21: Moisture (%) of different brands of Dhaka Cheese

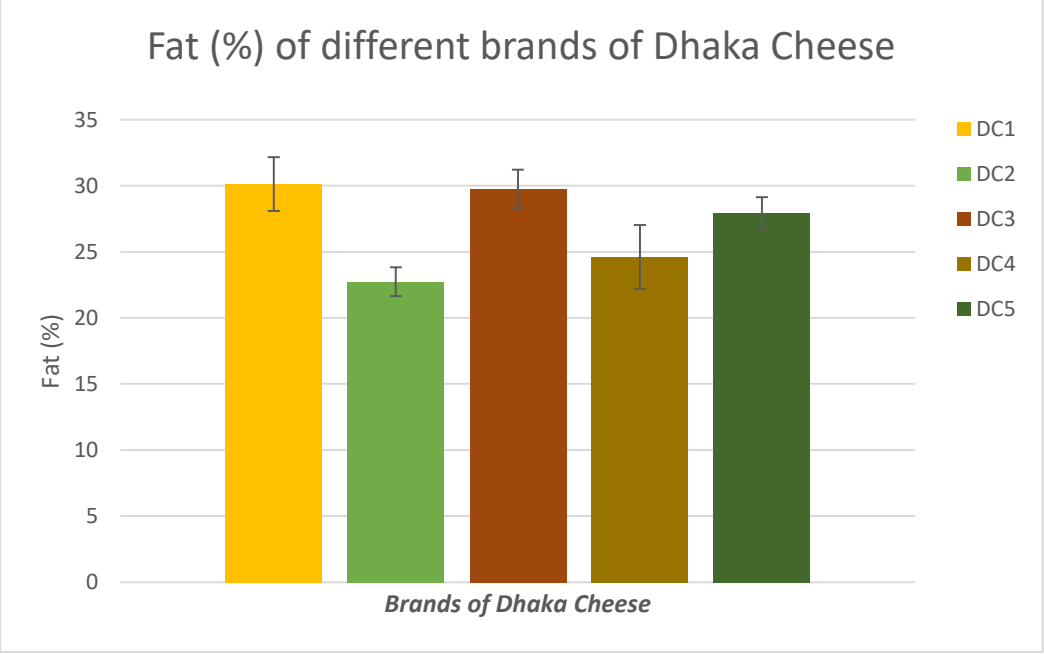


Figure 22: Fat (%) of different brands of Dhaka Cheese

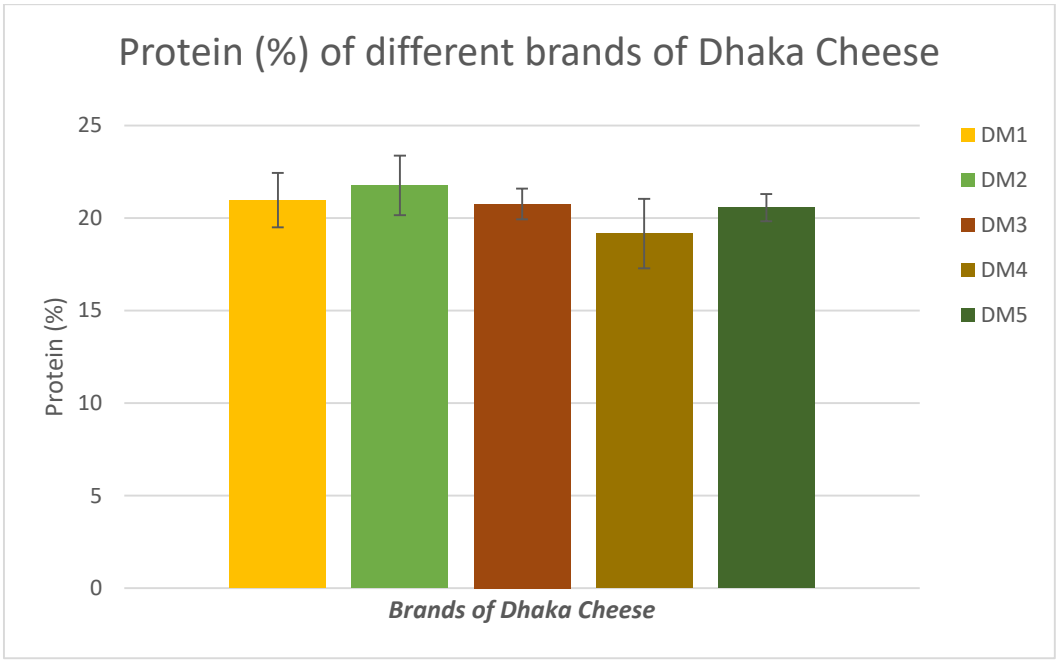


Figure 23: Protein (%) of different brands of Dhaka Cheese

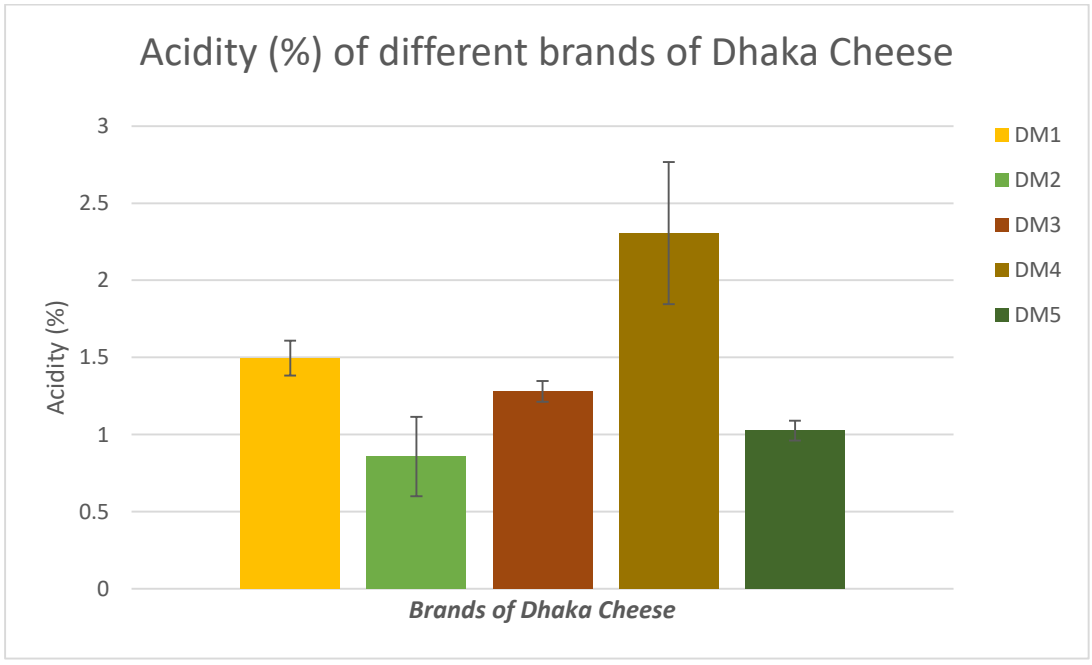


Figure 24: Acidity (%) of different brands of Dhaka Cheese

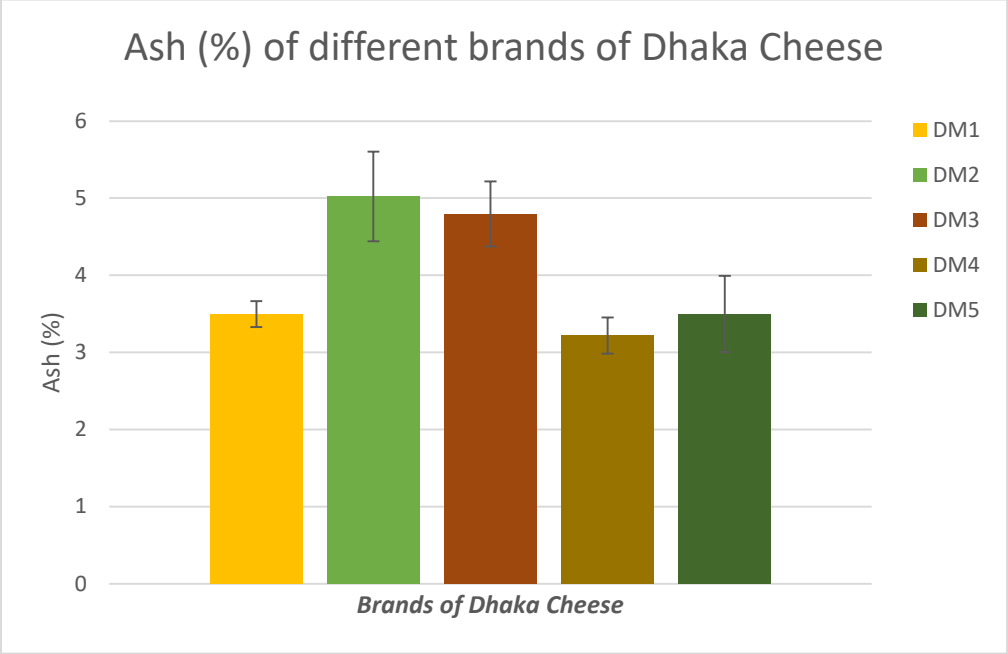


