Microbiological status of ice cream and tamarind juice (tok) collected from various locations of Dhaka city

A DISSERTATION SUBMITTED TO BRAC UNIVERSITY FOR PARTIAL FULLFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF BACHELOR OF SCIENCE IN MICROBIOLOGY

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AUGUST 2016

DECLARATION

This is to declare that the research work aggregating the results reported in this thesis entitled **"Microbiological status of ice cream and tamarind juice (tok) collected from various locations of Dhaka city"** has been carried out under the supervision of Dr. Mahboob Hossain, Associate Professor, Microbiology program, Department of Mathematics and Natural Sciences, BRAC University. It is further declared that the research work reported here is authentic and submitted in the partial fulfillment for the degree of Bachelors of Science in Microbiology, BRAC University, Dhaka. Any reference to work done by any other person or institution or any material obtained from other sources have been duly cited and referenced.

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Dedicated to…….
My Family

ACKNOWLEDGEMENT

First of all, I express my gratitude towards **Prof. A. A. Ziauddin Ahmad**, Chairperson, Department of Mathematics and Natural Sciences, BRAC University, for his kind cooperation, support and constant supervision. I am also grateful to **Prof. Naiyyum Choudhury**, coordinator of Biotechnology and Microbiology program, Department of Mathematics and Natural Sciences, BRAC University for all his cooperation and active support.

I would also like to express my gratefulness and deepest gratitude to my academic supervisor **Dr. Mahboob Hossain**, Associate Professor, Department of Mathematics and Natural Sciences, BRAC University for his dynamic supervision, worthwhile suggestions, motivation and encouragement all through this study.

I am thankful to all the teaching assistant and laboratory assistants for their help and cooperation in doing my research work. Finally I would like to extend my thankfulness to the members of my family and friends for their encouragement and supports in the hardest times throughout this study.

Abstract

The project was designed to take on a study on the microbiological status of the ice cream and tamarind juice (tok) samples taken from different shops and street vendors that are located in different areas of Dhaka city. Qualitative microbiological analysis of a total twenty samples was done and microbes were identified by the standard biochemical tests. The quantitative analysis and biochemical test results showed that the samples contained a number of microorganisms of which 11 isolates were identified. Cultural and biochemical examinations revealed the presence of *Proteus mirabilis, Streptococcus spp, Alcalegenes spp, Bacillus spp, Klebshiella spp, Staphylococcus spp, Micrococcus luteus, Proteus vulgaris, Enterobacter spp, Staphylococcus aureus* and *E. coli.* The highest microbial load was observed in Cornetto cone ice cream of I3 ($1.6X10^3$cfu/g) and tok sample collected from Shipahibag ($1.5X10^4$cfu/ml). Mango cup ice cream from I4 and tok sample from BRAC University showed the lowest microbial count; $6.3X10^2$cfu/g and $2.35X10^3$cfu/ml respectively. This study emphasized that the hygienically maintained food retained the best quality attributes required for consumer's acceptability and safety.

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LIST OF ABBREVIATIONS

mg	Milligram
g	Gram
ml	Milliliter
l	Liter
min	Minute
sec	Second
h	Hour
d	Day
^{0}C	Degree Celsius
µm	Micrometer
e.g.	For example
et al.	And others
pH	Negative logarithm of hydrogen ion concentration
%	Percentage
spp.	Species
cfu	Colony forming unit
NA	Nutrient Agar
MAC	MacConkey Agar
MFC	Mannitol Salt Agar
XLD	Xylose Lysine Deoxycholate
FAO	Food and Agriculture Organization of the United States
IDFA	International Dairy Food Association
USDA	United States Department of Agriculture
NCASS	Nationwide Caterers Association
MRCA	Market Research Cooperation of America
WHO	World Health Organization
BRAC	Bangladesh Rural Advancement Committee

CHAPTER 1: INTRODUCTION

1. Introduction

1.1 Definition and ingredients of ice cream

Ice cream is one of the most famous frozen food around the whole world. It is a sweet frozen food which is usually made from dairy products (such as: milk and cream) and often combined with fruits or other ingredients and flavors. Ice cream is normally sweetened with sucrose, corn syrup, cane sugar, beet sugar or other sweeteners. Typically, flavorings and colouring agent are added in addition to stabilizers. Generally, it is eaten as a snack or dessert. Ice cream may be served in dishes (for eating with a spoon) or in cones (which are licked). It can be served with other desserts, such as apple pie. Vanilla, chocolate, strawberry, mango – these are some classic flavors of ice cream that are commonly seen, where the total number of ice cream flavors is above 50 and the number is increasing day by day. This large variety of flavor includes natural and artificial flavor, fruit, nuts, and bulky inclusions such as: chocolate chunks and candies etc. in various types of ice cream.

