Investigation of selective food-borne pathogens and quality evaluation of Dahi Marketed at Chattogram Metropolitan Area of Bangladesh

Roll No. 0118/04

Registration No. 491

Session: 2018-2019

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

Department of Dairy and Poultry Science

Faculty of Veterinary Medicine

Chattogram Veterinary and Animal Sciences University

Chattogram-4225, Bangladesh

DECEMBER, 2019

Dedicated To My

Beloved Family

Authorization

I hereby declare that I am the sole author of this thesis. I also authorize the Chattogram Veterinary and Animal Sciences University (CVASU) to lend this thesis to other institutions or individuals for the purpose of scholarly research. I further authorize CVASU to reproduce the thesis by photocopying or by other means in total or in part, at the request of other institutions or individuals for the purpose of scholarly research.

I, the undersigned, and author of this work, declare that the electronic copy of this thesis provided to the CVASU Library, is an accurate copy of the print thesis submitted, within the limits of the technology available.

.....

Setara Akter

December, 2019

Investigation of selective food-borne pathogens and quality evaluation of Dahi Marketed at Chattogram Metropolitan Area of Bangladesh

Roll No. 0118/04

Registration No. 491

Session: 2018-2019

This is to certify that we have examined the above Master's thesis and have found that the thesis is complete and satisfactory in all respects, and that all revisions required by the thesis examination committee have been made

.....	.....
Professor Dr. A. K. M. Humayun Kober	**Assistant professor DR. Nasima Akter**
Supervisor	**Co-Supervisor**

.....

Professor Dr. A. K. M. Humayun Kober

Head & Chairman of the Examination Committee

Department of Dairy and Poultry Science

Faculty of Veterinary Medicine

Chattogram Veterinary and Animal Sciences University

Khulshi, Chattogram-4225, Bangladesh

December, 2019

Acknowledgements

First and foremost , I praises and thanks to the Almighty "ALLAH '' for His showers of blessings throughout my research work to complete the research successfully for the degree of Master of Science (MS) in Dairy Science under the Department of Dairy and Poultry Science, Chattogram Veterinary and Animal Sciences University (CVASU) .

I would like to express my heartfelt gratitude, profound appreciation and indebtedness to my research supervisor Dr. A. K. M. Humayun Kober, Professor and Head, Department of Dairy and Poultry Science, CVASU for his cordial help to conduct this experiment and also for overall supervision, valuable suggestions, critical but constructive criticisms, helpful comments which made easy for successful completion of this thesis.

I am indebted to my co-supervisor DR. Nasima Akter, Assistant Professor, Department of Dairy and Poultry Science, CVASU for her valuable advice, constant guidance and encouragement during the experiment.

I sincerely thank to the Coordinator of Advance Studies and Research for providing research fund to complete my research work.

I would like to expresses my gratitude to all technical and non-technical staffs of the Poultry Research and Training Centre (PRTC), CVASU for their cordial helps during the research period.

I sincerely thank to all the teaching and technical staffs of the Department of Dairy and Poultry Science for their help.

I am extremely grateful to my parents, friends and family whose blessings and sacrifices and encouragement in every steps of my life can never be repaid.

The Author

December, 2019

Table of Contents

List of Abbreviations

Abbreviation	Elaboration
EMB	Eosin Methylene Blue
XLD	Xylose lysine deoxycholate agar
Mac	Mac conkey agar
Sp.	Species
No.	Number
E. coli	*Escherichia coli*
°C	Degree centigrade
Kg	Kilogram
Mg	Milligram
gm	Gram
<	Less than
>	Greater than
=	Equal
cfu	Colony forming unit
Fig	Figure
e.g	Example
et al.	And his associates
etc.	Et cetera
%	Percentage
i.e.	That is
Sig.	Significance
Ref.	Reference
FAO	Food and Agriculture Organization
WHO	World Health Organization
FDA	Food and Drug Administration
UHT	Ultra High Temperature
APHA	American Public Health Association

MS	Master of Science
CVASU	Chattogram Veterinary and Animal Sciences University

List of Tables

List of Figures

Abstract

Fermented milk products are part of the diet in many parts of the world and are consumed on a regular basis. Enhancing the nutritional and therapeutic properties of traditional fermented milk can improve the global public health. Dahi quality and safety is an increasingly important public health matter. Dahi might be contaminated with pathogens due to poor packaging, unhygienic handling and inadequate transport system. Presence of these organisms are critical for the safety of fermented products during consumption. Measures should be taken to interrupt the transmission of pathogens to dahi at the household level. So, the aim of this study was to isolate the zoonotic microorganisms (*Escherichia coli and Salmonella sp*) and evaluate the quality of dahi samples marketed at Chattogram metropolitan area. A total of fifty (50) dahi samples (local and branded) were collected from July to October 2019, in different shops of Chattogram metropolitan area and tested for the presence of *E. coli* and *Salmonella sp* as well as for the total viable count of bacteria. Collected samples were also examined for their chemical quality (acidity, fat and protein percentage). All the samples were inoculated on respective selective bacteriological media for primary isolation of the pathogens of interest. Then the positive isolates were subjected to different biochemical tests for further confirmation. At the same time some qualitative tests of the dahi samples were also performed followed standard laboratory procedure. Significant differences were found in microbial (total viable count) and chemical (percentage of acidity, fat, protein) quality of dahi samples. The average percentage of fat, protein and acidity collected dahi samples ranges from 3.43±0.18 to 3.58±0.11, 4.58±0.34 to 5.13±0.17 and 0.87±0.02 to 0.75±0.03, respectively. The total viable count of all samples ranges within the standard level. All the samples were negative for *E. coli* and *Salmonella sp*, that indicates proper hygienic handling, preparation, marketing and storage of dahi. Since all the collected dahi samples of this study were negative for *E. coli and Salmonella sp*, so it can be said that the dahi samples that were collected from different retail shops of Chattogram metropolitan area were safe for human consumption.

Key words: Dahi, *E. coli, Salmonella sp*, Public health

Chapter-I

Introduction

Fermented dairy foods play an important role in the human diet. Dahi is a popular indigenous fermented milk product throughout South Asian countries such as Bangladesh, India, Nepal, Pakistan, Sri Lanka, etc. Curd (Dahi) and yoghurt are two different fermented dairy product, differing in the method of preparation. But in our country, both curd and yoghurt are termed as Dahi. The word curd is used in Indian English to refer to traditional probiotic yoghurt, while the term yoghurt refers to the pasteurized variety. Dahi is easy to digest and has been found to reduce the risk of cardiovascular problems and cancerous tumors, besides strengthening general immunity (Sinha and Sinha 2000). Yoghurt, a western counterpart of dahi, besides its nutritive value, is believed to be effective in both the prevention and treatment of various illnesses such as gastrointestinal disorders, heart diseases and tumor development, in man as well as animals (Deeth and Tamime 1981). Several health benefits of dahi and yoghurt have been reported (Arvind et al. 2009; Pala et al. 2011; Yadav et al. 2008).

People who regularly consume dahi gets a lot of benefit from it (Fig.1). Considering the benefits of eating yoghurt, from regulating bowel movements and promoting a healthy gut to fighting infections, we should incorporate this super food into our diet every day. It contains all the nutrients present in milk except a little variation in lactose content. People who has lactose intolerance syndrome can easily digest dahi. Dahi has also a special social value as being served and consumed in all festivals and occasions. Mainly two types of dahi are available in local markets here, sweetened / mistidahi (sugar added) and

Figure 1: Benefit of dahi consumption

sour dahi and both are prepared by a traditional method using previously made dahi (starter). Dahi, especially sweet dahi, is one of the most popular fermented milk products of Bangladesh.

The nutritive value of milk and products rely on their cleanliness, purity and wholesomeness. Although, fermented milk products are safer foods i.e. disease producing organisms cannot survive there in high acidity, still if contamination occurs then in most cases yeasts and molds and sometimes coliform organisms can grow somewhat, if this is having proper nutrition. According to US public health milk and milk products should not contain more than 20,000 bacteria/gm and 10 coliform bacteria/gm (Bhowmick et al., 2006). That is why; a desirable standard for the manufacture of dahi should be established according to the average consumers of Bangladesh. Traditional method invariably involves production on a small scale, either in the consumer's household or in the sweetmeat-maker's shop in urban areas. In the household , milk is heated to boiling temperature until volume is reduced up to 15-20% and 8-10% sugar added (sweetened dahi), cooled down to body temperature, inoculated 2-3% starter and poured into earthenware and kept for curd formation overnight by wrapping woolen cloth or straw or jute bag to maintain warmth. In the shops, the method is more or less the same and dahi is usually set in suitable containers (earthenware/ glass bottles/plastic cups) of the required capacity (Dey et al., 2011). Most of the producers are used earthenware for setting dahi to firmness rather than glass bottles/ plastic cups. Earthenware is assisting to absorb and evaporate a little amount of moisture from the dahi resulted more firmness. The quality and color of dahi are varies from shop to shop and area to area depending by using the different proportions of starter culture and color additives. Various means and methods are adopted in its preparation that results in variations among the quality of products. Although, dahi is prepared without any care of quality control and hygienic conditions and contain a lot of contaminants, which may be health hazardous spontaneously. In Bangladesh, dahi are sold almost in open markets and kept on shelf at ambient temperature. On the other hand, A few sellers of city areas are kept their products in refrigerators for prolonging storage and other kept their products at room temperature which is prone to deterioration of both chemical and microbial quality of dahi (Fig. 2).

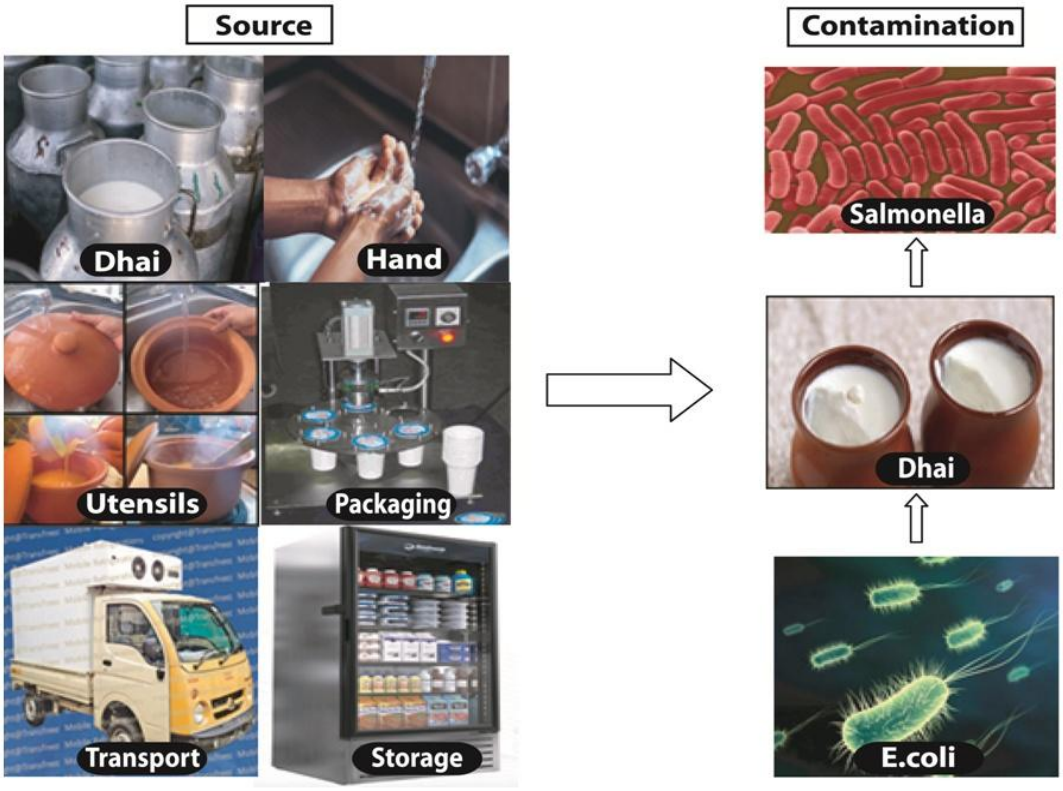


Figure 2: Source of contamination

Dahi quality and safety is an increasingly important public health issue. Nowadays, the topics "food quality" and "food safety" are very close and two important issues in the food sector, due to the globalization of the food supply and the increased complexity of the food chain. The consumers need to purchase safe products that do not involve any kind of risk for health. On one hand, the aim of the "food safety" is to avoid health hazards for the consumer: microbiological hazards, pesticide residues, misuse of food additives and contaminants, such as chemicals, biological toxins and adulteration. On the other hand, "food quality" includes all attributes that influence the value of a product for the consumer; this includes negative attributes such as spoilage, contamination with filth, discoloration, off-odors and positive attributes such as the origin, color, flavor, texture and processing method of the food.