Figure 25: Ash (%) of different brands of Dhaka Cheese

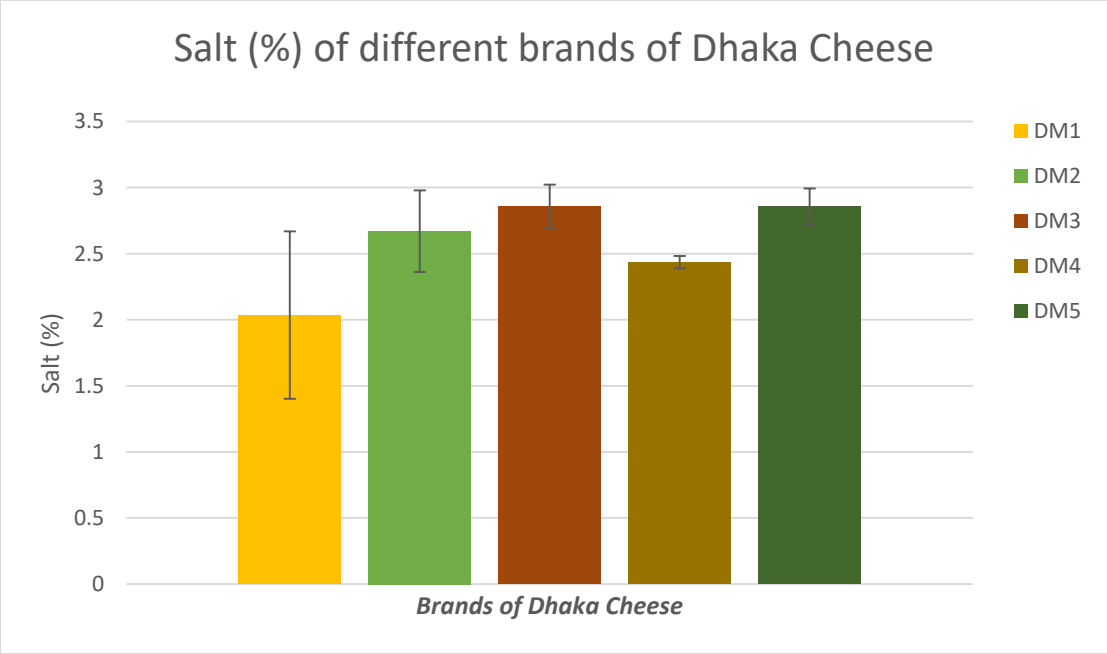


Figure 26: Salt (%) of different brands of Dhaka Cheese

Ash content in food samples mainly denotes different salts (ionic compounds) persisted in food samples. On the other hand common salt (NaCl) is an important ingredients of Dhaka cheese. So, comparison of Ash (%) and NaCl (%) is demonstrated in Figure 27.