There is a wide range of ingredients and formulations (recipes) to make ice cream. Milk fat (milk or cream or butter) provides creaminess and richness to ice cream and contributes to its melting characteristics. The total milk solids component of ice cream includes both the fat and other solids. The nonfat solids play an important role in the body and texture of ice cream by stabilizing the air that is incorporated during the freezing process. Sources of nonfat solids include milk, cream, condensed milk, evaporated milk, dry milk and whey. Sweeteners are used to provide the characteristic sweetness of ice cream. Sweeteners (sugar and corn syrups) also lower the freezing point of the mix to allow some water to remain unfrozen at serving temperatures. A lower freezing point makes ice cream easier to scoop and eat, although the

addition of too much sugar can make the product too soft. Stabilizers like: alginates (carageenan), gums (locust bean, guar), and gelatins are also used in ice cream to add viscosity and to control ice crystallization. Over time, during frozen storage, small ice crystals naturally migrate together and form larger ice crystals. Stabilizers help to keep the small crystals isolated and prevent the growth of large crystals, which would cause ice cream to become coarse, icy and unpleasant to eat. However, emulsifiers used in ice cream include egg yolks and mono- and diglycerides are used as helper to keep the milk fat evenly dispersed in the ice cream during freezing and storage. A good distribution of fat helps stabilize the air incorporated into the ice cream and provide a smooth product.

1.2 Definition and ingredients of tamarind juice (tok)

Tamarind juice (tok) is another most famous roadside food item around Bangladesh and India. Tok is the specially prepared tamarind juice that is used to eat chotpoti (cooked chickpeas with spices, potato, chili and onion) and fuchka (a crispy shell that is used to eat chotpoti). It is normally eaten with chotpoti and fuchka as snacks. Tok can be divided into two types: sweet tok and sour tok. People prefer between the two types according to their individual taste. As the combination of both tok and chotpoti create great flavor, these food items are seen to sell in every street of Dhaka city. From children to men, everyone enjoys a lot by having this street food item at any time. Besides the street vendors, some fast food shops also sell this food item.

Generally, tok is a little bit different than the normal tamarind juice. It is not only more diluted, but also more flavorful than the regular tamarind juice. Addition of various spices like: dry roasted cumin powder, salt, dry roasted red chili powder, chopped onion and pepper altogether

make the normal tamarind juice into tasty tok. For providing more flavors, a little amount of sugar, jaggery or honey is also mixed with the genuine recipe and turns the sour tok into sweet one. Very few snacks are more popular in Bangladesh than chotpoti and fuchka with tok.

1.3 Nutritional fact of ice cream and tok

Though ice cream is not typically eaten for its nutritional value, there is several health benefits associated with this frozen treat. Since ice cream is a dairy product like: milk or yogurt, it contains some of the same vitamins and nutritional content. Calcium found in dairy products, like: ice cream is beneficial for strong and healthy bones. Regular calcium intake from ice cream and other dairy products can also reduce the risk of osteoporosis, a disease related to an increase in bone fractures. Calcium is not only good for the bones and teeth, but also it plays a part in weight loss. Studies have shown a correlation between reduced weight and weight gain prevention with an adequate, daily intake of calcium. When the body is not receiving an adequate amount of calcium, it causes fat cells to enlarge by storing fat. Michael Zemel, Ph.D, of the University of Tennessee, Knoxville, relates a lack of calcium to the creation of fat producing hormones and a slowing effect of fat breakdown leading to weight gain.

Moreover, ice cream contains small amount of protein, a macronutrient that is important for parts of the body. Those who exercise regularly can use ice cream as an after workout snack to help with muscle building and recovery. In addition to healthy fruits, nuts, vegetables and dietary supplements, ice cream can be transformed into a great recovery shake or smoothie after a strenuous workout session. Ice cream typically contains vitamins A, vitamin B2 and vitamin B12 etc. Though it provides us some nutrients, most traditional ice creams are loaded with sugar and

saturated fats. They also have high calorie. Certain types of ice creams also include added toppings like: milk-chocolate, peanut-butter cups, fudge and other forms of candy, which increase their calorie and sugar content. These characteristics can completely negate the health benefits of ice cream, and can lead to major health risks like diabetes, high cholesterol and obesity. A table containing nutrient value of vanilla ice cream is shown below here:

Ice cream, vanilla ▾			
Amount Per 1 serving 1/2 cup (66 g) ▾			
Calories 137			
			% Daily Value*
Total Fat 7 g			10%
Saturated fat 4.5 g			22%
Polyunsaturated fat 0.3 g			
Monounsaturated fat 2 g			
Cholesterol 29 mg			9%
Sodium 53 mg			2%
Potassium 131 mg			3%
Total Carbohydrate 16 g			5%
Dietary fiber 0.5 g			2%
Sugar 14 g			
Protein 2.3 g			4%
Vitamin A	5%	Vitamin C	0%
Calcium	8%	Iron	0%
Vitamin D	1%	Vitamin B-6	0%
Vitamin B-12	5%	Magnesium	2%

Table 1.1: Nutritional value of ice cream

Source: United States Department of Agriculture (USDA) SR-21

Tamarind juice (Tok) contains certain health benefiting essential volatile chemical compounds, minerals, vitamins and dietary fiber. It is a rich source of non-starch polysaccharides (NSP) or dietary-fiber such as gums, hemicelluloses, mucilage, pectin and tannins. 100g of it provides 5.1 or over 13% of dietary fiber. NSP or dietary fiber in the food increases its bulk and augments bowel movements thereby help prevent constipation. The fiber also binds to toxins in the food thereby help protect the colon mucus membrane from cancer-causing chemicals. In addition, tok helps to bind to the bile salts (produced from cholesterol) and decrease their re-absorption in the colon; thereby help in expulsion of bad or LDL cholesterol levels from the body.