The contamination of food products with microorganisms presents a problem of global concern, since the growth and metabolism of microorganisms can cause serious food borne intoxications and a rapid spoilage of the food products. Thus, the acceptance and safety of

a food product for the consumers depends in great part on the presence and nature of microorganisms. Besides molds and yeasts, bacteria are the principle responsible for various types of food spoilage and food borne intoxications. It has to be mentioned that a food product naturally contains an indigenous microbiota that can include spoilage and/or pathogenic bacteria species. Depending on the preservation method these species can proliferate and adulterate the product. However, most bacterial contamination occurs during processing and manipulation of the food products.

The presence of *Escherichia coli* and *Salmonella sp* in Dahi represents an internationally accepted human health concern. Although *Salmonella* causes many food borne disease outbreaks, there is little evidence to support cross-contamination as a major contributing factor. However, the paramount importance of preventing cross-contamination and recontamination in assuring the safety of foodstuffs is well known. Sources and factors linked to cross-contamination and recontamination of *Escherichia coli* and *Salmonella sp* in foods are reviewed in detail. Those foods which are not submitted to lethal treatment at the end of processing or which do not receive further treatment in the home deserves special attention. *Escherichia coli* and *Salmonella sp* cross-contamination and recontamination episodes have been connected to the following factors: poor sanitary practices and equipment design, deficient control of ingredients etc.

Knowledge concerning the microbiological quality of commercialized dahi is valuable because the consumption of contaminated food may cause food-transmitted diseases (FTDs), thus, representing a public health problem. The quality and safety of milk products are determined by the presence of indicator bacteria. Hence, milk and milk products easily favors the growth and multiplication of pathogenic bacteria which may serve as vehicle for the transmission of diseases to humans such as salmonellosis, diarrhea, food poisoning, tuberculosis etc. So microbiological assessments have an important role to play in the dairy industry to protect the public health and to reduce economic losses.

In this context, the present study was conducted to isolate the food-borne pathogens such as *Escherichia coli* and *Salmonella sp* and evaluate the quality of marketed dahi and the general sanitary practices prevailing during processing and handling of dahi .

Chapter - II

Review of Literature

Fermented dairy foods such as dahi or yoghurt contain a unique package of nutrients that are an essential part of a healthy eating plan. It is the food for which there seems to be no adequate substitute and is one of the widely consumed products. Milk products such yogurt, spicy yoghurt milk, butter milk are also rich in proteins, spices and minerals which made them widely expectable for their nutrient values. Milk and other fermented milk products is highly susceptible to contamination by microorganisms and it is also a suitable medium for the rapid growth and multiplication of bacteria at favorable temperatures (Megha S.V. and Annadurai B., 2014). That‘s why it is important to know what particular microorganisms can potentially contaminate milk products because in South-Asian countries, improper processing conditions of dairy products are very prevalent making the products unhygienic.

2.1. Difference between yoghurt and dahi

According to the PFA rules (1976), “dahi or curd is the product obtained from pasteurized or boiled milk by souring, natural or otherwise, by a harmless lactic acid or other bacterial culture.” Dahi may contain additional cane sugar. It should have same percentage of fat and solids-not-fat as the milk from which it is prepared.

Although yoghurt and dahi both are cultured or ripened dairy products still there are little differences between those. Yoghurt is prepared by using starter organisms *Streptococcus thermophilus* and *lactobacillus bulgaricus* in a proportion of 1:1, whereas dahi is prepared by using mixed culture of *Streptococcus lactis*, *Lactobacilhus bulgaricus*, *Streptococcus thermophilus*, *Streptococcus citrophilus*, *Lactobacillus plantarum* etc. Higher temperature (45°-50°C) and shorter (3-4h) incubation period is required for yoghurt making. On the other hand, lower temperature (37°-42°C) and long incubation period (8-15h) is required for dahi preparation. Comparatively yoghurt is softer and dahi curd is reverse of that. (Varnam and Sutherland., 1994)

2.2. Classification of dahi

Types of Yoghurt:

According to FAO/WHO (1973)

1. Natural yoghurt
   - Clean, slightly acidic, tart flavor
2. Set yoghurt
   - Fairly thick, flat surface with fruit & flavoring at base
3. Flavored yoghurt
   - Added sugar or artificial sweetner
4. Greek yoghurt
   - Deliciously thick & creamy texture
5. Yoghurt made with active cultures
   - Non-heat treated
   - Retain live & active cultures
6. Fruit Bottom Yoghurt
   - Fruit on the bottom is found
7. Yoghurt Drink
   - Stirred, diluted & mixed with flavors, fruit juices
8. Frozen Yoghurt
   - Blend of sugars, stabilizers, emulsifiers and flavors in natural stirred yoghurt
9. Stirred Yoghurt
   - Fermented in bulk with the fruit or flavoring stirred in
10. Labneh
    - Drained of whey to form a fresh 'yoghurt cheese'
    - Flavored and rolled into balls

Types of Dahi :

According to Indian Standard Institution (1973)

1. Plain Dahi
   - Normal dahi with characteristic flavor

2. Sweet Dahi
    - Have additionaly added friendly bacteria
    - Give added advantage to consumers
3. Sour Dahi
    - Dahi with 0.7% acidity
4. Probiotic Dahi
    - Dahi with 1% acidity

2.3. Food and nutritive value of dahi

It has been established that fermented milk products including dahi increases food and nutritive value as compared to the original milk.

Composition of whole milk dahi

The composition of dahi depends upon the type of milk used and the manufacturing conditions. The average composition of dahi from whole milk is as follows: water 85 to 88%, fat 5.00 to 8.00%, protein 3.20 to 3.40%, lactose 4.60 to 5.20%, ash 0.70 to 0.75%, lactic acid 0.50 to 1.10%, calcium 0.12 to 0.14%, and phosphorus 0.09 to 0.11% (Food Safety and Standards Regulations., 2011).

Composition of skimmed milk dahi

The composition of dahi depends upon the type of milk used and the manufacturing conditions. The average composition of dahi from skimmed milk is as follows: water 90 to 91%, fat .05 to .10%, protein 3.30 to 3.50%, lactose 4.70 to 5.30%, ash 0.50 to 1%, lactic acid 0.70 to .75%, calcium 0.12 to 0.14%, and phosphorus 0.09 to 0.11% (Food Safety and Standards Regulations., 2011).

Average Mineral and Vitamin Contents of Dahi

Dahi is more palatable due to its composition. The average mineral and vitamin contents of Dahi is as follows: Mineral matter 2.1 g, Calcium 149 mg, Phosphorus 93 mg, Vitamin A 102 i.u, Vitamin B1 49 µg, Riboflavin 157 µg, Nicotinic acid 86 µg, Biotin 23 µg, Pantothenic acid 183 µg, Folic acid 178 µg, Ascorbic acid 1.3 µg (Balasubramanian and Basu., 1985).

Akter et al., (2010) dahi is the curd resulting from lactic acid fermentation of milk. Dahi is the simplest way of preserving milk for human consumption in a tropical condition. The lactic acid produced during fermentation checks putrefactive changes while giving it an acid type pleasant aromatic taste, which is particularly refreshing in a hot climate.

Pazakova et al., (1999) studied on the sensorial evaluation of yoghurt produced from cow and goat milk. They reported that cow milk yoghurt is better than goat milk yoghurt.

Nergiz and Seckin., (1998) manufactured Torba yoghurt using a traditional method (a strained yoghurt produced from different groups of milk). Milk was heated to 90° C for 10 min. cooled to 45°C inoculated with 3% starter culture and incubated at 43°C to 4.7. The curd was cooled to 21°C and stored at 4°C for 12 hours.

Karthikeyan et al., (1998) evaluated in performance of 2 strains of yoghurt culture in different combinations in sweet cream buttermilk (SCBM) at various culture TS levels (9-24%) in terms of increasing acidity, rate of synerois, of curd and sensory quality of curd. They reported that acidity increased significantly with increase in TS content in the base mix up to 18%, the rate of synerosis of curd significantly and inversely decreased with increase in TS in SCBM was superior to that of control curd.

Salem et al., (1995) reported that the high-fat content frozen yoghurt (7 and 10%) fat had a good melting quality until 60 days of storage. The texture scores tended to be the same, or improved with increasing fat content of milk and decreased slightly after 30 day storage.

Hashimoto and Antunes., (1995) studied on the effects of heat treatment and utilization of 'ropy' lactic starters on rheological properties of milk yoghurt. They found that use of ropy lactic starters and heat treatment at 90°C for 75 min. gives a product with desirable physio-chemical characteristics and rheological properties.

Fernandez et al., (1994) recommended that culturing conditions for yoghurt preparation should be a temperature of 45°C and the addition of a starter culture at a proportion of 3-4% v/v.

Ahmed., (1992) manufactured zabadi from fresh milk with or without addition of 5 or 10% dried skim milk. He reported that zabadi manufactured from milk supplemented with dried

skim milk had lower and higher titratable acidity than that made from milk without dried skim milk. Addition of 5% dried skim milk greatly improved the quality of the zabadi produced from cow milk, while the quality of zabadi made from milk was improved when 10% dried skim milk was added.

Gabriel., (1990) manufactured of milk yoghurt. Whole or semi-skim milk is prepared by pasteurization, rapid cooling and homogenization. The starter is prepared from 2 strains of *Lactobacillus* and *Streptococcus* and a new starter is used for each batch, as it is claimed that this improves flavour. Culturing takes place for 2.5 - 4.25h at 42° - 48°C , followed by rapid cooling, stirring and then flavouring with sugar and/or natural fruit flavourings. The product is then packaged in pots and stored at 0-6°C.

Baltadjeva et al., (1989) produced typical bulgarian yoghurt with good flavour by concentrating goat milk to 16-18% TS (by ultrafiltration), pasteurizing it for 20 min at 90-92°C and incubating it for up to 180 min at 35°-37° or 41°- 43°C with *Streptococcus thermophilus* and *lactobacillus bulgaricus* in ratios of 2:1 to 5:1 and amounts of 1.5-30%.

Nikoiov., (1960) reported from his experiments that high quality yoghurt of typical flavour could be obtained with a mixed culture (rods: streptococci ratio during coagulation, 1: up to 15).

2.4. Chemical and Microbiological quality of dahi or yoghurt

Bozanic et al., (1998) prepared yoghurt from goat milk and from cow milk. They reported that yoghurt samples prepared from milk had a softer consistency and lower viscosity than those prepared from standard cow milk; they also had a higher acidity throughout the storage period. They also reported that sensory properties of samples of milk yoghurt were rated as being inferior to those of samples prepared from cow milk.

Ghaleb et al., (1998) separated Buffalo milk and the milk fat replaced by palm oil (PO) to produce recombined milk containing 5.50% fat and 8.75% SNF, which was used to make yoghurt and reported that free cholesterol content decreased more than esterified cholesterol. Acetaldehyde content, and titratable acidity of fresh and stored PO yoghurt

were similar to control. Syneresis was lower in PO yoghurt Sensory scores of fresh or stored PO yoghurt were acceptable.