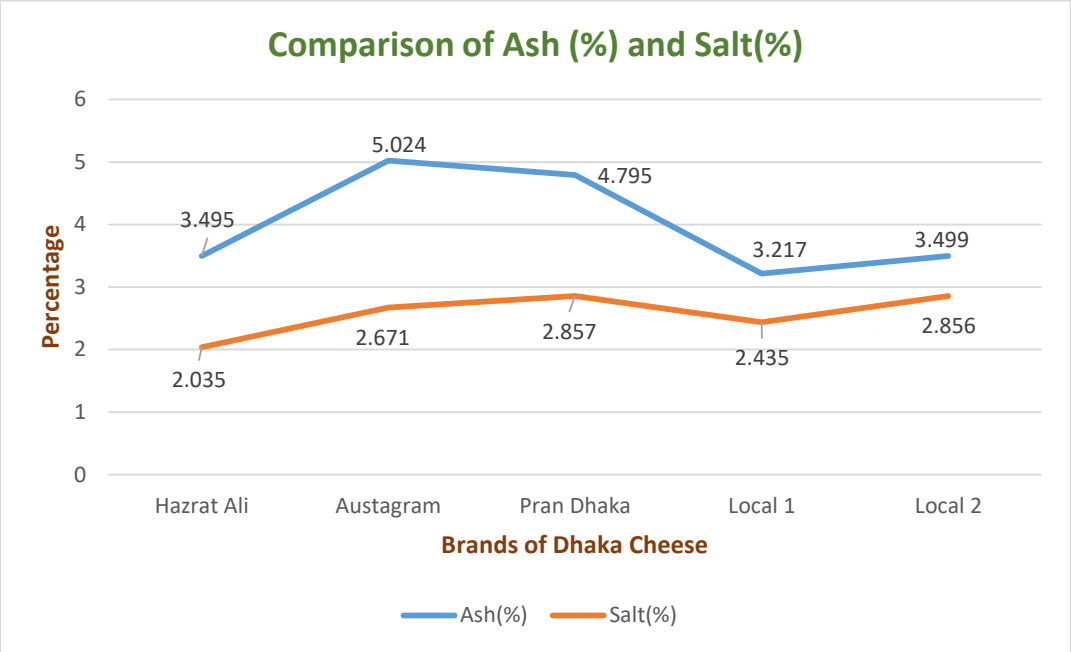


Figure 27: Comparison of ash (%) and salt (%) in different brands of Dhaka cheese

4.2.2 Chemical Analysis of Mozzarella Cheese

The results of chemical analysis of Mozzarella Cheese samples demonstrates in Table 5. Similar to Dhaka Cheese, no significant mean difference was noted among the samples of Mozzarella cheese in terms of Dry matter and moisture content. Significant mean difference was observed in Crude Protein content of Mozzarella cheese samples while no prominent difference was observed in fat content. In accordance with table 4, it is clear that Mozzarella Cheese samples contain less acidity percentage, ash and salt content than Dhaka Cheese.

Table 5: Chemical Analysis (Mean±SD) of Mozzarella Cheese:

Attributes	***MC1***	***MC2***	***MC3***	***MC4***	***MC5***	***P Value***	***Level of Sign.***
Moisture	48.13 ± 1.87	55.53 ± 5.50	49.99 ± 0.43	51.38 ± 4.37	47.46 ± 3.48	0.054	NS
Dry Matter	51.87 ± 1.87	44.47 ± 5.50	50.01 ± 0.43	48.62 ± 4.37	52.54 ± 3.48	0.054	NS
Fat	16.37 ± 1.68	18.11 ± 6.72	17.95 ± 5.28	22.00 ± 2.98	22.37 ± 1.47	0.094	NS
Crude Protein	28.20 ± 3.13	22.46 ± 1.71	24.17 ± 0.96	22.24 ± 0.95	25.70 ± 1.33	0.00002	*
Acidity	0.71 ± 0.22	1.47 ± 0.54	0.96 ± 0.39	1.69 ± 0.32	0.72 ± 0.02	0.00005	*
Ash	3.25 ± 0.24	2.20 ± 0.15	3.27 ± 0.06	1.90 ± 0.15	3.39 ± 0.03	2.47×10^{-10}	*
Salt (NaCl)	0.91 ± 0.24	0.30 ± 0.08	1.05 ± 0.24	0.30 ± 0.07	1.56 ± 0.33	5.28×10^{-6}	*

NS= Not statistically significant; * statistically significant at $p \leq 0.01$

From the data of Table 5 it is clear that there are notable difference in chemical compositions of different brands of Dhaka cheese. Moisture (%), Fat (%), Protein (%), Acidity (%), Ash (%), Salt (%) of different brands of Dhaka cheese are represented graphically in Figure 28, Figure 29, Figure 30, Figure 31, Figure 32 and Figure 33 respectively.