Tok is also rich in tartaric acid. Tartaric acid gives sour taste to food besides its inherent activity as a powerful antioxidant. Thus it helps human body to stay protected from harmful free radicals. Tamarind juice contains many volatile phytochemicals such as: limonene, geraniol, safrole, cinnamic acid, methyl salicylate, pyrazine and alkylthiazoles. Together, these compounds account for the medicinal properties of tamarind. This prized condiment spice is a good source of minerals like: copper, potassium, calcium, iron, selenium, zinc and magnesium. Potassium is an important component of cell and body fluids that helps control heart rate and blood pressure. Iron is essential for red blood cell production and as a co-factor for cytochrome oxidases enzymes. In addition, it is also rich in many vital vitamins, including thiamin (36% of daily required levels), vitamin-A, folic acid, riboflavin, niacin, and vitamin-C. Many of these vitamins play roles as antioxidant, as well as co-factor for enzyme metabolism inside the body. Not only that, tamarind juice has been used in many traditional medicines as a laxative, digestive, and as a remedy for biliousness and bile disorders. This spice condiment is also used as emulsifying agent in syrups, decoctions, etc., in different pharmaceutical products. Though tok contains lots of nutrients that are highly beneficial for our health, it has some drawbacks too. A large portion of the calories

can make this food harmful as the calories come from added sugars. A table containing nutrient value of tamarind juice is shown below here:

Tamarind			
Amount Per 1 fruit (3" x 1") (2 g) ▾			
Calories 5			
			% Daily Value*
Total Fat 0 g			0%
Saturated fat 0 g			0%
Polyunsaturated fat 0 g			
Monounsaturated fat 0 g			
Cholesterol 0 mg			0%
Sodium 1 mg			0%
Potassium 13 mg			0%
Total Carbohydrate 1.3 g			0%
Dietary fiber 0.1 g			0%
Sugar 1.1 g			
Protein 0.1 g			0%
Vitamin A	0%	Vitamin C	0%
Calcium	0%	Iron	0%
Vitamin D	0%	Vitamin B-6	0%
Vitamin B-12	0%	Magnesium	0%

Table 1.2: Nutritional value of tok

Source: United States Department of Agriculture (USDA) SR-21

1.4 Foodborne illness caused by ice cream and tok

Both ice cream and tok contain many important elements that are beneficial for human health, but these food items can also hamper to human health too. Various types of foodborne illness can be occurred from these food items easily. Foodborne illness can be define as an infection or irritation of the gastrointestinal (GI) tract caused by food or beverages that contain harmful bacteria, parasites, viruses, or chemicals. A survey shows that, each year, an estimated 48 million people in the United States experience a foodborne illness. Not only that, foodborne illnesses caused 128,000 hospitalizations and about 3,000 deaths in the United States annually. Common symptoms of foodborne illness include vomiting, diarrhea, abdominal pain, fever and chills. Many different disease-causing microbes or pathogens can contaminate foods; thus, there are many different types of foodborne illnesses. *Bacillus cereus* that can cause diarrhea and vomiting, *Clostridium perfringens* which is one of the most common causes of food poisoning can cause diarrhea and abdominal cramps. *Staphylococcus aureus* is a kind of bacteria that festers when food isn't properly refrigerated. It can cause nausea, loss of appetite, abdominal cramps and mild fever. Finally, *Salmonella spp* can affect the intestinal tract and cause diarrhea, fever and abdominal cramps. Most foodborne diseases are infections caused by a variety of bacteria, viruses, and parasites. Other diseases are poisonings caused by harmful toxins or chemicals that have contaminated food.

So, different types of illness can definitely occur by consuming contaminated ice cream. Generally, people think that, ice cream is hygiene because it is a frozen and packaged food item. However, ice cream can be contaminated through various reasons even before packaging. Lack of proper environment in the factory, hygiene, water supply, clean packaging materials,

contaminated ingredients, hygiene of workers, contamination in refrigerator or during storage period etc. are some reasons that can lower the quality of ice creams. Convener of Students' Wing of JCILPS (Joint Committee on the Inner Line permit system) of India, M Angamba said that ice-cream is one of the favorite food items of children. The ice-cream produced from unhygienic factories could have serious impact on the health of children. Thus, if high levels of bacteria can be found from ice creams, then it is very risky to eat those and from that, illness can take place really fast.

Similarly, tok is also not safe enough to consume. It is a street food and street food is not totally hygienic. The street vendors take money; exchange notes with multiple customers, cook and serve food. He rarely (if ever) wears gloves and hurries about his business, pushing pots, pans and buckets, all at the same time wash his hands. Not only that, he does not even use gloves during preparing the tok. As per the Food Safety and Standards Authority of India (FSSAI), "Following an incubation period of about 3-4 days, a variety of gastrointestinal symptoms appear, ranging from mild to severe bloody diarrhea, mostly without fever. The one causing infection now is a highly virulent mutated strain". The ingredients of the tok can be unhygienic too and can create contamination in tok. Sometimes, people take away tok in their home with chotpoti and fuchka. In that time, vendors parcel the tok in the polythene bag. This polythene bag is a carrier of loads of germs and bacteria as no one clean this bag at all. Consequently, after having the tok from that bag makes a clear path for sickness and microorganisms can without doubt done their job on consumer. Therefore, from the over discussed points, it can be said that, foodborne illness or sickness can simply happen to anyone, anytime from ice creams and tok.

1.5 Overall objectives

The overall objective of this study were-

- To characterize the microorganisms from ice cream and tok collected from various shops and food vendors respectively.
- Relate the result to assume the quality of these food items and further relate that with food safety and hygienic practices in Dhaka.