Table 1: Chemical standard of dahi

a) composition of cow milk and buffalo milk

Parameters	Cow Milk	Buffalo Milk
Moisture	85-88	82-85
Fat	3.5-4.5	6.0-8.0
Protein	3.0-3.5	3.5-4.0
Lactose	3.8-4.5	4.6-5.2
Ash	0.64-0.66	0.70-0.72
Lactic Acid	0.5-1.00	0.5-1.1

Source: Food Safety and Standards Regulations, 2011.

b) Composition of Whole milk and Skim yoghurt

Parameters	Whole Milk Yoghurt (%)	Skim Milk Yoghurt (%)
Moisture	85-88	90-91
Fat	5-8	0.05-0.1
Protein	3.2-3.4	3.3-3.5
Lactose	4.6-5.2	4.7-5.3
Lactic acid	0.5-1.11	0.5-1.11
Ash	0.7-0.75	0.7-0.75

Source: Food Safety and Standards Regulations, 2011.

c) Nutritive value of Dahi per 100 g

Parameters	Value (per 100 gm)
Energy	257 KJ
Fat	3.3 gm
Protein	3.5 gm

Carbohydrate	4.7 gm
Vitamin A	27 µg (3 %)
Riboflavin (vit B2)	0.14 mg (12%)

Source : Balasubramanian and Basu, 1985.

Table 2 : Microbiological standard of dahi

Parameters	**value (per g)**
Total viable count	$\geq 10^5$/g
Coliform	10/g (maximum)
yeast and mold count	100/g (maximum)

Source: Indian Standard Institution, 1973.

Giudici et al., (1996) worked on the role of galactose fermenting yeast in plain yoghurt spoilage. They concluded that the presence of galactose, resulting from lactose hydrolysis by lactic acid bacteria, is the major potential cause of the changes in plain yoghurt brought about by galactose-positive, non-lactose fermenting yeasts.

Bozanic et al., (1996) prepared yoghurt samples using sterilized goat milk and sterilized cow milk and a 1:1 mixture of sterilized goat and cow milks. Yoghurt samples were the stored at 8°C for 9 days. They found that yoghurt made from the 1:1 mixture of milks tended to have the highest viscosity throughout the 9 day storage period, while cow milk yoghurt had the lowest. Goat milk yoghurt also had the lowest lactic acid content, while cow milk yoghurt had the highest. There were no significant differences between yoghurt samples with respect to sensory properties.

Jawalekar et al., (1993) prepared yoghurt form standardized cow (4% fat, 8.5% SNF) milk. They found that buffalo yoghurt had higher viscosity and curd tension and lower syneresis score than had corresponding cow yoghurt.

Noeman and Shalabv., (1992) were used to study the effect of culturing on the chemical composition of the two products. Lactose content decreased to approximately 4.22 and 4.07 g/l00g respectively compared with 4.73g/100g in the non cultured milk. Vitamin B

content for both cultured products showed relatively minor changes, except for folic acid in zabady which increased.

Kehagias et al., (1992) studied on effect of on the yield and solids recovery of strained yoghurt form goat and cow milk. They found that yield and recovery of milk solids were higher at the lower range (4.3 - 4.1). Researched milk was richer than a standard milk in all constituents. However, the Intron penetration force of the yoghurt gel form experimented milk was lower compared with that from standard milk (measured just after fermentation). The yield and the recovery of milk solids in strained yoghurt form experimented milk was higher than the yield and the recovered milk solids form standard milk.

Cardoso et al., (1991) reported that firmness of yoghurt made from buffalo whole milk (6.3% fat and 4.7% protein) was most acceptable when milk was pasteurized at 75°C for 5 min. Average chemical and microbiological composition was; 6.1, 4.68, 16.06 and 1.0 (percentage of milk fat, protein, total solid and acidity respectively) and 10 coliforms/ml. Yoghurt could be stroed satisfactorily at 10°C for 7 days. Zabady and acidophilus milk made from buffalo semi-skim milk (3% fat).

Mehanna and Hefnawy., (1988) worked on cow, buffalo and mixed milk that had been heat treated at 90°C for 5min, and then refrigerated for 2 days, was inoculated with 2% yoghurt culture and incubated at 40°C for 4h. Rate of increase in acidity and decrease in during incubation was similar in milk form all 3 treatments. During refrigerated storage of the yoghurts for 7 days, no significant differences observed in chemical composition and organoleptic score of the yoghurt, but some differences ($P<0.05$) were noted in curd tension.

Salji et al., (1987) assessed the production processing and quality of dairy products in the Western Province of Saudi Arabia. They reported that microbiological analyses of fluid milks were of good microbial quality, but some contamination of yoghurt with coliforms yeasts or molds was noted. This was traced to the use of polluted water in 3 small-scale plants.

Ranganadham and Gupta., (1987) evaluated the sensory characteristics of dahi and yoghurt. They recommended that when evaluating the products, Judges should note the

type and condition of the container, aroma, colour and appearance, body and texture and flavour. Good quality dahi should have a creamy-white to creamy- yellow colour, a smooth, glossy appearance, a mildly acidic flavour and a weak-gel texture.

Manjunath and Abraham., (1986) used goats' milk to make yoghurt by standard methods and compared with cows' milk yoghurt and goats' milk yoghurt, showed an increased rate of lactic acid production and decreased rate of acetaldehyde production, 'otolytic activity was similar for both yoghurts. Goats' milk yoghurt had a softer body, was whiter in colour and less wheying off was observed, Members of a taste panel could not distinguish between the 2 Yoghurts (flavour and taste), and the characteristic flavour of goats' milk was masked in goats milk yoghurt which had a typical yoghurt flavour.

Jasjit et al., (1979) studied a comparative assessment of the antibacterial acitivity of pure and mixed-strain cultures of *Streptococcus thermophilus* and *lactobacillus bulgaricus* against *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas fragi* and *Micrococcus flavus* was made using cows' milk and buffaloes' milk. Antibacterial activity of yoghurt culture was greater in buffaloes' milk than in cows' milk.

Singh and Jasjit., (1979) prepared yoghurt from (i) cows' and (ii) buffaloes" milk using a 16-18 h old culture of *Streptococcus thermophilus* (Hst) and *Lactobacillus bulgaricus* (W) in equal proportion. Yoghurt made from (ii) was rated' higher on the hedonic scale than the yoghurt from (I).

Duitschaever., (1978) reported that microscopic appearance of goat's milk yoghurt and of the *streptococci* in it was different from that of cows' -milk yoghurt. The goats' milk yoghurt was less viscous than that made from cows' milk, and when unstirred was less firm; when stirred, it showed no wheying off during subsequent storage at 4°C. Both products had similar amino acid profiles. In organoleptic tests, there were a 32% preference t'or goats' milk yoghurt (72% when sugar and /or flavor was added).

Lusiani et al., (1974) worked on microbiological quality of yoghurt in connection with storage times and temperatures. Through a long investigation authors concluded that yoghurt has a shelf life of approx. 1 month if stored continuously at refrigeration temperature.

Sreenivasan and Ranganathan., (1972) observe that when market samples of dahi prepared by lactic acid fermentation, of milk are used as starters, the contaminating yeasts proliferate during daily transfer of previous days dahi and impart an off-flavor to the product after storage for 72 h.

2.5. Standards and Standardization of yoghurt or dahi

Ahmed et al., (2015) carried out an investigation on quality evaluation of stirred yoghurt flavoured with Guddaim fruit. The ingredients and fruit puree were collected from different places of Khartoum, Sudan. Yoghurt manufactured with Guddaim fruit was produced in their university laboratory. In their research, all the yoghurt samples were stored at 4°C for 10 days and chemical, microbiological and sensory characteristics were carried out at 10-day intervals. Dilution was done maintaining a ratio of 1:10 (v/v) and used for total viable bacteria (TVB), coliform bacteria, *Staphylococcusaureus* and yeasts and molds counts. For coliform bacterial count Mac Conkey agar medium was used and plates were incubated at 32°C for 48 hr (Christen et al., 1992). Also, Mannitol salt agar medium was used for *Staphylococcus aureus* count and the plates were incubated at 32°C for 48 hr (Flowers et al., 1992). During their study coliform bacteria count ranged between Log 3.15 cfu/gm in the control and Log 3.69 cfu/gm in Treatment 1 (5% v/v Guddaim fruit extract), and the count showed a decrease in day 7 (Log 3.06 cfu/gm) before they increasing to the end. On the other hand, *S. aureus* count was found to be Log 3.70 cfu/gm in Treatment 3 (10% v/v Guddaim fruit extract) and Log 4.44 in the control. The count was increased during storage from Log 4.07 cfu/gm at day 1 to Log 4.16 cfu/gm at day 10 with a slight decrease in day 7 (Log 3.90 cfu/gm). It was suggested that the environmental contamination and heat treatment during preparation of yoghurt might be the reason for the presence of these microorganisms.

Das et al., (2015) evaluated the microbial load and the quality of the eighty-seven dairy samples collected from different locations of Dhaka city. The study considered the total viable count, total coliform count and total fungus count of the different dairy samples. The coliform count of the yoghurt was 9.5×10^3cfu/g which was undesirable according to the FDA standard. On the other hand, the mean viable count of the borhani was significantly higher than raw and UHT milk.

American Public Health Association (1983) set the standards for both coliforms and yeasts in yoghurt at less than 10 colonies per ml. and in only, one sample out of four.

Spanish Standard (1976) specified requirements applicable to the following types of yoghurt for the Spanish market: ordinary yoghurt (> 2% milk fat, > 8.5%S.N.F.), skim-milk yoghurt (< 0.5% milk fat, > 8.5% S.N.F.), yoghurt with fruit, juices and /or other natural products (must contain 70% yoghurt by weight). In all the fermentating organisms *Streptococcus thermophilus* and *L. bulgaricus* must be viable and abundant in the final product

Indian Standard Institution (1973) prescribed the types, requirements, method of sampling and test for fermented milk products (plain and flavoured) i.e. (i) sweet, dahi produced using a culture of *streptococcus lactis, S. diacetilactis, V. diacetilactis* either alone or in combination with or without *Leuconostoc . spp.*, (ii) sour dahi, using cultures as above but with *Lactobacillus bulgaricus* and /or *S. thermophilus*, and (iii) yoghurt, produced using cultures of *S. thermophilus* and /or *L. bulgaricus*. Requirents are: maximum acidity (as lactic acid), 0.7, 1.0 and 0.8% by mass for (i), (ii) and (iii) respectively, maximum yeast and mould count 100/g, maximum coliform 10/g, and osatase test negative.

Eschmann., (1968) reported referring the swiss standards that fermented milk products in Switzerland should be negative PR (Phosphates Reaction), lactic acid bacteria should present in at least 16c' dilution and frozen bacteria not more than 50000/ml on retailing.

2.6. Diseases caused by the microbes

Milk, as well as other milk products such as butter milk, yoghurt milk, and butter can contain a large number of microbes with greater diversity and serves as a great growth medium (Ruegg P. L., 2003). Bacteria can be the causative agent of microbial spoilage of milk products. Because of low pH of most of the milk products, bacteria such as *Shigella spp., Pseudomonas spp., Salmonella spp.,* can grow easily in them. There are other types of pathogenic bacteria present in the environment and they can cause various diseases to humans

Escherichia coli is a gram negative bacterium which can be found in lower intestine of warm blooded animals. A large variety of *E. coli* is present but most them are not infectious. They are harmless. *E. coli* are also opportunistic microbes. Some types of *E. coli* such as *E. coli* 0157:H7 can create intestinal infection. Some other strains can cause bloody diarrhea, severe kidney failure which can cause death. Humans and animals can be infected by eating foods or drinking water which is already contaminated with *E. coli*.