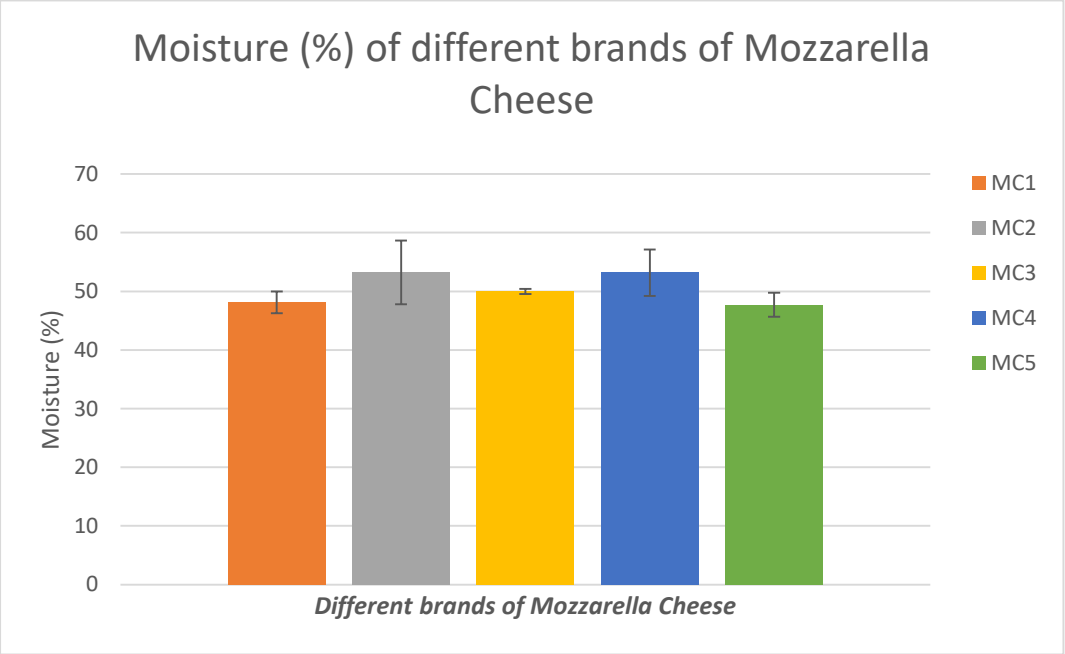


Figure 28: Moisture (%) of different brands of Mozzarella Cheese

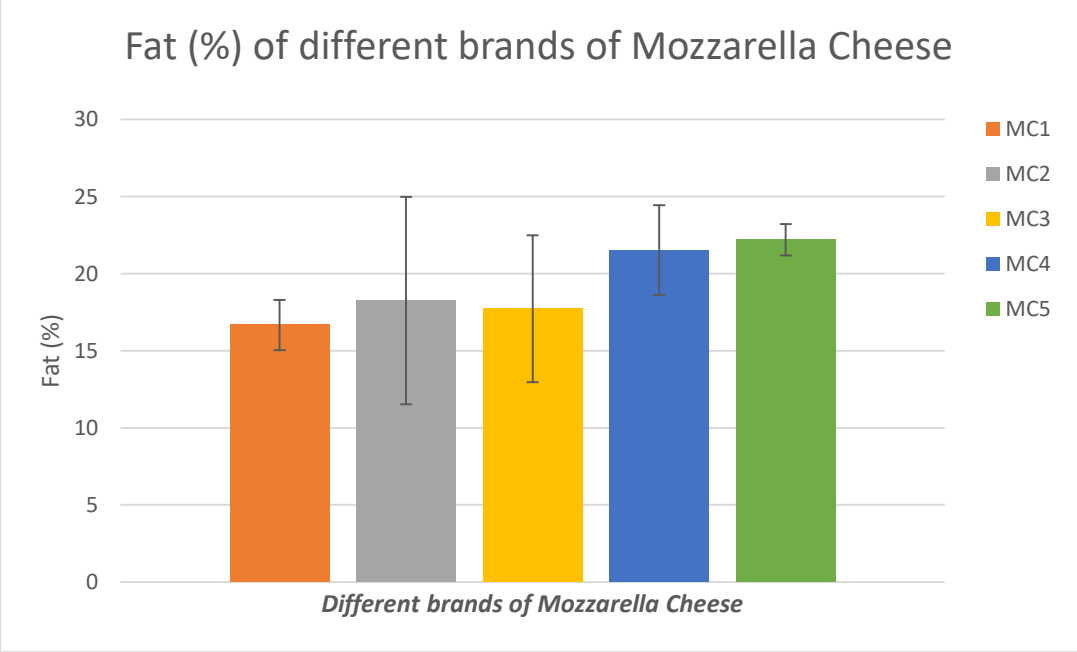


Figure 29: Fat (%) of different brands of Mozzarella Cheese

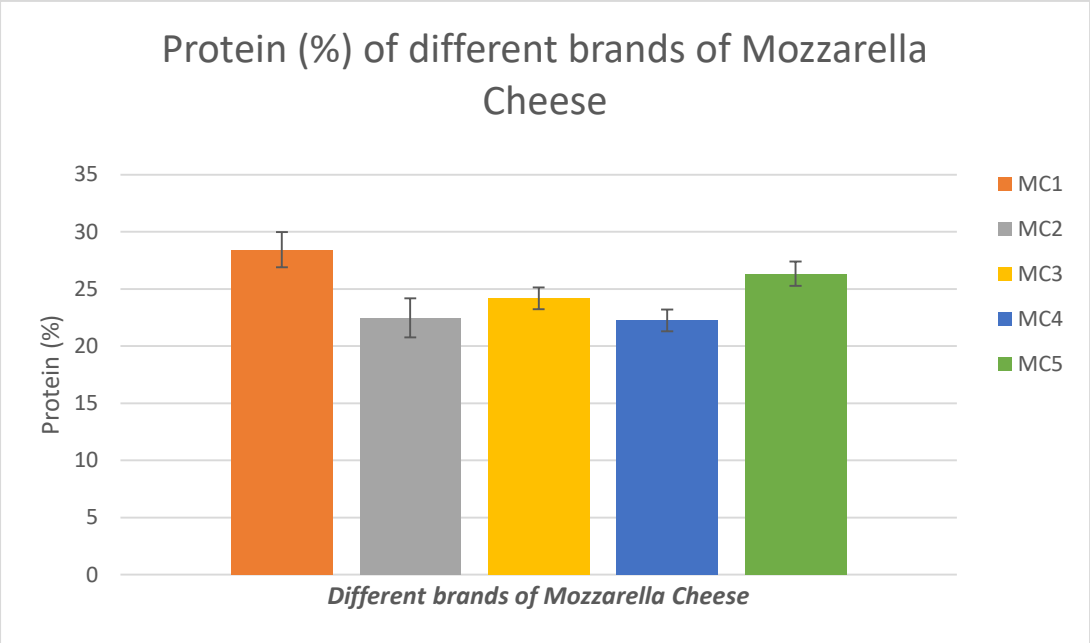


Figure 30: Protein (%) of different brands of Mozzarella Cheese

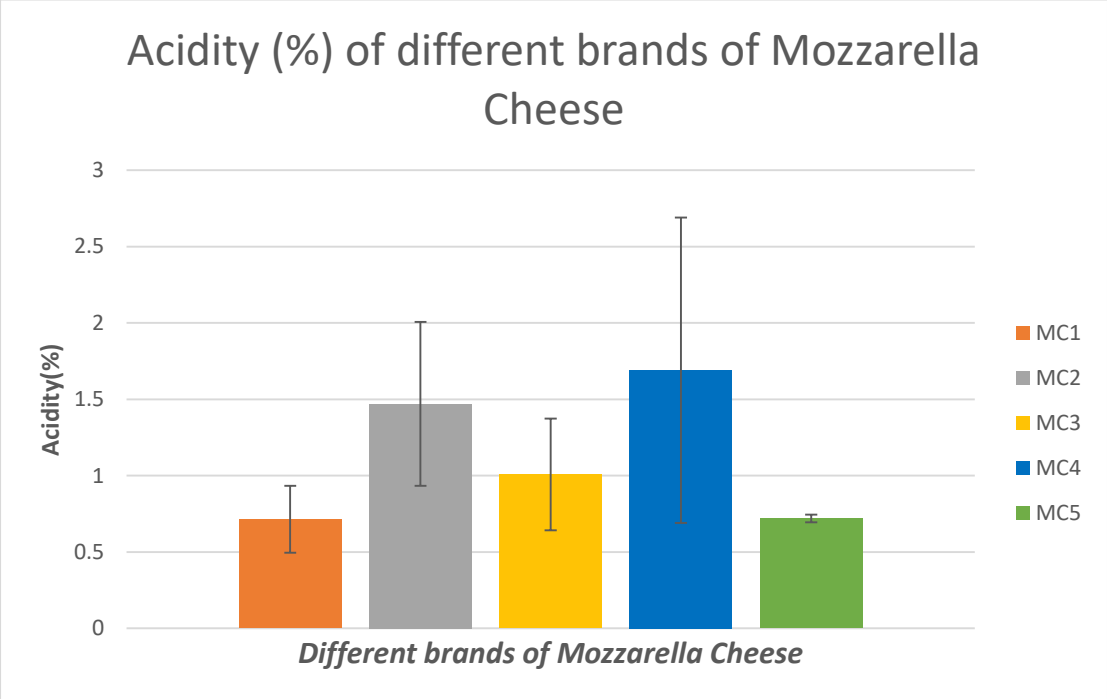


Figure 31: Acidity (%) of different brands of Mozzarella Cheese

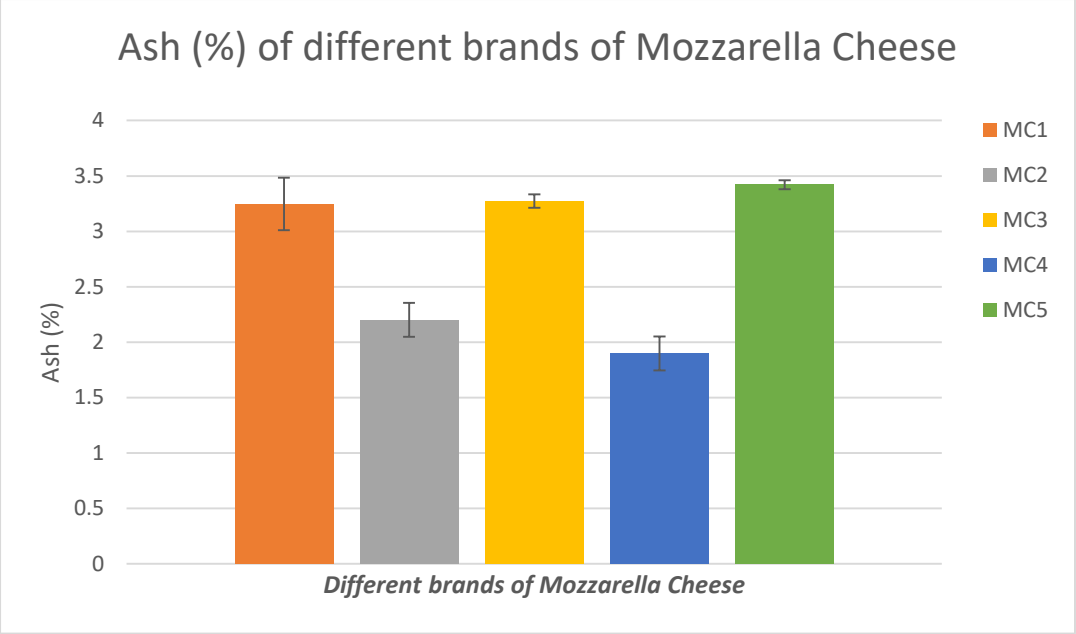