CHAPTER 2: LITERATURE REVIEW

2. Literature review

2.1 Popularity of ice cream

Ice cream is such a food item which is almost irresistible to anyone and for that, its popularity is quite astonishing. The average American consumes almost 22 pounds of ice cream per year. U.S. ice cream companies made more than 872 million gallons of ice cream in 2014. The popularity of ice cream is on all type of people (IDFA, 2012). Children age from two through 12 and adults age from 45 plus, eat the most ice cream per person. Not only that, about 1.53 billion gallons of ice cream and related frozen desserts were produced in the U.S. in 2012 (IFDA, 2012). "Ice cream is thought of as a treat that appeals to the young," says David Fencl of MRCA Information Services. "Surprisingly, we find the over-55 age group consuming both premium-brand and regular ice cream at nearly 50 percent above the national average", he says.

According to a recent survey of International Ice Cream Association member companies, vanilla remains the most popular flavor among their consumers. Companies said that Chocolate Chip Mint and Cookies and Cream were the next most popular flavors, (IDFA ice cream company survey, 2012). The popularity of ice cream plays role on global economical sector too. The majority of ice cream and frozen desserts are marketed regionally. More than 66.7 percent of U.S. ice cream and frozen dessert manufacturers say they market their products regionally, with 16 percent marketing nationally. The international market accounts for 10 percent of the market for U.S. companies, (IDFA ice cream company survey, 2012). Premium ice cream, which tends to have lower amount of aeration and higher fat content than regular ice cream, is the most popular product with consumers according to a recent survey of U.S. ice cream manufacturers. In the survey, 79.3 percent cited premium ice cream as the most popular product made while 10

percent said that novelties are most popular. Novelties are defined as separately packaged single servings of a frozen dessert – such as ice cream sandwiches and fudge sticks, (IDFA ice cream company survey, 2012). Since public have ice cream a lot, so they should know the drawbacks of having it too.

2.2 Popularity of tok (as street food)

Tok with chopoti and fuchka is one of the most prominent street foods across Dhaka, Bangladesh. According to Food and Agriculture Organization study in 2007, 2.5 billion people worldwide eat street food every day. A total dietary study among 37 male and ten female students, ranging in age from 18 to 24 years, was conducted in Bogor. The economic levels of the participants' households varied but all students had diets consisting largely of street food. Using diary recordings, total daily food consumption data were collected for a 14-day period. Sixty-three percent of the students' monthly expenditures were allocated to street foods. The study found that street foods constituted the largest part of total energy intake (78 percent), accounting for 82 and 79 percent, respectively, of total protein and iron intake. These data may indicate that street foods play a major role in the overall diet for students in Indonesia (Street Food Project Report No. 3, 1990).

Like ice cream, street foods are also very demanding as these are enough cheap to the customers. In Africa and Asia, urban households spend 15 to 50 percent of their food budgets on street foods (Cohen *et al*. 1986). Nevertheless, in the US the food truck market has grown 12.4% over the last five years, to a reported 4,130 trucks today, generating a $1.2 billion industry, (Catering News ME, 2015). The street food industry currently directly employs around 1,500 people in the UK

and supports around 500 SME's, predominantly in London where the scene first started and probably the same number in supporting industries such as agriculture. It has the capacity and demand able to support ten times this number of businesses and employees, (NCASS, 2013).

2.3 Foodborne illness

Food-borne diseases are a major public health concern worldwide. WHO defines food-borne disease (FBD) as "disease of infectious or toxic nature caused by, or thought to be caused by, the consumption of food or water". Although FBD has decreased in recent years, it is still higher than Healthy People 2020 goals. An estimated of 600 million almost where 1 in 10 people in the world fall ill after eating contaminated food and 420,000 die every year, resulting in the loss of 33 million healthy life years (WHO, 2015). Not only that, children under 5 years of age carry 40% of the foodborne disease burden, with 125,000 deaths every year, (WHO, 2015).

Street foods play a major role behind the food borne illness. Unsafe food causes many acute and life-long diseases, ranging from diarrheal diseases to various forms of cancer (WHO, 2015). WHO also estimates that food borne and waterborne diarrheal diseases taken together kill about 2.2 million people annually, 1.9 million of them are children (WHO, 2010). According to Dr. Ritika Samaddar, Dietetics, Max Hospital, "*E. coli* is a kind of bacteria that can cause diarrhoea or gastroenteritis. Gastroenteritis is a condition in which your stomach or intestines are inflamed. *E. coli* can be found in street food, cut fruits or any contaminated food. High temperature makes the situation worse because it multiplies these bacteria, making you more prone to falling ill".

The immune system of children is not strong enough, so they can fall into sick by having contaminated ice cream or tok. According to Bangladesh health and injury report on children

under 5 in 2005, five children every year died in diarrhoea (Bangladesh Health and Injury Survey Report, 2005). Unsafe food containing harmful bacteria, viruses, parasites or chemical substances, causes more than 200 diseases, ranging from diarrhea to cancers (WHO, 2015). Diarrheal diseases are the most common illnesses resulting from the consumption of contaminated food, causing 550 million people to fall ill and 230,000 deaths every year, (WHO, 2015).