Salmonella spp. are gram negative, rod-shaped (bacillus) bacterium. *Salmonella enterica* can cause four diverse clinical signs: bacteremia, gastroenteritis, asymptomatic carrier state and enteric fever. It is more common in youngsters less than 5 years old. Gastroenteritis is also known as food poisoning showing the symptoms such as vomiting, sudden nausea, abdominal cramps, headache, diarrhea and high fever. In Asia non-typhoid salmonellosis is more common especially in industrialized countries. Their primary hosts are animals such as cattle, wild birds, flies as well as pets. The final hosts are human. Humans are the only known hosts for *Salmonella Typhi*. Humans only get infected when they consume contaminated water and foods such as meat, milk, egg products, milk products which are contaminated with infected faeces as well as infected animals. Certain host carries the bacteria for years

Pseudomonas spp. is gram negative bacteria. Morphologically they are vibrios and enteric bacilli. They are motile bacterial species because they have peritrichous flagella. *Pseudomonas spp.* is aerobes in nature and can be found widely in water, soil, skin and almost all the manmade environments. *Pseudomonas aeruginosa* can cause cystic fibrosis, urinary tract infection and also other external and internal of human body. *Pseudomonas spp.* is also opportunistic bacteria. They can remain silent inside host body but whenever they get the chance they can create worst situation. *Pseudomonas spp*. is predominate in raw milk and play a major part in milk spoilage (Muir et al., 1979; Griffiths et al., 1987).

Staphylococcus aureus are gram positive, non-motile, small round shaped cocci. They can be frequently found in respiratory tract, nose and skin. Pathogenic strains can cause infection by producing a cell surface toxin protein that binds with the antibodies and deactivates them. *Staphylococcus aureus* can spread by skin-to-skin contact, objects such as towels, cloths and other equipment that are used by the infected person. They can lay

dormant in host body for several years undetected. Staphylococcal scalded skin syndrome is a severe skin infection which can be seen in new born babies (Akbar and Anal., 2013).

Klebsiella is a gram negative, rod-shaped, non-motile bacterium. This species can be found everywhere in nature. *Klebsiella* is an opportunistic bacterium and primarily they attach those people who are immunocompromised and suffering from different disease such as diabetes. *Klebsiella* pneumonia is the most known pathogen among all the other species which cause human disease. *Klebsiella spp*. also can cause septicemia, intensive care unit infection, and urinary tract infection.

From this section of review it is clear that numerous scientists around the globe have tried to know the physical, chemical and microbiological quality of dahi or yoghurt prepared from different source of market milk under various condition. Very limited work has been conducted in our country regarding the microbiological quality particularly isolate the food-borne pathogens such as *Escherichia coli* and *Salmonella sp* and evaluate the quality of commercialized dahi.

Chapter - III

Materials and Methods

3.1. Study area

This experimental attempt was made to isolate the *E. coli* and *Salmonella sp* from dahi available in local market of Chattogram Metropolitan Area.

Figure 3: Map of Chattogram Metropolitan showing study area

3.2. Study period

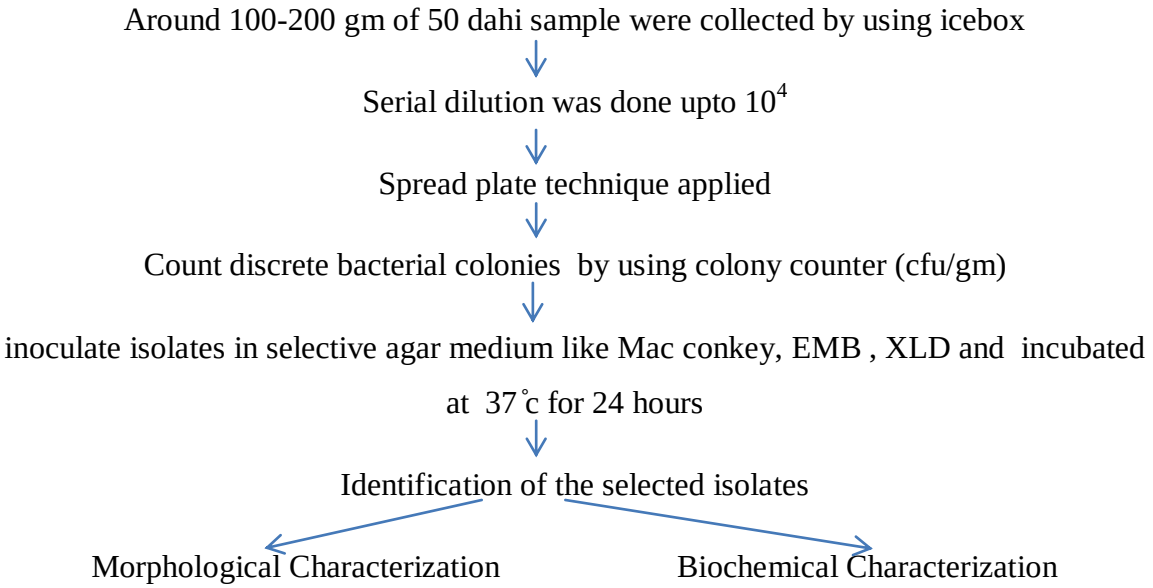
The experiment was conducted for a period of 6 months starting from July to December, 2019. Samples were collected from shops at weekly basis.

3.3. Materials

3.3.1. Media: Bacteriological parameters (*Salmonella* and *E. coli*) were determined by the methods as described in the "Standard Methods for Examination of Dairy Products" by APHA (1983). Xylose lysine deoxycholate agar and Mac conkey agar, Eosin methylene blue agar were used for *Salmonella* and *E. coli* count respectively. Buffered peptone water was used as pre-enrichment medium for the growth of bacteria. Plate count agar was used for evaluate the total viable bacteria count by using colony counter.

3.3.2. Chemical Analysis: Different specific biochemical media were prepared for different biochemical tests. Acidity and protein percentage was determined by titrating with 0.1 N sodium hydroxide solution and by Kjeldahl method respectively by using the procedure of Aggarwala & Sharma (1961). Fat percentage was determined by Gerber method.

3.4. Flowchart of the study design



Source: Standard Methods for examination of Dairy products by APHA, 1983.

3.5. Methods

3.5.1. Collection of dahi

A total of fifty (50) dahi samples (local and branded) were collected randomly from different sweetmeat shops and retail shop of the local markets under stringent hygienic conditions. Among them, three popular branded dahi such as Banoful, Fulkoli, Genuine were collected from market. Samples were brought to experimental site with the help of a icebox, maintaining the temperature 4-5°C. The samples size ranged from 100-200g packet in small size plastic pots. After collection, samples were stored in refrigerator at 4°C until analysis.

a) Local dahi

b) Branded dahi

Figure 4: Local and Branded dahi sample

Figure 5: Collection of dahi sample

3.5.2. Chemical analyses (Dahi)

The following parameters were determined in the laboratory:

- Acidity content (%)
- Fat content (%)
- Protein content (%)

Determination of Acidity percentage

Nine gram of sample was taken in a conical flask and 9 ml of distilled water was added to the sample and was mixed thoroughly with the help of a glass stirrer. 3-4 drops of phenolphthalein indicator was added to the sample. 0.1 N NaOH solution was taken in the

burette and was added drop by drop to the sample and stirred until a pink color was persisted for a short time. Finally the ml of NaOH required was noted and the calculation was made using the following formula:

Percentage of acidity = (ml of NaOH x 0.09 x strength of NaOH/ Weight of dahi) x 100

Where 0.09 is the gram of lactic acid equivalent to each ml of normal strength of NaOH. 17.6 ml dahi is equivalent to 18 grams of milk and 0.1 was the normality of NaOH used.

Determination of Fat percentage

17.5 ml of diluted sulfuric acid was added into the butyrometer. Nine gram of well mixed dahi sample was weighed and was taken into a butyrometer with the help of 9 ml pipette 9 ml of soft water was added with it and mixed thoroughly. Then 3 ml of normal amyl alcohol was added and mixed for at least 1 minute. After that, the opening was closed by the lock stopper and shaken well until the disappearance of white particle. The butyrometer with the sample was centrifuged for 5 minutes in a Gerber's centrifuge machine. Holding the butyrometer in vertical position and reading of fat column was recorded.

Determination of crude protein of Dahi sample

The determination of CP actually involves the determination of nitrogen content of the sample, which is multiplied by the factor 6.38.

Therefore, %CP= % N x 6.38

% of Nitrogen = (ml of 0.1N HCI solution used for titration x Normality of the acid x ml equivalent weight of Nitrogen/ Weight of the sample) x l00

Two grams of dahi samples whose dry matter percentage was known, transferred in a kjeldahl flask. One gram of mixed catalyzer was added to the flask. Then 20 ml of commercial concentrated sulphuric acid was poured gradually into the sample in the kjeldahl flask. The flask was kept in the digestion chamber in inclined position and was heated gently until frothing ceases. Boiling was continued (briskly boiling) for 30 minutes until the solution becomes clear. After digestion, the digested solution was cooled by air and 100 ml of distilled water was added to the digested sample. Some zinc granules were

added to prevent bumping. The digested solution was then neutralized by gradual adding of 80 ml of 40% NaOH solution without agitation. The flask was immediately connected to digest bulb on condenser and then tip of condenser was immersed in an Ehrlenmeyer flask containing 20 ml of 2% boric acid solution. The flask was rotated to mix the content thoroughly and was heated until all ammonia was distilled. The distillated sample was titrated with standard HC1 solution (0.1 N) using mixed indicator (Methyl blue+methyl red) and the percent of nitrogen was calculated.

3.5.3. Bacteriological analysis

Serial dilution

Test tubes containing 9 ml of physiological (0.9% NaCl) saline water were autoclaved before use. Tenfold serial dilution of the dahi samples were prepared in autoclaved saline water. Initially, 1 gm of dahi was mixed with 9 ml of saline water in a test tube in order to dilution 10^{-1} and mixed with 9 ml of saline in it by repeated pipetting in order to make tenfold dilution. Again, 1 ml from the 10^{-1} test tube was transferred to 10^{-2} labeled test tube and mixed with 9 ml saline solution in it by repeated pipetting. This action was repeated for the test tubes labeled as 10^{-3}, and 10^{-4}.

Spread plate technique

After finishing serial dilution, four plate count agar plates were labeled as 10^{1}, 10^{2}, 10^{3} and 10^{4}. From each of the diluted sample test tubes 0.02 ml of sample from the test tubes labeled 10^{1}, 10^{2}, 10^{3} and 10^{4} were added on the respective plates and the drops were spread using spread plate technique with a spreader. All plates were then incubated at 37°C for 24-48 hours. After incubation, the plates having colonies were counted and noted down.

Morphological characterizations of bacteria

Buffered peptone water , Mac conkey, EMB , XLD agar were prepared and autoclaved at 121°C, 15 psi and cooled at water bath to 45-50°C . This buffered peptone water was used as enrichment media for *Escherichia coli* and *Salmonella sp*. The media was then dispensed into sterile petridish while liquid and left for solidifying. Using sterile technique,

Mac conkey, EMB, Xld agar plate were streaked by picking a loop full of colony culture with an inoculating loop by means of three quadrant streak plate method to obtain isolated discrete colonies. The plates were then incubated at 37°C for 24 hours. After the incubation period the growth patterns of the bacteria were evaluated for size, pigmentation, form, margin, elevation and texture (Cappuccino and Sherman, 2005).