Figure 32: Ash (%) of different brands of Mozzarella Cheese

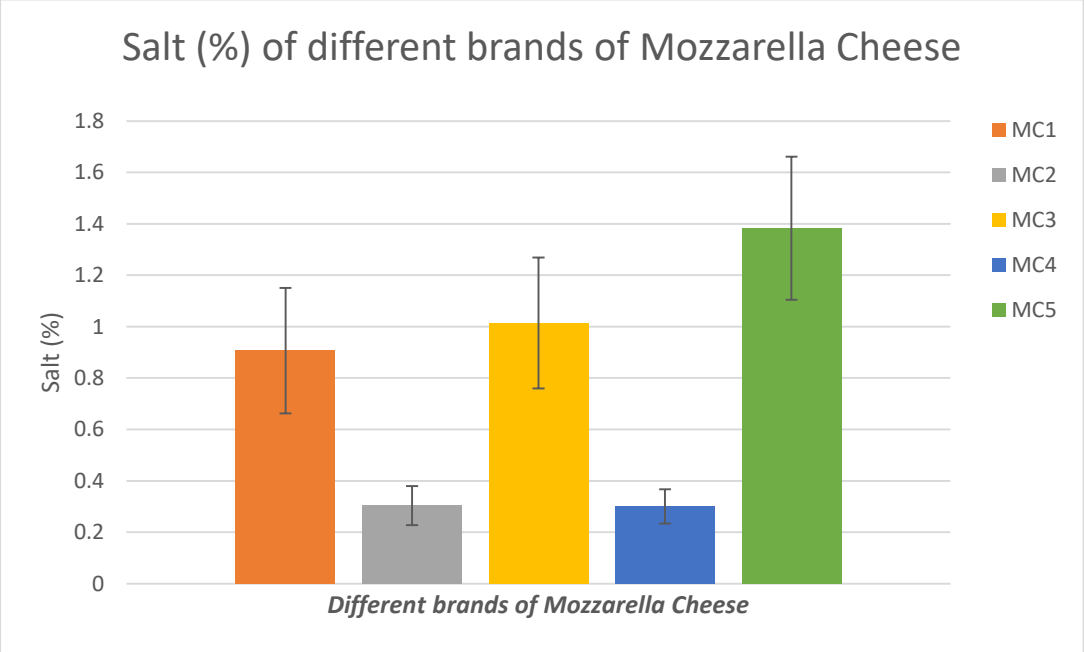


Figure 33: Salt (%) of different brands of Mozzarella Cheese

Ash content in food samples mainly denotes different salts (ionic compounds) persisted in food samples. On the other hand common salt (NaCl) is an important ingredient of cheese including Mozzarella cheese. So, comparison of Ash (%) and NaCl (%) is demonstrated in figure 34.