A survey involving 135 street foods in Iloilo, the Philippines found that more than forty street food items caused diarrhea among the study participants (Tinker *at el,* 1987). Hence, it can be said that, most street foods contain lots of microorganisms. *Staphylococcus aureus* is one of the most common pathogens causing several outbreaks, (Veras et al. 2008). Shiga toxin-producing *Escherichia coli* are a group of bacteria strains capable of causing significant human disease (Physician, 2000). In 2004, *Enterococcus* genus took the place of fecal coli forms as the new federal standard for water quality and public beaches in Hawaii USA (Yang, 2008).

A variety of reasons are working behind the contamination of food. A lack of knowledge among fast food vendors about the causes of food-borne disease is a major risk factor. Poor hygiene, inadequate access to potable water supply and garbage disposal, and unsanitary environmental conditions such as proximity to sewers and garbage dumps further exacerbate the public health risks associated with street foods (FAO, 1998). Traditional processing methods that are used in preparation, inappropriate holding temperatures and poor personal hygiene of food handlers are some of the main causes of contamination of street-vended food (Mensah et al, 2002; Barro*et al,* 2006). Despite the availability of food safety strategies for public health and economic development in many countries, food safety policies, plan of action and legislation have not been implemented especially in developing countries (Addo, 2007).

2.4 Lack of hygiene practice

General people are not concern about the safety of their food at all. Lack of knowledge about handling and packaging food items are decreasing the quality more. As a result, the contamination of food gets the opportunity to turn into foodborne illness in the human body. To prevent this straight forward way of occurring diseases, the role of hygiene practice should bring forward openly to everyone. Only then, the vendors and workers will be more responsible and consuming ice cream or any street food items (tok) will be safer than the present situation.

CHAPTER 3: MATERIALS AND METHODS

3. Materials and Methods

3.1 Working laboratory

In general, the research was performed at the Microbiology research Laboratory on (UB02 18th floor) of the Department of Mathematics and Natural Sciences, BRAC University from December 2015 to June 2016.

3.2 Sources, collection and transportation of samples

3.2.1 Ice cream samples

Ice cream samples were collected from distinct confectionary stores that are located in various places of Dhaka city. Four different brands of ice creams were selected for this study. The brands are:

1) I1
2) I2
3) I3
4) I4

In total ten ice cream samples were taken from these four brands for the research. Each sample was collected by using the laboratory glass bottle (DURAN® double walled, wide mouth bottle GLS 80® of 500mL) from every store. The period of transportation was variable, from ten minutes to two hours depending on the location of the individual store. After bringing the samples into the laboratory, the glass bottle was moved into refrigerator immediately. Before examination, all samples were left at room temperature for some time period until melted.

3.2.2 Tok samples

Tok samples were collected from various street food vendors located in diverse places like: Mohakhali, Khilgaon, Dhanmondi etc. area of Dhaka city. The total number of tok samples was ten. Like the ice cream sample, tok was collected within the laboratory glass bottle (DURAN® double walled, wide mouth bottle GLS 80® of 500mL) from every vendor. The transportation period was also variable, from ten minutes to two hours depending on the location of the individual street food vendor. The glass bottle carrying tok was shifted into the laboratory refrigerator straight away like ice cream. The samples were left at room temperature for some time to reach the normal temperature of tok.

All the samples collected for this study were carefully handled during the transportation to the laboratory in aseptic condition. Since aseptic care was taken during transportation, the samples were superior enough for the bacteriological analysis.

3.3 Preparation of samples

Before starting experiment, all samples were mixed properly via the analog vortex mixer machine (VWR collection). Mainly the tok samples were then kept for 1-2 min. As a result, the solid elements of the tok assembled at the bottom of the glass bottle and created some clear portion at the top. 1mL of each sample was taken aseptically with a sterile micropipette and transferred carefully into the test tube having 9mL sterile saline. Thus 1:1 dilution of the samples was obtained. Then, using the vortex machine again, the mixture was mixed properly. Later different serial dilutions ranging from 10^{-1} to 10^{-4} were prepared according to the standard method (ISO, 1995). Thus, the samples were studied in quantitative and qualitative method.

3.4 Preparation of agar media plates

Large and small agar plates were sterilized in hot air oven. Respective media recipe ingredients for agar medium were chosen and mixed in respective portion. For the agar medium, the solvent used was distilled water. After that, the agar media were mixed properly by applying heat and then were autoclaved at 121^0C for 15 min for sterilization. The hot agar media was then cool down to 50^0C and waited until it solidified and then poured into agar plates inside laminar airflow. All the agar plates were kept at 4^0C for further use.

3.5 Detection of total microbial count

For the determination of total bacterial count, 200µl of each tenfold diluted sample was added to transfer to nutrient agar (NA) plate. It has a pH of approximately 6.8 at 25^0C. For each dilution, two test plates containing NA agar were used. Then inoculation was performed using spread plate technique. All the agar plates were incubated at 37^0C temperature or 24 hours and 48 hours respectively. White, yellowish white, off white, pink etc. coloured colonies were observed on nutrient agar media. The total bacterial count was calculated according to ISO (1995). The results of the total bacterial count were expressed as the number of organism or colony forming units per milliliter (cfu/mL) for all samples.

3.6 Detection of total coliform count

For the determination of total coliform count, 200µl of each tenfold diluted sample was transferred to MacConkey agar plate. It has a pH of approximately 7.1. For each dilution, two test plates containing MacConkey agar were used. Inoculation was done using spread plate technique. All the agar plates were incubated at 37^0C temperature for 24 hours and 48 hours

respectively. Pink or red and colourless colonies were observed on MacConkey agar media. Then the total coliform count was calculated according to ISO (1995). The results of the total coliform count were expressed as the number of organism or colony forming units per milliliter (cfu/mL) of all samples.