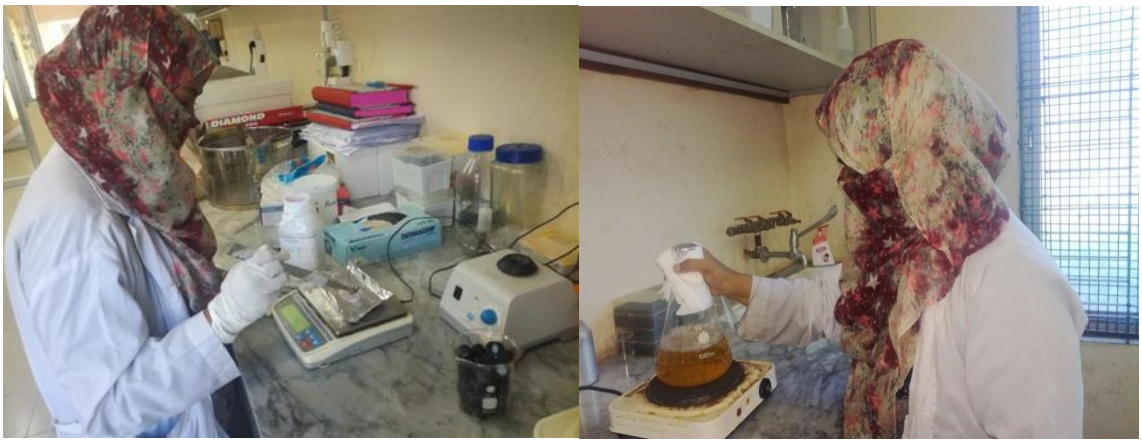


Figure 6: Agar media preparation

3.5.4 Biochemical characterization of the bacteria

Several biochemical tests were carried out in order to have a presumptive identification of the potential bacteria chosen before. Most of the methods were done according to the microbiology laboratory manual (Cappuccino and Sherman., 2005). The biochemical tests performed were Indole production test, Citrate utilization test and Urease test,

Citrate utilization test

Citrate utilization test was done to differentiate among enteric organisms on the basis of their ability to ferment citrate as a sole source of carbon by the enzyme citrate permease. Simmons citrate agar slants of 2 ml in each vials were prepared by autoclaving at 15 psi 121°C. Using sterile technique, small amount of the experimental bacteria from 24-hours old pure culture was inoculated into the vials by means of a streak inoculation method with an inoculating needle and the vials were incubated for 48 hours at 37°C (Cappuccino and Sherman., 2005). Growth of organism and change of color from green to blue in positive sample and no color change obtain in negative sample (Figure 7). Bacteria which give

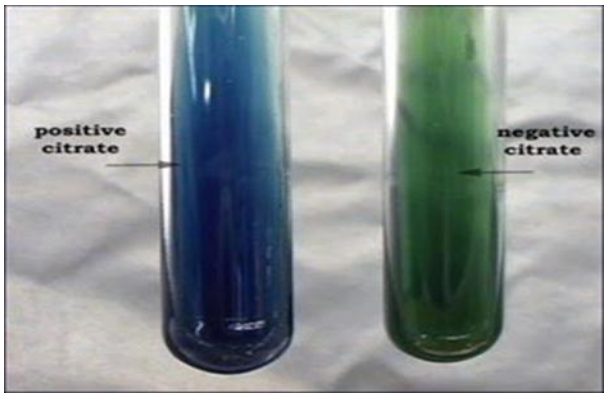
positive results for the citrate test include *Salmonella, Enterobacter sp* etc. *E. coli, Shigella sp* show negative result.

Indole Production test

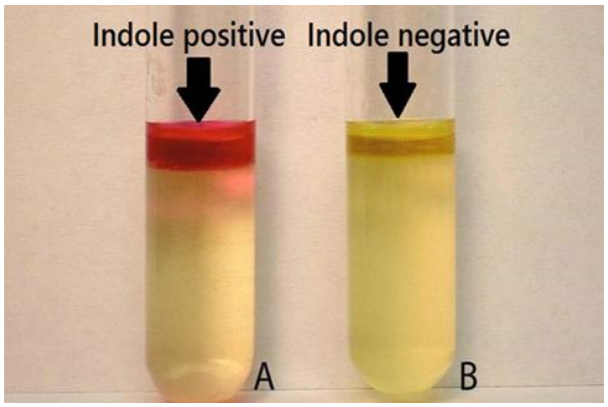
Indole production test was done to determine the ability of the bacteria to degrade the amino acid tryptophan by the enzyme tryptophanase. Tryptophan broth of 5 ml in each test tube was prepared by autoclaving at 15 psi 121°C. Using sterile technique, small amount of the experimental bacteria from 24 hours old pure culture was inoculated into the tubes by means of a loop inoculation method with an inoculating loop and the tubes were incubated for 48 hours at 37°C. In order to test for indole production, 5 drops of Kovac's reagent was added directly into the tubes (Cappuccino and Sherman., 2005). Cherry red ring form in the upper layer in case positive sample and no ring form in the upper layer in negative sample (Figure7). Bacteria which give positive results for the citrate test include *E.coli, Proteus sp* etc. and *Salmonella, Klebsiella sp* show negative result.

Urease test

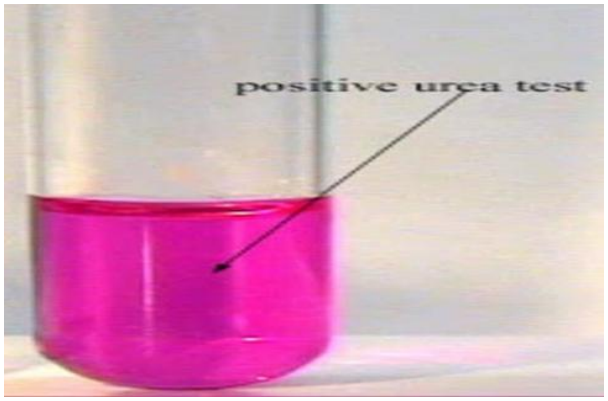
Urease test was done to simultaneously determine the ability of the bacteria to hydrolized urea by means of the enzyme urease. Urease media was prepared by autoclaving at 15 psi 121°C. The media was cooled to about 50-55°C and 100ml of urea glucose solution was added aseptically to 900 ml base medium. After that, 6ml solution was transferred to each sterile test tube and allowed to form a semi solid medium. Using sterile technique, small amount of the experimental bacteria from 24 hours old pure culture was inoculated into the tubes by means of a stab inoculation method with an inoculating needle and the tubes were then incubated for 24 hours at 37°C (Cappuccino and Sherman., 2005). In positive sample magenta to pink color change obtained in media and no color change found in negative sample (Figure 7). Bacteria which give positive results for the citrate test include *Bacillus, Proteus sp* etc. *E. coli* and *Salmonella sp* show negative result.



a) Absence of growth and no color change obtain in slant



b) Absence of pink to red color ring (cherry ring) in the upper layer



c) Development of bright pink color colonies

Figure 7 : Results of biochemical tests ; a) Citrate test; b) Indole production test; c) Urease test

3.6. Statistical analysis

The data obtained were imported, stored and coded according to recorded information in the data sheet using the Microsoft Excel – 2007 program for statistical analysis. A descriptive statistics was performed for chemical and microbial parameters according to different samples. The result was presented as mean with the standard error and p-value <0.05 was considered significant.

Chapter – IV

Results

4.1 Chemical analysis

The results of chemical analyses (Acidity, fat and protein percentage) of local and branded dahi samples (n=50) from study areas are shown in Table 3.

4.1.2. **Fat**

The average percentage of fat of branded Sweetmeat shops and retail shops made dahi were 3.43 ± 0.18 and 3.58 ± 0.11 respectively (Table 3). Statistical analysis showed that fat content of different dahi samples differ significantly ($p<0.05$). Maximum fat percentage was seen in local dahi (3.58 ± 0.11) and minimum fat percent was seen in branded dahi. Among the collected branded dahi samples minimum fat percentage obtained in banoful dahi (3.43 ± 0.18) and maximum fat percentage obtained in Genuine dahi (3.57±0.11) (Table 4).

4.1.3. Acidity

Acidity percentage for dahi samples of branded and local were 0.87 ± 0.02 and 0.75 ± 0.03 respectively (Table 3). Significant differences were found ($p<0.05$) in respect of acidity content of the samples. The highest acidity was that of local dahi at 0.87 ± 0.02 and the lowest acidity was that of Banoful dahi at 0.75 ± 0.03. On the other hand, highest acidity percentage (3.57± 0.11) obtained in Fulkoli dahi (Table 4) among the collected branded dahi samples .

Table 3: Chemical analysis of local and branded dahi collected from Chattogram Metropolitan Area

Parameters	Brand	Mean ± SE	Range	
			maximum	minimum
Fat (%)	Branded	3.43 ± 0.18	3.90	3.00
	Local	3.58± 0.11	3.90	3.10

Acidity (%)	Branded	0.75 ± 0.03	0.85	0.63
	Local	0.87 ± 0.02	0.95	0.80
Protein (%)	Branded	4.58 ± 0.34	5.33	3.5
	Local	5.13 ± 0.17	5.33	3.6

Table 4: Chemical analysis of branded dahi collected from Chattogram Metropolitan Area

Parameters	**Brand**	**Mean ± SE**	**Range**	
			maximum	**minimum**
Fat (%)	Bonoful	3.43 ± 0.18	3.90	3.00
	Fulkoli	3.55 ± 0.20	4.10	2.90
	Genuine	3.57± 0.11	3.80	3.10
Acidity (%)	Bonoful	0.75 ± 0.03	0.85	0.63
	Fulkoli	0.83 ± .02	0.92	0.78
	Genuine	0.77± .06	0. 87	0. 66
Protein(%)	Bonoful	4.58 ± 0.34	5.33	3.50
	Fulkoli	4.0 ± 0.36	5.00	3.00
	Genuine	5.13 ± 0.17	5.33	3.60

4.1.4. Protein Percentage

The average percentage of protein of branded dahi and local dahi were 4.58 ± 0.34 and 5.13 ± 0.17 respectively (Table 3). Statistical analyses showed that there were significant dilference ($P<0.05$) within the protein content of different groups of dahi.

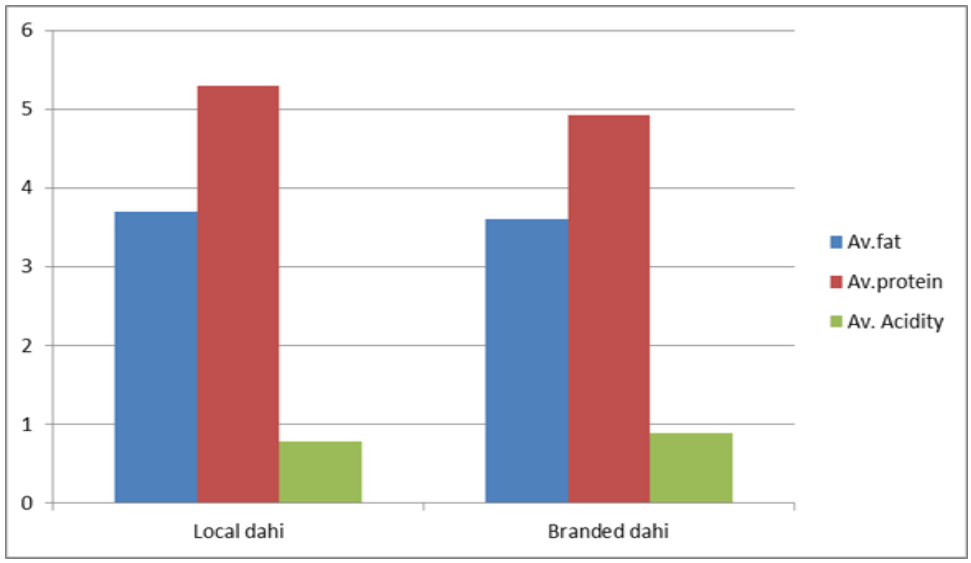


Figure 8: Fat , protein and acidity percentage of dahi

4.2. Microbial count

4.2.1. Total Viable Count (TVC)

The TVC is the number of bacteria in a sample that can grow and form countable colonies on standard plate count agar after being held at 37°C for 24 hours. Statistical analysis showed no significant difference ($p>0.05$) among the total viable counts of the Dahi samples from the collected areas. The highest total viable bacterial count $3x10^5$ cfu/gm was found in branded dahi and lowest total bacterial count was $1.2x10^5$ cfu/gm, which had been collected in local area (Table 5). The results of the total viable count of all samples was within the standard level ($\geq 10^5$).