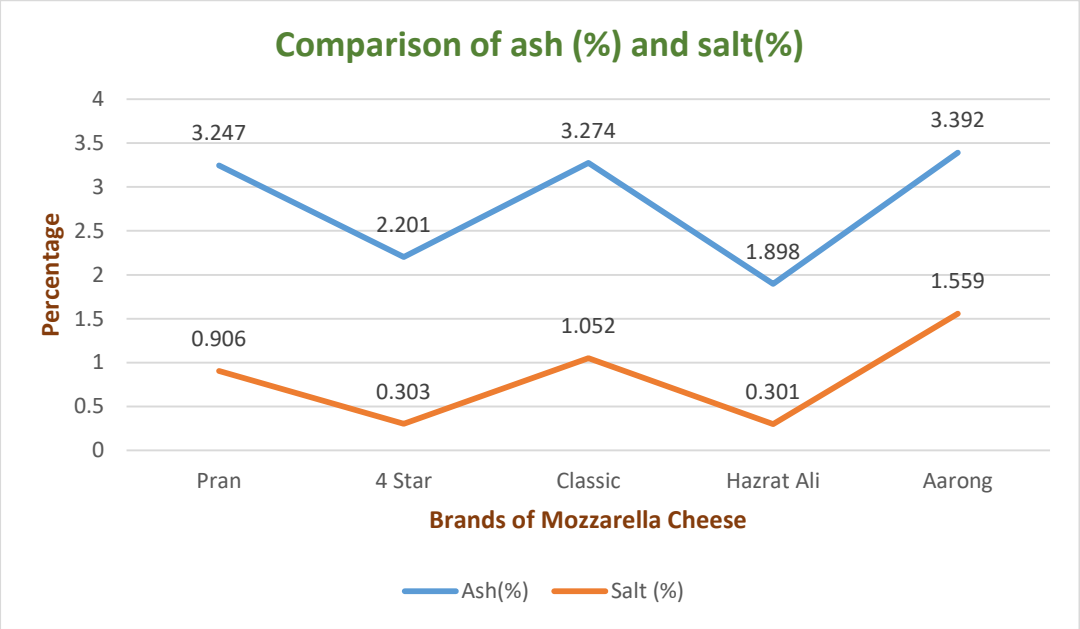


Figure 34: Comparison of ash (%) and salt (%) in different brands of Mozzarella cheese

Comparison between in terms of Dhaka cheese and Mozzarella cheese in terms of chemical composition are demonstrated in Table 6. Dhaka cheese samples contain higher fat and salt content than Mozzarella cheese. In contrast, Mozzarella cheese samples reported to contain higher protein content than Dhaka cheese samples.

Table 6: Comparison of Chemical composition (Mean ± SD) of Dhaka cheese and Mozzarella Cheese:

	DHAKA CHEESE	MOZZARELLA CHEESE
MOISTURE	47.360 ± 5.523	51.032 ± 4.546
DM	52.640 ± 5.523	48.968 ± 4.546
FAT	27.031 ± 3.358	19.272 ± 4.328
PROTEIN	20.644 ± 1.502	24.726 ± 2.681
ACIDITY	1.393 ± 0.562	1.120 ± 0.511
ASH	4.006 ± 0.850	2.808 ± 0.659
SALT	2.571 ± 0.432	0.781 ± 0.470

Graphical comparison of chemical compositions of Dhaka cheese and Mozzarella cheese is represented in figure 35.

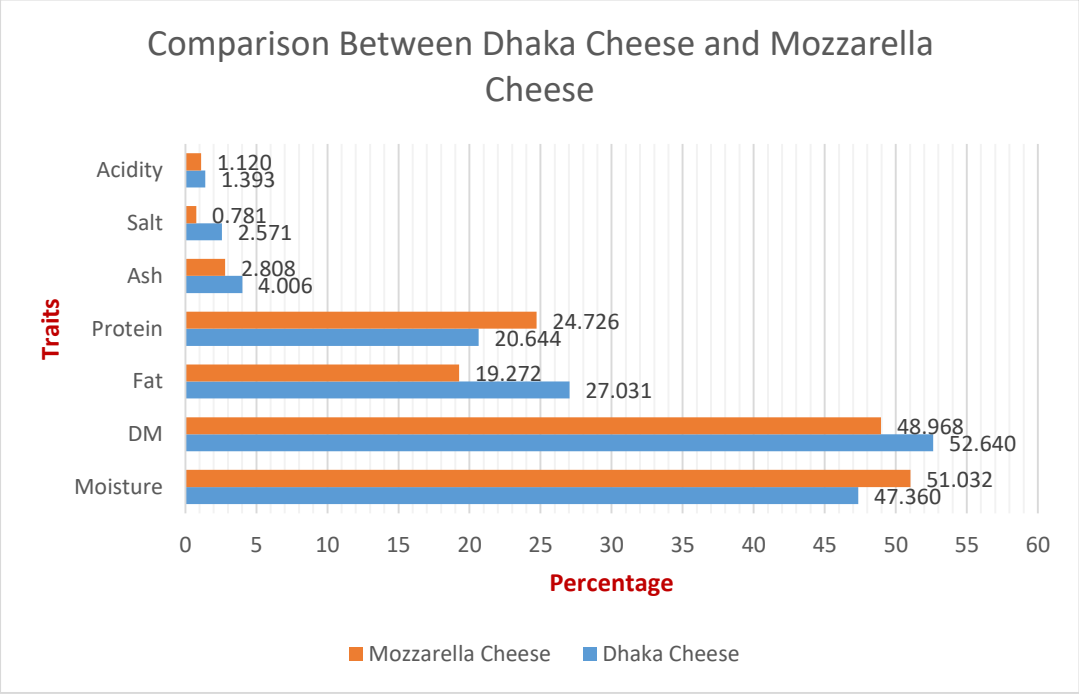


Figure 35: Comparison of chemical composition of Dhaka Cheese and Mozzarella Cheese

Microbial Analysis

4.3 Microbial Analysis

Result was shown as colony forming unit (cfu) in per gram or cfu/g.

4.3.1 Coliform Count of Dhaka Cheese

Except for one, all Dhaka Cheese samples were found to have a higher number of Coliform bacteria. *DC_4* had the highest coliform colony count (2.00×10^4cfu/g), while plates containing *DC_5* had no coliform colony growth. Among the samples where coliform growth was observed, *DC_1* cheese had the lowest coliform colony (1.50×10^2 cfu/g).