3.7 Detection of total Staphylococcal count

For the detection of *Staphylococci*, 200µl of each tenfold diluted sample was transferred to Mannitol salt agar (MSA) agar plate. For each dilution, two test plates containing MSA were used. Inoculation was performed using spread plate technique. All the agar plates were incubated at 37^0C temperature for 24 hours and 48 hours respectively. The colour of this media is mainly red. The following changes are shown if any of the following bacterial growth is found:

Gram +ve *Staphylococcus*: fermenting mannitol: Media turns yellow (ex. *S. aureus*)

Gram +ve *Staphylococci*: not fermenting mannitol. Media does not change colour (ex. *S.epidermidis*)

Gram +ve *Streptococci*: inhibited growth

Gram -ve: inhibited growth

Generally white and yellowish colonies were observed on MSA media. The total Gram-Positive bacteria count was calculated according to ISO (1995). The results of the total Gram-Positive bacteria count were expressed as the number of organism or colony forming units per milliliter (cfu/mL) of the samples.

3.8 Detection of *Salmonella* and *Shigella* Species

For the detection of *Salmonella* and *Shigella spp,* 200µl of each tenfold diluted sample was transferred to xylose lysine deoxycholate (XLD) agar. It has a pH of approximately 7.4, leaving it with a bright pink or red appearance due to the indicator phenol red. Inoculation was performed using spread plate technique. The agar plate was incubated at 37^0C temperature for 24 hours and 48 hours respectively. Sugar fermentation lowers the pH and the phenol red indicator registers this by changing to yellow. Most gut bacteria, including *Salmonella*, can ferment the sugar xylose to produce acid; *Shigella* colonies cannot do this and therefore remain red. After exhausting the xylose supply, *Salmonella* colonies will decarboxylate lysine, increasing the pH once again to alkaline and mimicking the red *Shigella* colonies. *Salmonella* metabolize thiosulfate to produce hydrogen sulfide, which leads to the formation of red colonies with black centers and allows them to be differentiated from the similarly coloured *Shigella* colonies. Other Enterobacteria such as *E. coli* ferment the lactose and sucrose present in the medium to an extent that prevent pH reversion by decarboxylation and acidify the medium turning it yellow.

***Salmonella* species:** red colonies, some with black centers. The agar itself will turn red due to the presence of *Salmonella* type colonies.

***Shigella* species:** red colonies.

Coliforms: yellow to orange colonies.

Pseudomonas aeruginosa: pink, flat, rough colonies. This type of colony can be easily mistaken for *Salmonella* due to the colour similarities. The result of the total *Salmonella* and *Shigella* sp count were expressed in colony formation, colony colour and colour changing capability of the media.

3.9 Isolates from spread plates

For isolating from each spread plates, exceptional colonies were taken from the media plates and were streaked on the Nutrient agar plate using four way streaking technique. All the sample plates were incubated in 37^0C for 24 hours and then preserved in 4^0C.

3.10 Long-term preservation

For long-term preservation, bacterial culture was grown in Tryptophan salt agar (T_1N_1). First, T_1N_1 was taken in a sterile cryovial and was being autoclaved at 121^0C for 15 min for sterilization. Then the cryovial was left to cool. After inoculation, it was stored at room temperature.

3.11 Microscopic observation of isolates

For evaluation of microscopic character, pure colony of each isolate was picked and Gram staining was performed according to Hacker's modified method (Doetsch, 1981). The size, shape and gram reaction properties of isolates were carefully observed in a microscopic field.

3.12 Morphological characteristics of isolates

Colony morphology of various isolates were examined from the plates (according to 'Microbiological Laboratory Manual' by Cappuccinos and Sherman, 1999) and recorded on the basis of size, form, pigmentation, margin, elevation and opacity.

3.13 Biochemical identification

Different biochemical tests were performed according to the methods described in Microbiology

Laboratory Manual (Cappuccino *et al*., 2005). The biochemical tests carried out were:

1) Nitrate reduction test

2) Triple sugar iron test

3) Catalase test

4) Oxidase test

5) Carbohydrate fermentation (Dextrose, Sucrose, Lactose) test

6) Citrate utilization test

7) Motility test

8) Urease activity test

9) Methyl-red test

10) Voges- Proskauer test

11) Starch hydrolysis test

12) Gelatin hydrolysis test

Nitrate reduction test

The nitrate reduction test is a test to differentiate between bacteria based on their ability or inability to reduce nitrate (NO_3^-) to nitrite (NO_2^-) using anaerobic respiration.

1. Nitrate broth was inoculated with an isolate from each sample plates and incubates for 48hours.

2. Then reagent A and reagent B were mixed carefully. If the bacterium produces nitrate reductase, the broth will turn a deep red within 5 minutes at this step.

3. If no colour change is observed, then the result is inconclusive. If addition of small amount of zinc does not change the broth colour, then it means the organism contained nitrate reductase.

Triple Sugar Iron (TSI) test

This test was performed to assess the mode of sugar utilization. This test is done by stabbing the butt of the media and streaking the bacteria over the slant of Triple Sugar Iron (TSI) agar media.

1) To inoculate, isolated colony from the respective agar plate was picked with a cool, sterile needle, stabbed into the TSI, (Himedia, India) containing dextrose, lactose and sucrose butt.