Table 5: Total bacterial count of collected dahi sample marketed in Chattogram Metropolitan Area

Samples no.	Sample	Total viable count cfu/gm	*E. coli* count	*Salmonella* count
1	Banoful	2.8×10^4	Nil	Nil

2	Fulkoli	4.5×10^3	Nil	Nil
3	Genuine	3.0×10^5	Nil	Nil
4	Local	5.0×10^2	Nil	Nil
5	Local	6.8×10^4	Nil	Nil
6	Local	5.0×10^2	Nil	Nil
7	Local	2.1×10^5	Nil	Nil
8	Local	4.1×10^2	Nil	Nil
9	Local	5.5×10^3	Nil	Nil
10	Local	1.1×10^4	Nil	Nil
11	Local	4.4×10^3	Nil	Nil
12	Local	5.1×10^3	Nil	Nil
13	Local	1.8×10^4	Nil	Nil
14	Local	1.6×10^5	Nil	Nil
15	Local	3.2×10^4	Nil	Nil
16	Local	6.2×10^2	Nil	Nil
17	Local	4.5×10^3	Nil	Nil
18	Local	2.3×10^4	Nil	Nil
19	Local	2.0×10^3	Nil	Nil
20	Local	1.2×10^5	Nil	Nil
21	Local	2.3×10^2	Nil	Nil
22	Local	2.9×10^5	Nil	Nil
23	Local	1.8×10^2	Nil	Nil
24	Local	3.6×10^3	Nil	Nil
25	Local	6.4×10^4	Nil	Nil
26	Local	4.3×10^3	Nil	Nil

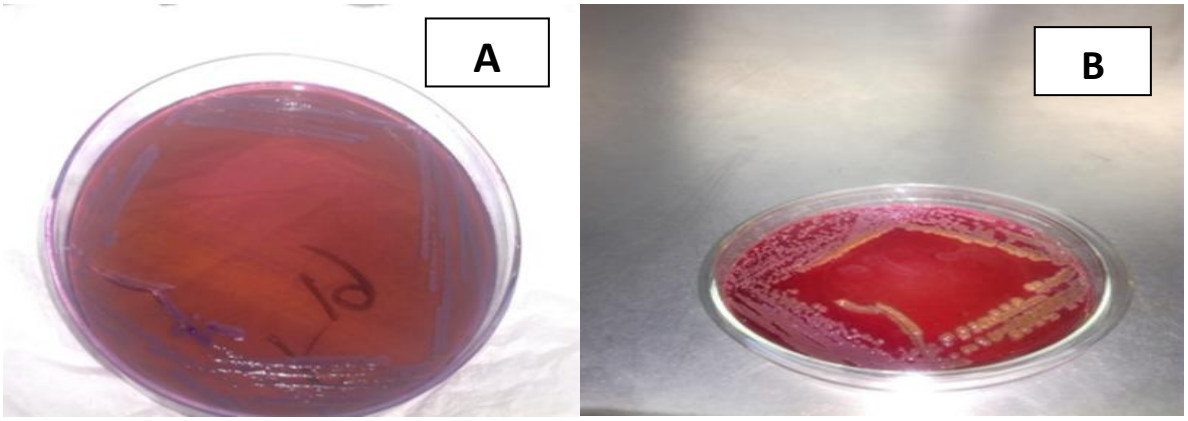
27	Local	1.1×10^{3}	Nil	Nil
28	Local	2.0×10^{4}	Nil	Nil
29	Local	5.2×10^{5}	Nil	Nil
30	Local	2.1×10^{2}	Nil	Nil
31	Local	6.2×10^{2}	Nil	Nil
32	Local	5.5×10^{3}	Nil	Nil
33	Local	3.7×10^{2}	Nil	Nil
34	Local	2.5×10^{4}	Nil	Nil
35	Local	3.1×10^{2}	Nil	Nil
36	Local	9.0×10^{3}	Nil	Nil
37	Local	8.6×10^{4}	Nil	Nil
38	Local	1.9×10^{5}	Nil	Nil
39	Local	1.5×10^{3}	Nil	Nil
40	Local	2.36×10^{2}	Nil	Nil
41	Local	2.4×10^{5}	Nil	Nil
42	Local	1.8×10^{4}	Nil	Nil
43	Local	4.0×10^{2}	Nil	Nil
44	Local	7.3×10^{3}	Nil	Nil
45	Local	3.2×10^{4}	Nil	Nil
46	Local	2.6×10^{5}	Nil	Nil
47	Local	1.8×10^{3}	Nil	Nil
48	Local	1.04×10^{4}	Nil	Nil
49	Local	5.2×10^{2}	Nil	Nil
50	Local	3.9×10^{3}	Nil	Nil

The table 5 showed the total viable count of marketed dahi which is range from $1.2x10^5$ to $3x10^5$ cfu/gm. On the other hand, *E. coli* and *Salmonella sp* count were nil.

Figure 9: Total viable bacterial count under colony counter

4.2.2. *E. coli and Salmonella sp* count

The presence of *E. coli and Salmonella sp* in dahi samples indicate the contamination of dahi during their production and handling which may cause public health problems. In this study no bacterial growth obtained in selective media like Mac conkey, EMB for *E. coli* and XLD for *salmonella sp*. Pink color colonies ; Greenish metallic sheen and black centered red colonies were absent in A) Mac conkey, B) EMB and C) XLD agar respectively. The data for *E. coli and Salmonella sp* count has not statistically tested as because all samples possess nil count (Table 5).



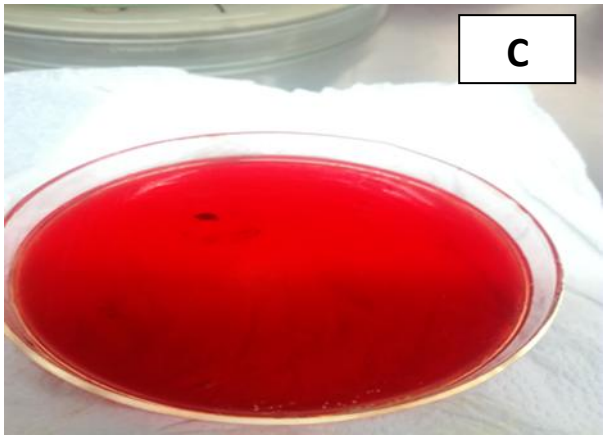


Figure 10: Absence of microbial growth in selective media

4.3. Biochemical Characteristics of bacterial isolates of different samples

Suspected microorganisms were collected from the different samples. The selection of the microbes was done from different culture media according to their growth, morphology and visual form. Then the microorganisms were compared with the suspected one and selected one was then sub-cultured for biochemical tests. To obtain more specific and desired result different types of selective growth media were used to isolate specific microorganisms. All the biochemical tests were described with details in chapter III. The test results of the selected microorganisms are noted down with a chart (Table 6). During the study after all the biochemical tests have been done it was found that *E. coli* and *salmonella sp* was absent in all collected samples.

Table 6 : Biochemical characteristics of sample in different biochemical test

Samples no.	**Sample**	**Citrate reaction**	**Indole reaction**	**Urease reaction**	**Organism interpretation (*E.coli*/ *Salmonella*)**
1	Banoful	−	−	−	absent
2	Fulkoli	−	−	+	absent
3	Genuine	+	+	+	absent
4	Local	+	−	+	absent

5	Local	+	+	+	absent
6	Local	−	−	+	absent
7	Local	+	+	+	absent
8	Local	+	+	+	absent
9	Local	−	−	−	absent
10	Local	−	−	+	absent
11	Local	+	+	+	absent
12	Local	−	−	+	absent
13	Local	+	+	+	absent
14	Local	−	−	−	absent
15	Local	−	−	+	absent
16	Local	+	+	+	absent
17	Local	−	−	+	absent
18	Local	+	+	+	absent
19	Local	−	−	−	absent
20	Local	+	+	+	absent
21	Local	−	−	+	absent
22	Local	+	+	+	absent
23	Local	−	−	−	absent
24	Local	+	+	+	absent
25	Local	−	−	−	absent
26	Local	+	+	+	absent
27	Local	+	+	+	absent
28	Local	−	−	+	absent
29	Local	+	+	+	absent
30	Local	+	+	+	absent
31	Local	−	−	−	absent
32	Local	−	−	+	absent
33	Local	+	+	+	absent
34	Local	−	−	+	absent

35	Local	+	+	+	absent
36	Local	+	+	+	absent
37	Local	−	−	+	absent
38	Local	−	−	−	absent
39	Local	+	+	+	absent
40	Local	−	−	+	absent
41	Local	+	+	+	absent
42	Local	−	−	−	absent
43	Local	+	+	+	absent
44	Local	−	−	+	absent
45	Local	+	+	+	absent
46	Local	−	−	−	absent
47	Local	+	+	+	absent
48	Local	−	−	+	absent
49	Local	+	+	+	absent
50	Local	−	−	−	absent
'−'= Negative, '+'= Positive ; *E. coli* only indole positive; *Salmonella* only citrate positive; Both *E. coli* and *Salmonella* urease negative.					

Chapter-V

Discussion

The present study was conducted to isolate the pathogenic organism such as *E. coli and Salmonella sp* from retail shop dahi. Effectiveness of hygienic practices and sanitary status in the production of dairy food products are reflected by its total bacterial count, presence of *E. coli and Salmonella* organisms. These are commonly used methods for evaluation of quality control of food products. A variety of diseases can potentially be transmitted through milk and other milk products. The pathogenic agent source can be cow or humans associated with the production and handling of the foods and it can be transmitted into other milk products (Pelczar., 2007).

The present study is agreed by Rashid and Miyamoto., 2005 that the fat percentage of dahi of Bangladesh was ranged from 3.00 to 4.75%. Although, the fat percentage should be higher because during heating it loses some moisture but total percentage is not increase due to use of skim milk powder.The variation in fat content between different dahi samples might be caused by lacking of quality control or standardization of milk for dahi production, adulteration of milk etc.

The Highest acidity of local dahi might be due to improper incubation, postproduction handling and long storage period while branded dahi samples might be produced under proper incubation condition and temperature or maintained a low temperature after production.

The result agreed with the findings of Chakraborty., (1998) and Ali., (1998) who found that milk dahi contained 4.22% and 4.44% protein a respectively in low protein findings and the findings of Chakraborty who found protein 5.1% in buffalo milk dahi incase of high protein finding. Concentration of milk to about 60%-70% of original volume due to prolonged heating has positive effect on protein content of dahi.

The present study did not detect any *Salmonella species*. Similar results were recorded in previous studies. In previous study was conducted by Nassib TA et al., (2003) where they investigated Egyptian dairy products using various detection media. During the study, all

the samples were tested to confirm the presence of *Salmonella sp*. This might be due to growth of other organisms which suppressed the growth of *salmonella species*.

In a previous study Malek et al., (2015) conducted a research study and they included matta (plain butter milk), sweetened yogurt, lassi and borhani (spicy yoghurt milk) as the samples. In their study, all the samples exhibited the presence of bacterial contamination. Among specific pathogens they spotted *Staphylococcus spp.* and *Klebsiella spp*. Similar result was also found in current study. After several biochemical tests, presence of *Staphylococcus spp.* and *Klebsiella spp*. were confirmed in samples of spicy yoghurt milk and plain butter milk collected from food markets in Dhaka city. However, in the study of Malek et al., (2015) they have found *Vibrio spp*. at a very high rate in the samples which is dissimilar with present study. Present investigation also showed presence of other pathogenic microorganisms such as *Salmonella spp., Pseudomonas spp.* which does not correspond with the study conducted by Malek et al., (2015).