Table 7: Microbiological Analysis of Dhaka Cheese (in cfu/g):

	Batch 1	**Batch 2**	**Batch 3**	**Average**	**P Value**	**Level of Sign.**
DC_1	1.50×10^2	4.00×10^2	5.00×10^2	3.50×10^2	0.002	*
DC_2	1.65×10^3	3.00×10^2	5.00×10^2	8.16×10^2		
DC_3	2.00×10^2	5.00×10^2	2.00×10^2	3.00×10^2		
DC_4	2.00×10^4	7.00×10^3	1.05×10^4	1.25×10^4		
DC_5	NG	NG	NG	0		

NG= No growth; * statistically significant $p \leq 0.01$

4.3.2 Coliform Count of Mozzarella Cheese

Unlike Dhaka Cheese samples, almost all Mozzarella Cheese samples, with the exception of one, were reported to have no coliform growth on VRB media. Only *MC_4* brand samples were found to have colony growth (0.9-1× 10^2 cfu/g).

Table 8: Microbiological Analysis of Mozzarella Cheese (in cfu/g):

	Batch 1	**Batch 2**	**Batch 3**	**Average**	**P Value**	**Level Of Sign.**
MC_1	NG	NG	NG	0	1.37×10^{-12}	*
MC_2	NG	NG	NG	0		
MC_3	NG	NG	NG	0		
MC_4	9.00×10^1	1.00×10^2	1.00×10^2	9.66×10^2		
MC_5	NG	NG	NG	0		

NG= No growth; * statistically significant $p \leq 0.01$

CHAPTER 5: DISCUSSION

In this study, a total of 30 samples (5 brands each from both Mozzarella Cheese and Dhaka Cheese and 3 different batches under every brand) were taken for analysis. Physical and chemical analysis was performed based on the respective AOAC methods, while sensory analysis was conducted using a scorecard.

Sensory Analysis:

As per our study, scores of sensory attributes were on color 8.43 ± 0.90; finish and appearance 11.83 ± 1.72; body 11.93 ± 1.15, texture 12.23 ± 1.40, flavor and aroma 38.1 ± 1.54. Mizan *et al.*, (2010) conducted a sensory evaluation of mozzarella cheese based on some attributes- flavor and taste, body and texture, finish and color. In their study, the average score of flavor and taste, body and texture, finish and color were 40.1, 26.3, 12.5, and 8.2 respectively which are higher than our observations. According to the study of Zedan *et al.*, (2014) score on the appearance of freshly prepared mozzarella cheese was almost similar to our study, and the score on appearance 28 days after storage was slightly lower than our observations. A slight deviation was observed between our observations and the observations of Zedan *et al.*, (2014) in terms of body & texture, and flavor.

Chemical Analysis:

A study was carried out by Parvin *et al.*, (2008) on physiochemical characteristics of Dhaka Cheese. In the study, they revealed that moisture, ash and acidity content on zero (0) days of ripening and revealed moisture (%) of Dhaka cheese 40.8 to 41.1, which is lower than our study. As moisture (%) was lower in the study, DM (%) of examined sample would be higher than our observed value. Miah and Quddus (1970) stated that DM content of commercial Dhaka cheese was 60.7% while enumerated DM content of Dhaka cheese ranging from 55.7 to 61.2% as per study of Habib *et al.*, (2012). The observations of the authors on DM content are slightly higher than our observation.

The estimated fat (%) of Dhaka cheese according to our study was 27.031 ± 3.358, which is lower than observations of previous studies (Habib *et al.*, 2012; Miah and Quddus, 1970). According to our study, the ash (%) and acidity (%) of Dhaka cheese were lower and higher respectively, than the study conducted by Parvin *et al.*, (2008).

Parvin *et al.,* (2008) found total nitrogen content of 3% and 3.8% in their study which is equivalent to Crude Protein (CP) 19.14% and 24.24%, respectively. In our study, we found CP (%) 20.64 ± 1.50 somewhat similar to the result stated by Parvin *et al.,* (2008). On the contrary, another study conducted by Habib *et al.,* (2012) reported containing higher protein content in Dhaka cheese than our study.

As per the research of Miah and Quddus, (1970) salt content in commercial Dhaka cheese was ranging from 4.30 to 5.12%, which is almost significantly higher than our observed value range (1.882 ± 0.574 to 2.857 ± 0.166).

A study conducted by Goyal *et al.,* (2007) on traditional Paneer where estimated fat content 18-25 %, acidity content 0.40-0.72%, protein content 11.85-16.26%, ash content 1.00-1.15%, moisture content 54.04-59.25%. All the observations except the moisture content of Goyal *et al.,* (2007) were lower than our observations regarding Dhaka cheese.

A study on chemical analysis of Mozzarella cheese was conducted by Mizan *et al.,* (2010), El-owni and Oswan (2009), Jana (1992) and Pizaia *et al.,* (2003). The enumerated value of moisture (%), protein (%), and acidity (%) of collected mozzarella cheese samples of our study is higher and dry matter (%), fat (%), and salt (%) are lower than the observations of Mizan *et al.,* (2010), El-owni and Oswan (2009), Jana (1992), Pizaia *et al.,* (2003). Zedan *et al.,* (2014) conducted a study on Mozzarella cheese produced from different milk sources; in the study, they found moisture content 48.172-49.654 %, fat content 17.9-21.7 %, protein content 22.534-26.362 %. Among the observations, protein and fat content are in line with this study while moisture content was slightly lower than the observed result.

According to the criteria of Codex standard for cheese, our tested samples of Dhaka cheese and Mozzarella cheese can be categorized as semi-hard cheese based on MFFB (moisture-free fat basis) percentage (BSTI, 2008). Comparing our observation with the Codex standard for cheese (1978) it can be said that Dhaka cheese is a full-fat cheese variety, at the same time Mozzarella cheese is a medium-fat cheese variety in terms of fat in dry matter.

Microbial Analysis:

In our study, only one of five brands of Mozzarella Cheese was reported to have coliform colony growth; at the same time, the growth of coliform bacteria occurred in four out of five brands of Dhaka Cheese. Our study revealed that coliform colony was found in samples of 5 brands out of 10 tested brands. The rate of finding coliform colonies among the samples was 50% which was relatively higher than the study of Nur *et al.,* (2021). In their study Nur *et al.,* (2021) stated that four out of five cheese samples collected from Dhaka were free from coliform growth, the remaining sample was reported to have 2.3×10^3 cfu/ml. In accordance with Khan *et al.,* (2014). Total coliform count in cheese samples collected from different super shops of Dhaka market had huge microbial load ranging from 1×10^2 CFU/ml to 2.5×10^7 CFU/ml. On the other hand, the total coliform count of cheese as per our study lay between 0.9×10^2 cfu/g to 2×10^4 cfu/g. It is somewhat identical to the observations of (Khan *et al.,* 2014; Nur *et al.,* 2021).