2) Incubated with caps loosened at 37°C for overnight and examined after 24 hours for carbohydrate fermentation, CO_2 and H_2S production.

3) A yellow (acidic) colour in the butt indicated that the organism being tested capable of fermenting all the three sugars, whereas red (alkaline) color in the slant and butt indicated that the organism being tested is a non-fermenting.

4) Detection of H_2S production identified by black precipitation in the butt of the tube.

5) CO_2 gas production was indicated by splitting and cracking of the medium.

Catalase test

Catalase is an enzyme that splits H_2O_2 into water and O_2. This test is performed to differentiate between groups of microorganism on the basis of catalase production.

1) A small amount of bacterial colony was transferred from the respective agar plate to a surface of clean, dry glass slide using a clean toothpick.

2) A drop of the catalase reagent (Hydrogen Peroxide) was placed on to the slide and mixed.

3) A positive result gave a rapid evolution of oxygen within 5-10 seconds and was proven by bubbling reaction.

4) A negative result showed no bubbles or only a few scattered bubbles.

Oxidase Test

Oxidase test was performed to differentiate between enteric and non-enteric bacteria.

1) A loopfull of bacteria from the nutrient agar plate was streaked onto a piece of filter paper (Whatman, 1MM).

2) A few drops of oxidase reagent (*N,N,N′,N′*-tetramethyl-*p*-phenylenediamine) were added onto the streaked bacteria on the filter paper.

3) Positive reactions turned the bacteria from violet to purple within 1 to 30 seconds. Delayed reactions should be and was ignored.

Carbohydrate fermentation (Dextrose, Sucrose, Lactose) test

When carbohydrates are fermented by bacteria, they produce acidic products. A change in pH can be detected when fermentation of a given carbohydrate has occurred. Acids lower the pH of the medium, which turns the media to yellow colour. When bacteria do not ferment the carbohydrate, the media remains red.

1) The Durham tubes were inserted in an inverted position into all the tubes fully filled with broth (lactose, dextrose and sucrose).

2) Each labeled carbohydrate broth (lactose, dextrose and sucrose) was inoculated aseptically with each of the seven bacterial cultures.

3) After inoculation into a particular sugar, the loop was sterilized in order to avoid cross contamination of the tube with other sugars.

4) The tubes were incubated for 24 hours at 37^0C.

5) Following incubation, the tubes showed one of the following results: acid production, acid and gas production or no fermentation at all.

6) The presence of acid and gas changes the medium into a yellow colour indicating a positive result.

7) Gas production can be detected by the presence of small bubbles in the inverted durham tubes.

8) The broth retaining the red color is an indication of the absence of fermentation.

Citrate utilization test

Simmons citrate agar shows the ability of organisms to utilize citrate as a carbon source. Simmons citrate agar contains sodium citrate as the sole source of carbon, ammonium dihydrogen phosphate as the sole source of nitrogen, other nutrients, and the pH indicator bromthymol blue.

1) Colourless bacterial colonies were picked from the respective agar plate by a straight wire and inoculated into the slope of Simmon's citrate agar (Oxoid ltd, England) and incubated overnight at 37^0C.

2) If the organism had the ability to utilize citrate, the medium would change from green to prussion blue colour; a negative slant would have no growth of bacteria and would remain green.

Motility test (Indole activity test)

Some bacteria have the ability to propel themselves through liquids by means of flagella. These long fibers of protein are found on many bacteria, including most supported by this simulation. In semi-solid agar media, motile bacteria 'swarm' and give a diffuse spreading growth that is easily recognized by the naked eye. Non-motile bacteria generally give growths that are confined to the stab-line, have sharply defined margins and leave the surrounding medium clearly transparent. Motile bacteria typically give diffuse; hazy growths that spread throughout the

medium rendering it slightly opaque. Motility test is also used for the species differentiation of gram positive cocci.

1) The test was carried out in motility indole urea semisolid media.

2) One suspected isolated colony was touched with a straight wire and was stabbed carefully into down the tubes without touching the bottom.

3) Following incubation, the tubes were observed for the presence of motile organisms which will disperse through the medium leaving the stab line spread and make the tube turbid.

4) Production of cherry red reagent layer after addition of Kovac's reagent in MIU medium demonstrates that the substrate tryptophan has been was hydrolyzed which indicates indole positive reaction.

Urease activity test

Urea is the product of decarboxylation of amino acids. Hydrolysis of urea produces ammonia and CO_2. The formation of ammonia alkalinizes the medium, and the pH shift is detected by the colour change from the light orange of phenol red at pH 6.8 to pink at pH 8.1. Rapid urease-positive organisms turn the entire medium pink within 24 hours. Weakly positive organisms may take several days, and negative organisms produce no colour change or yellow as a result of acid production**.**

1) The motile indole urea media was inoculated with the sterile wire containing the organism from the culture.

2) Then the media was kept in incubator for 24-48 hours at 37°C.

3) After the incubation period, the tubes containing the media were observed for colour change.

4) If the media showed pink colour, then that indicates urease positive, otherwise yellow colour of the media indicates the negative result.

Methyl red (MR) test

Methyl Red (MR) test determines whether the microbe performs mixed acids fermentation when supplied with glucose. Types and proportion of fermentation products produced by anaerobic fermentation of glucose is one of the key taxonomic characteristics which help to differentiate various genera of enteric bacteria. The large amounts of produced acid after inoculation show a significant decrease in the pH of the media (below 4.4). This is visualized by using pH indicator, methyl red (p-dimethyl aminoaeobenzene-O-carboxylic acid), which is yellow above pH 5.1 and red at pH 4.4.