Another study was done by Khan et al., (2008) on raw milk of balk tank milk before it is processed to other milk products such as butter milk and curd. In their study, they found that the bulk tank milk was highly contaminated by *Escherichia coli* and *Pseudomonas aeruginosa species*. The presence of these pathogenic isolates was also confirmed in the after product where the milk was used. Like the study of Khan et al., (2008), in present research study *Escherichia coli* and *Pseudomonas spp*. were found in plain butter milk and spicy yoghurt milk. So, it can lead to an assumption that the contamination occurred from the beginning of the food processing.

Ahmed et al., (2015) conducted a study on flavored yoghurt and investigated the microbial quality. The samples were prepared and stored. The study was carried out to identify the cause of contamination and the microorganisms responsible. They found that all the samples were highly contaminated with *Staphylococcus aureus*. According to their study, it was suggested that environmental contamination during preparation and handling of the products might be the reason of the contamination. In current research study *Staphylococcus aureus* was found in spicy yoghurt milk samples. This result was similar to the previous finding because the contamination might have happened while handling the food. *Staphylococcus aureus* is widely found in respiratory tract, nose and skin. Therefore,

food those are exposed or frequently touched could be contaminated by it. This result was also supported by Aly et al., (2004) where *Staphylococcus aureus* count got higher due to frequently exposure. In another study by Belickonva et al., (2001), *S. aureus* count was reported in yoghurt milk and strawberry flavored yogurt milk.

Another study was conducted place by Beukes et al., (2000) where they investigated dairy products and fermented milk products. During the study, all the samples were tested to confirm the presence of *Staphylococcus aureus*, *Salmonella spp*., *Pseudomonas spp*. and *Listeria monocytogenes*. In there study, the IDF reference method (International Dairy Federation, 1995) was used for detection of *Salmonella species* with XLD (Xylose Lysine Deoxycholate) medium as selective solid media. *Staphylococcus aureus* was detected by using the reference method of IDF (International Dairy Federation., 1990). The present study was also similar to the previous one. Presence of *Staphylococcus aureus* and *Salmonella spp*. was confirmed from the dairy product samples. However, *Pseudomonas spp.* was not found in any sample from the study conducted by Beukes et al., (2000) but in this present study, presence of *Pseudomonas spp.* was confirmed.

The finding of the present study indicate absence of organism in the sample it can be occured due to proper hygienic practices, high boiling temperature during dahi preparation, use of antibiotics.

There was no research conducted on the versatile qualities of retail dahi market of Chattogram metropolitan area. The present study was undertaken to explore the feasibility of chemical and microbiological qualities of available marketed dahi which related to public health concern.

Chapter-VI

Conclusion

In conclusion, this study showed that all the collected dahi samples were negative for *E. coli and Salmonella sp*. The average percentage of fat, protein and acidity of collected dahi samples ranges from 3.43±0.18 to 3.58±0.11, 4.58±0.34 to 5.13±0.17 and 0.87±0.02 to 0.75±0.03, respectively. The total viable count of all samples ranges within the standard level. All the samples were negative for *E. coli and Salmonella sp*, that indicates proper hygienic handling, preparation, marketing and storage of dahi. So it can be said that the dahi samples that were collected from different retail shops of Chattogram metropolitan area were safe for human consumption. Presence of these organisms is critical for the safety of fermented milk products. Measures should be taken to prevent the transmission of pathogens into dahi at the household as well as plant level. A comprehensive research work recommended to set a standard commercial production of dahi in Bangladesh. Government should take proper steps by the help of BSTI to increase the quality of dahi all over the Bangladesh.

Limitations

The present study showed that all the collected dahi samples from Chattogram metropolitan area were negative for *Escherichia coli* and *Salmonella sp*. On the basis of the current study following limitations are given as below:

(1) Sample size of this investigation was not representative to the population due to short period of the study.

(2) Timing was not sufficient for such type of study.

(3) Lack of funding Antibiotic susceptibility test and PCR was not performed.

(4) Follow up was not completed for each and every case.

References

Ahmed, M.S.O., Mohamed O.M. Abdalla., Nahid M.T. Fawi., GamarEldin E. Mohamed and Gubara E.M. Ahmed (2015). Quality evaluation of stirred yoghurt flavored with guddaim (grewia tenax) fruit. *Asian Journal of Agriculture and Food Sciences*, 3(1): 2321-1571).

Akbar, A. and Anal, K.A. (2013). Prevalence and antibiogram study of *Salmonella* and *Staphylococcus aureus* in poultry meat. *Asian Pacific Journal of Tropical Biomedicine*, 3(2):163–168.

Akter, N., Nahar, A., Islam, M.N. and Al-Amin, M. (2010). Effects of different level of starter culture and sugar on manufacturing characteristics of MistiDahi (Sweet Yoghurt). *Journal of Bangladesh Agricultural University,* 8(2): 245–252.

Arvind., Sinha, P.R., Singh, N.K. and Kumar, R. (2009). Effect of Acidophilus casei dahi (Probiotic curd) on lipids in 1,2-dimethylhydrazine induced intestinal cancer in rats. *International Journal of Probiotics and Prebiotics*, 4(3):195–200.

Aly, S.A., Galal, E.A. and Elewa, N.A. (2004). Carrot yoghurt: sensory, chemical, microbiological properties and consumer acceptance. *Pakistan Journal of Nutrition*, 3(6): 322-330.

Ali, M.D.Y. (1998). A comparative study on the quality of dahi (yoghurt) available in Mymensing town. M.S. Thesis, Dept. of Dairy Sci. Bangladesh Agricultural University, Mymensing.

Ahmed, T.K. (1992). Comparative studies on zabadi made from cow and milk with or without skim milk powder supplement. *Sudan Journal of Animal Production*, 5: 93-102.

Aggawala, A.C. and Sharma, (1961). A Laboratory Manual of Milk Inspection. Bombay, Calcutta, New Delhi, India.

American Public Health Association (APHA) (1983). Standard Methods for the Examination of Dairy Products ('12thEd). American Public Health Association. Inc.1740 Broadway, New York. 34-62, 224-242.

Bhowmick, K.B., Saha, M.L. and Khan, M.R. (2006). Microbial Study of Some Milk with Special Reference to Coliform Bacteria. *International Journal of Dairy Sciences*, 1(1): 57-62.

Beukes, E.M., Bester, B.H. and Mostert, J.F. (2001). The microbiology of South African traditional fermented milks. *International Journal of Food Microbiology*, 63(3): 189–197.

Belickova, E., Tkacikova, L., Naas, T.H., Vargova, M., Ondrasovic, M.,O., Ondrasovicova Obsitnikova, D. and Toth, L. (2001). Staphylococci plate counts in foods of milk origin. *Veterinary Medicine-Czeck*, 46(1): 24-27.

Bozanic, R., L. Tratnik and O. Marie (1996). The influence of sterilized and cow milk on the quality of yoghurt during storage. *All 'ekarstvo*, 46(4): 239-250.

Baltadjeva, M., M. Tollorolcvu., P. Panayatov., M. Baltadzllicva and M. Choroleeva (1989). Production of yoghurt from milk. *Quademi-dellInstituto-Lattiero-caseario-di-Thiene*, 18:636-68, 72-75.

Balasubrarnanian, S.C. and K.P. Basu (1985). Indian Dairy man. 7: 99. (Cited from Rangappa K.S. and K.T. Achaya 1974. Indian Dairy products. Asia Publishing House. Bombay, p. 130).

Cappuccino, J. G. and Sherman, N. (2005). Microbiology: A Laboratory Manual. Peason new international edition.

Chakraborty, M. (1998). A study on the preparation of Dahi from whole milk of cow, buffalo and their different proportionate mixtures M.S. thesis. Dept, of Dairy Sci. Bangladesh Agricultural University, Mymensingh. *Dairy Research*, 8(3): 105- 168.

Christen, G.L., P.M. Davidson., J.S. McAllister and L.A. Roth (1992). Coliform and Other Indicator Bacteria. In Standard Method for the Examination of Dairy Product, Marshall, T.R. (Ed.). American Public Health Association, Washington, DC., pp: 247-267.

Cardoso, F., C. Iniguez and R. Morgado (1991). Effect of heat treatment on firmness of yoghurt made from buffalo milk. *RevistaCubana-de-Alimentacion-Y Nutrition*, 5(2): 111-117.

Das, S., Hasan, G.M.M.A. and Parveen, S. (2015). Evaluation of Microbial Load And Quality of Milk &Milk Based Dairy Products. *Octa Journal of Biosciences*, 3(1):1-4.

Dey, S., Iqbal, A., Ara, A. and Rashid, M. H. (2011). Evaluation of the quality of Dahi available in Sylhet Metropolitan City. *Journal of Bangladesh Agricultural University*, 9(1): 79–83

Deeth, H.C. and Tamime, A.Y. (1981). Yoghurt: nutritive and therapeutic aspects. *Journal Food Protection*, 44:78–86

Duitschaever, C.L. (1978). Yoghurt from milk. *Cultured-Dairy-Products*, 13(4): 20-23

El-Kenany, Y.M., J. Seatmov and F.S. Ibrahim. (1996). Improving the self-life of yoghurt. *Anaals of Agricultural Science Moshtohor*, 34(1): 335-343.

Eschmann, K. H. (1968). Establishment of reference methods and microbiological standards for the Swiss Food Legislation Book. (*Dairy Science* Abstract. 30: 375).

Food Safety and Standard Regulations (2011). Food Safety and Standards Authority of India, New Delhi, 1st august, 2011.

Fernandez, S.G., M. B. Serra., M.A. Casas., J.M.L Bes., J.1Z. Pol., E.T. Gay., A.R. Pico and P.B. Lopez (1994). Rheological changes during the processing of cultured milk Yoghurt. *Alimentaria*, 31(254): 41-48.

Flowers, R.S., Andrews, W., Donnelly, W.C. and Koenig, K.E. (1992). Pathogens in Milk and Milk Products. In Standard Methods for the Examination of Dairy Products, 16th edition, Ed., R.T. Marshal. Washington, DC: American Public Health Association, 8: 103-212.

FAO / WHO (1973). Code of Principles Concerning Milk and Milk Products, CX 5/70, Rome, Italy.

Ghaleb, H.M., N.M. Hanafy and A.A. El-Ghandour (1998). Some trials to produce yoghurt of low cholesterol content. (2): Bacterial cholesterol assimilation.

Giudici, P., G. Masini and C. Caggia. (1996). The role of galactose fermenting yeast in plain yoghurt spoilage. *Annali di Microbiologiaed Enzimologia (Italy),* 46(1): 11-19.

Gabriel, J.M. (1990). Process for manufacturing liquid yoghurt from goat milk. French patent Application. FR263 6506 Al.*C.F. Dairy Science,* 52(11):7819.

Griffiths, M.W., J.D. Phillips and D.D. Muir (1987). Effect of low-temperature storage on the bacteriological quality of raw milk. *Food Microbiology*, 4: 285-291.

Hashimoto, E.M. and L.A.F. Antunes (1995). The effect of heat treatment and ropy starter on the rheological characteristics of milk yoghurt. *Ciencia-e- Technologia-de-Alinoerrtvs*, 15(3): 225-261.

International Dairy Federation.(1995). Milk and Milk Products. Detection of *Salmonella* (IDF Standard 93B: 1995). IDF, Brussels.

International Dairy Federation.(1995). Milk and Milk Products. Detection of *Listeria monocytogenes* (IDF Standard 143A: 1995). IDF, Brussels.

International Dairy Federation.(1990). Milk and Milk-based Products. Enumeration of *Staphylococcus aureus* (IDF Standard 145: 1990). IDF, Brussels.

Indian Standards Institution (1973). Specification for fermented milk products. Indian Standards, (Dairy Science, Abstract. 38: 757).