No previous study was found on the microbial count of Dhaka cheese to the best of our knowledge. Goyal *et al.,* (2007); Singh and Singh (2000) conducted a study on Paneer, a traditional unripened variety of soft cheese. Among the samples of Dhaka cheese in our study average, Total Coliform Count was 2.6×10^3 cfu/g which was significantly higher than the observations of Goyal *et al.,* (2007). Singh and Singh (2000) estimated the coliform count of Paneer at 103.05 cfu/g, 1010.39 cfu/g respectively for laboratory-made samples and market samples which is much higher than our study. The coliform count of marketed Paneer was 1.19×10^2 cfu/g (Goyal *et al.,* 2007) which was significantly lower than our count.

As per our study, the lowest coliform count in Dhaka cheese was enumerated 1.5×10^2 cfu/g, whereas the highest count was 2×10^4 cfu/g among the samples where coliform growth occurred. Our observations are somewhat similar to the observations of Esho *et al.,* (2013); where they reported that 19.8% of total cheese samples were containing coliform growth with a minimum coliform colony count of 25 cfu/g while a maximum count was 3.0×10^6 cfu/g.

In our study, only one of five brands of Mozzarella Cheese was reported to have coliform colony growth; where the coliform colony count was 0.97×10^2 cfu/ml. Both, percentage of positive samples and total coliform count of mozzarella cheese samples of our study were lower than the study of Garbaj *et al.*, (2007). Laslo and Gyorgy (2018) enumerated the average coliform count of mozzarella cheese 5×10^3 cfu/g which is much higher than our study. This difference could be resulted from either use of milk with higher coliform count for cheese making or contamination while processing cheese.

CHAPTER 6: CONCLUSIONS

The study is conducted to evaluate marketed cheese quality in Bangladesh. The quality evaluation is performed in terms of sensory, chemical, and microbiological aspects of cheese samples. This study was mainly carried out on two cheese varieties, which are traditional Dhaka cheese and globally familiar Mozzarella cheese. In accordance with our study, the color, finish, and flavor score of Dhaka cheese is higher compared to Mozzarella cheese. In contrast, the score on body and texture of Mozzarella cheese is relatively higher than Dhaka cheese. No significant difference is observed in sensory attributes among the Dhaka cheese of different brand samples. But, a significant difference is noted in finish and texture among Mozzarella cheese brands. Dhaka cheese is reported to have higher fat and salt content than mozzarella cheese, whereas mozzarella cheese contains higher crude protein content. A significant difference is found among the brands of Dhaka cheese in fat, acidity, ash, and salt content. In the case of mozzarella cheese, a significant difference is observed in crude protein, acidity, ash, and salt content. Dhaka cheese samples contain a higher bacterial load than the samples of Mozzarella cheese. Fecal coliform is also reported in some Dhaka cheese samples. In this study, a higher bacterial load than the FAO standard is recorded in some samples. This study will help the consumers to know nutritional value of cheese as well as hygienic status of cheese manufactured in Bangladesh.

LIMITATIONS

A significant number of constraints arose throughout the study period. These could be considered before conducting a study in the future-

- No molecular technique was implemented to identify the micro-organisms.
- The sample size of the study was relatively small.
- Sensory evaluation was performed by judges, there were chances of personal biases.
- The potentiometric titration method could not be applied to determine NaCl (%) in cheese due to the lack of a potentiometer with a mercury (l) sulfate electrode.
- Throughout the study endpoint of titration was determined by visual power, no automated technique or potentiometer was used to determine the endpoint.

RECOMMENDATIONS

Cheese is a globally popular food with high nutritive value. Cheese is consumed either directly or as a supporting ingredient in cooking. So, cheese is directly involved with public health. In our study, evidence of coliform bacteria is found. For that reason, proper hygienic practices should be maintained in every step from milking to the packaging of final products. Further study can be introduced to determine the fecal coliforms loads in cheese samples of Bangladeshi market.

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Appendix 1

SCORE CARD FOR EVALUATING PHYSICAL QUALITY OF CHEESE

Sample No:

Batch No:

Judge No:

Date of Judging:

Remarks:

Name of Attribute	***Criteria***	***Full Marks***	***Obtained mark***	***Defects***
Color	• Uniformed color • Not artificially colored	10		☐ Faded/bleached ☐ High/unnatural mottled ☐ Seamy ☐ Uneven/ wavy

Name of Attribute	***Criteria***	***Full Marks***	***Obtained mark***	***Defects***
Finish and Apperance	• Smooth • Neat, clean, unbroken appearance	15		☐ Cracked surface ☐ Misshapen surface ☐ Mouldy surface ☐ Huffed surface

Name of Attribute	***Criteria***	***Full Marks***	***Obtained mark***	***Defects***
Body	• Slightly elastic • Firm but not hard when crushed between fingers	15		☐ Dry/ hard body ☐ Crumbly body ☐ Curdy body ☐ Greasy body ☐ Watery body ☐ Soft body

Name of Attribute	*Criteria*	*Full Marks*	*Obtained mark*	*Defects*
Texture	• Compact • Continuous and homogenous • Free from openings, holes, cracks and fissures	15		□ Gas/Pin holes □ Mechanical holes □ Fish eye/ yeast holes

Name of Attribute	*Criteria*	*Full Marks*	*Obtained mark*	*Defects*
Flavor and aroma	• Pleasing aroma • Mildly salted in taste • Causes a pleasant sensation within mouth • After swallowing taste resembling the flavor of sweet nuts	45		□ Sour flavor □ Bitter flavor □ Mouldy flavour

BIOGRAPHY

Myself, Sujoy Barua, son of Dilip Kumar Barua and Rupna Barua, I was born in a little village in Chattogram, Bangladesh. I completed my S.S.C. from Chittagong Government High School in 2011 and my H.S.C from Noubahini School and College, Chittagong in 2013. I completed my graduation on Doctor of Veterinary Medicine (DVM) from CVASU in 2019. Currently, I am a Post Graduate student of Dairy science at the Department of Dairy and Poultry sciences, Faculty of veterinary medicine, Chittagong Veterinary and Animal Sciences University (CVASU). I intend to pursue my future research on dairy product marketing and dairy microbiology.