Jawalekar, S.D., U.M. Ingle., P S. Waghmare and P.N. Zanjad (1993). *Indian Journal of Dairy Science*, 46(5): 217-219.

Jasjit, S., A. Khanna., H. Chander and J. Singh (1979). Antibacterial activity of yoghurt strarter in cow and buffalo milk. *Journal of Food-protection*, 42(8): 664-665.

Khan, M.T.G., Zinnah, M.A., Siddique, M.P., Rashid, M.H.A., Islam, M.A. and Chowdhury, K.A. (2008). Physical and Microbial Qualities of Raw milk Collected from Bangladesh Agricultural University, Dairy Farm and the Surrounding Villages. *Bangladesh Journal of Veterinary Medicine*, 6: 217-221.

Karthikeyan, S., H. K. Desai and K.G. Upadhyay (1998). Evaluation of starter culture and level of total solids in sweet cream butter milk for chakka making. *Indian Journal of Dairy and Bioscience,* 9: 49-54.

Kehagias, C., L. Kalavritinos and C. Triadopoulou (1992). Effect of on the yield and solids recovery of strained yoghurt from and cow milk. *Cultured Dairy Products*, 27(3,10): 12-14.

Luisiani, G., P. Salvadori and B. Bianchi-Salvadori (1974). Microbiological evaluation of yoghurt in connection with storages times and temperatures. *Lati*,54(5311532): 53-59.

Laxminarayana, H., V.K.N. Nambudripad., N.V. Lakshmi., S.N. Anantaramiah and V. Sreenivasamurthy (1952). Studies on dahi II. General servey of the quality of market dahi. *Indian Journal of Veterinary Science. Animal Husbandry,* 22(1): 13.

Malek, M., Akter, J., Ahmed, T. and Uddin, Md.A. (2015). Isolation and quantification of microorganisms from some common milk products within Dhaka city, Bangladesh. *Stamford Journal of Microbiology*, 5(1): 13-17.

Marjan, S., Das, K.K., Munshi, S.K. and Noor, R. (2014). Drug-resistant bacterial pathogens in milk and some milk products. *Nutrition and Food Science*, 44 (3): 241-248.

Michael, M.J., Pelczar, J.R., Chan, E.C.S. and Nobel, R.K. (2007). Microbiology-5th edition, Tata McGraw-Hill Publishing Company Limited, New Delhi.

Mehanna, N.M. and S.A. Hefnawy (1988). Effect of thiocyanate-lactoproxidase- hydrogen peroxide system on the manufacture and properties of yoghurt. *Egypt. Journal of Dairy Science*, 16(1): 55-63.

Manjunath, N. and M.J. Abraham (1986). Yoghurt from milk. *Asian Journal of Dairy Research*,5(2): 103-107.

Muir, D.D., Phillips, J.D. and Dalgleish D.G. (1979). Lipolytic and proteolytic activity of bacteria isolated from blended raw milk. *International Journal of Dairy Technology*, 32(1): 19–23.

Nassib, T.A., El-Din, M. Z. and El-Sharoud, W. M. (2003). Assessment of the presence of *Salmonella spp*. in Egyptian dairy products using various detection media. *Letters in Applied Microbiology*, 37(5): 405-409.

Nergiz, C. and A.K. Seckin (1998). The losses of nutrients during the production of strained (Torba) yoghurt. *Food Chemistry*, 61(1-2): 13-16.

Nikolov, N., (1960). Study of Yoghurt. DOI Prom 21(8): 31-34 (*Dairy Science*. Abs, 22: 6451).

Noeman A.A. and S.U. Shalaby (1992). A comparative study between Zabdy and acidophilus milk. *Egypt. Journal of Food Science*.20:Sup 43-51.

Pala, V., Sieri, S., Berrino, F., Vineis, P., Sacerdote, C., Palli, D., Masala, G., Panico, S., Mattiello, A., Tumino, R., Giurdanella, M.C., Agnoli, C., Grioni, S. and Krogh, V. (2011). Yogurt consumption and risk of colorectal cancer in the Italian EPIC cohort. *International Journal of Cancer*, 129(11):2712-2719

Pazakova, J., O. Burdova., P. Turek and A. Laciakova (1999). Sensorial evaluation of yoghurt produced from cow, ewe and milk. *Czech Journal of Food Science*, 17(1): 31-34.

PFA (1976). Prevention of Food Adulteration Act Ministry of Health and Family Welfare, New Delhi.

Ruegg, P.L. (2003). Practical Food Safety Interventions for Dairy Production. *Journal of Dairy Science*, 86: E1–E9.

Rashid, M.H. and Miyamoto, T. (2005). Quality evaluation of traditional fermented milk "Dahi" in Bangladesh. *Milk Science*, 54(1): 29-36.

Ranganadham, M. and S.K. Gupta (1987). Sensory evaluation of dahi and yoghurt. *Indian Dairyman*, 39(10): 493-497.

Ray, H.P. and R.A. Srinivasan (1972). Use of microorganisms for production of indigenous fermented milk products (Sweetened dahi). *Journal of Food Science and Technology*, 9: 62.

S.V. Megha and B. Annadurai (2014). Isolaton and Identification of proteolytic bacteria from raw milk samples. *Global Journal of Bio-Science and Biotechnology*, 3(4): 391-397.

Sinha, P.R. andSinha, R.N. (2000). Importance of good quality dahi in food. *Indian Dairyman*, 52:45–47.

Salem, S.A., I.A. Attlia., E. Gooda and M.S. Kamar (1995a). Studies on frozen yoghurt 2.Effect of using different levels of fat content Egypt. *Journal of Food Science*, 22(1): 13-25.

Salem, S.A., I.A. Attia., E. Gooda and M.S. Kamar(1995b). Studies on the frozen yoghurt. 3. Effect of using high levels of fat with different inoculaof starter. *Egypt. Journal of Food Science*, 22(1): 27-29.

Salji, J.P ; W.N. Sawaya., M. Ayaz and A. Mashhadi.(1987). Production, processing and quality assessment of dairy products in the Western Province of Saudi Arabic. *Milchwissenschaft*, 42(1): 27-31.

Singl, J., and S. Jasjit (1979). Manufacture of yoghurt from cow and buffalo-milk. *Indian-Dairyman*, 31(2): 117-119.

Spanish Standard (1976). Spanish Standard for Yoghurt. *Via Lacten*, 8: 31-33.

Sreenivasan, K.N. and B. Ranganathan (1972). Role of contaminating yeasts in the spoilage of dahi during storage. *Journal of Food Science and Technology*, 9(2): 69-72.

Varnam, H.A. and J.P. Sutherland (1994). Milk and milk Products: technology, chemistry and microbiology. *ASPEN Publishers Inc.* USA.

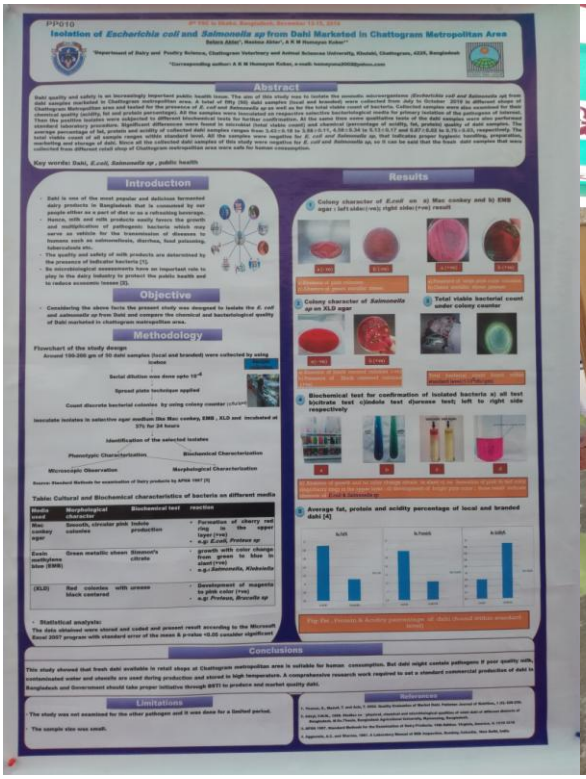
Yadav, H., Jain, S. and Sinha, P.R. (2008). Oral administration of dahi containing probiotic *Lactobacillus acidophilus* and *Lactobacillus casei* delayed the progression of streptozotocin-induced diabetes in rats. *Journal of Dairy Research*, 75(2):189–195.

.

Publication/presentation related this thesis

2019

(1) Setara Akter., Nasima Akter., A. K. M. Humayun Kober., (2019). Isolation of *Escherichia Coli* and *Salmonella sp* from Dahi marketed in Chattogram Metropolitan Area. 4th Young Scientist Congress'19. (Page-116) (Abstr- PP 10).



Appendix

Appendix I

Formula of various bacteriological media

A. Mac Conkey Agar

Peptone	20.00 g
Lactose	10.00 g
Bile salts	5.00 g
Sodium chloride	5.00 g
Neutral red	0.075 g
Agar	12.00 g
Distilled water	1000 ml

B. Eosin Methyl Blue (EMB) Agar

Peptone	10.00 g
Sucrose	5.00 g
Lactose	5.00 g
Dipotasssium phosphate	2.00 g
Agar	13.50 g
Eosin	0.04 g
Methylene blue	0.065 g
Distilled water	1000 ml

C.Xylose-Lysine-Deoxycholate Agar

Yeast extract	3.00 g
L-lysine	5.00 g
Lactose	7.50 g
Sucrose	7.50 g
Xylose	3.50 g
Sodium chloride	5.00 g
Sodium deoxycholate	2.50 g
Sodium thiosulfate	6.80 g
Ferric ammonium	0.80 g
Phenol red	0.08 g
Agar	15.00 g
Distilled water	1000ml

D. Physiological Saline

Sodium Chloride	9.00 g
Distilled water	1000ml

E. Simmon's Citrate Agar

Magnesium sulphate	0.20 g
Ammoniundihydrogen phosphate	1.00 g
Dipotassium phosphate	1.00 g
Sodium citrate	2.00 g
Sodium chloride	5.00 g
Bacto agar	15.00 g
Bactobromothymol blue	0.08 g
Distilled water	1000 ml

F. Triple Sugar Iron Agar

Bio-polytone	20.00 g
Sodium chloride	5.00 g
Lactose	10.00 g
Sucrose	10.00 g
Dextrose	1.00 g
Ferrous ammonium sulphate	0.20 g
Sodium thiosulphate	0.20 g
Phenol red	0.0125 g
Agar	13.00 g
Distilled water	1000 ml
Final pH	7.3

G. Motility Indole Urease (MIU) Agar

Tryptone	10.00 g
Phenol red	0.10 g
Agar	2.00 g
Sodium chloride	5.00 g
Distilled water	1000 ml
pH (at 25°C)	6.8 ± at 25°C

Reagents

Appendix II

Kovac's Reagent: (150 ml) To a reagent bottle, 150 ml of reagent grade isoamyl alcohol, 10 g of pdimethylaminobenzaldehyde (DMAB) and 50 ml of HCl (concentrated) were added and mixed. The reagent bottle was then covered with an aluminum foil to prevent exposure of reagent to light and stored at 4°C.

Urease Reagent: (50 ml 40% urea solution) To 50 ml distilled water, 20 g pure urea powder was added. The solution was filtered through a HEPA filter and collected into a reagent bottle. The solution was stored at room temperature.

Biography

Setara Akter is an MS student of Dairy Science under the Department of Dairy and Poultry Science at Chattogram Veterinary and Animal Sciences University (CVASU). She is the daughter of Md. Abul Kalam Ajad and Hazera Begum. She passed her SSC examination in 2009 and HSC examination in 2011. She had successfully completed her DVM degree and clinical training in Madras Veterinary College and Veterinary College and Research Institute (VCRI), Namakkal, India in 2017. She also successfully completed her MS 1st year theory courses.