1) The bacterium to be tested was inoculated into potassium phosphate broth (MR-VP broth), which contained dextrose, peptone and potassium phosphate and was incubated at 37°C for 24h.

2) Over the 24 hours the mixed-acid producing organism was expected to produce sufficient acid to overcome the phosphate buffer and remained acidic.

3) The pH of the medium was tested by the addition of five drops of MR reagent. Development of red colour was taken as positive. MR negative organism produced yellow colour.

Voges - Proskauer test

In this test, the active product in the medium formed by bacterial metabolism is acetyl methyl carbinol, a product of the butylenes glycol pathway. Pyruvic acid, the pivotal compound in the fermentative degradation of glucose, is further metabolized through various metabolic pathways, depending on the enzyme systems possessed by different bacteria. One such pathways result in

the production of acetion (acetyl methyl carbinol), a neutral-reacting end product. Organisms such as members of the *Klebsiella, Enterobacter, Serratia* group produce acetoin as the chief end product of glucose metabolism and form smaller quantities of mixed acids. In the presence of atmospheric oxygen and 40% potassium hydroxide, acetoin is converted to diacetyl, and alpha-naphthol serves as a catalyst to bring out a red complex.

1) Bacterium to be tested was inoculated into potassium phosphate broth (MR-VP broth) and incubated for 24 hours.

2) Barritt's reagent A was added to the test broth and shaken.

3) Barrit's reagent B was added and the tube was allowed to stand for 15 min.

4) Appearance of red colour was taken as a positive test, negative tube might be held for an hour after addition of reagents.

Starch hydrolysis test

Starch hydrolysis media is a simple media that contains beef extract, soluble starch and agar. Because starch is such a large molecule, it cannot be directly transported into a bacterial cell. A bacterium may secrete the extracellular enzyme alpha-amylase to cleave the glucosidic linkage of starch releasing individual glucose molecules. The presence of starch can be indicated by the development of a blue color; therefore, the absence of a blue color indicates starch hydrolysis.

1) A sterile swab or a sterile loop was used to pick a few colonies from pure culture plate. Streak plate method was used to inoculate the starch plate by covering the width of the plate.

2) Plates were incubated for 24-48 hours at 37°C.

3) 2-3 drops of 10% iodine solution directly added onto the edge of colonies.

4) Appearance of dark media with clear area surrounded by isolated colonies indicates that the starch has been hydrolyzed by amylase.

Gelatin hydrolysis test

Gelatin hydrolysis test is used to detect the ability of an organism to produce gelatinase (proteolytic enzyme) that liquefy gelatin. Hydrolysis of gelatin indicates the presence of gelatinases. This process takes place in two sequential reactions. In the first reaction, gelatinases degrade gelatin to polypeptides. Then, the polypeptides are further converted into amino acids. The bacterial cells can then take up these amino acids and use them in their metabolic processes. Gelatin hydrolysis test is helpful in identifying and differentiating species of *Bacillus, Clostridium, Proteus, Pseudomonas* and *Serratia spp*.

1) The isolates were inoculated by stabbing 4-5 times on the tube containing nutrient gelatin media.

2) Test tubes were incubated for 24-48 hours at 37°C.

3) After inoculation, the test tubes were moved from the incubator and placed in ice bath or refrigerator (4°C) for 15-30 minutes (until control is gelled) to check for gelatin liquefaction as gelatin normally liquefies at 28°C and above. Hence, in order to confirm that liquefaction was due to gelatinase activity, the tubes are immersed in an ice bath or kept in refrigerator at 4°C.

4) The test tubes were tilt to observe if gelatin has been hydrolyzed.

5) Partial or total liquefaction of the inoculated tube (control medium must be completely solidified) even after exposure to cold temperature of ice bath or refrigerator (4°C) indicates positive result.

6) Complete solidification of the inoculated tube even after exposure to cold temperature of ice bath or refrigerator (4°C) indicates negative result.

CHAPTER 4: RESULTS

4. Results

The main purpose of microbiological examination of ice cream and tok is to give an idea about the safety of these widely eaten food items from the public health standpoint. In addition, these food items will be of satisfactory quality, i.e., will consist of good original materials that have not deteriorated or become excessively contaminated during processing, packaging, storage, handling or marketing.

The practice that has been in effect for many years and continues to be followed is - to determine the sanitary quality of foods by their content of certain indicator organisms and pathogens. This research study is therefore undertaken to determine the total viable bacterial count, the presence of coliforms, gram-negative and gram positive bacteria, *Salmonella* and *Shigella spp* and detection of *Staphylococci* from the samples.

For the quantitative analysis and observation of both type of samples, Nutrient Agar (NA), MacConkey Agar (MAC), Mannitol salt agar (MSA) and Xylose lysine deoxycholate (XLD) agar plates were used. Sample were appropriately diluted and spread over agar plate and incubated at 37^0C for 24-48 hours.

Table 4.1:Total viable count (TVC) and enumeration of coliform, *E.coli*, *Staphylococci spp*, *Salmonella spp* and *Shigella spp* from various ice cream samples of Dhaka city

Sample No.	Location	Ice cream brand (flavor/name, type)	TVC (cfu/g)	Coliforms (cfu/g)	*Staphylococci spp* (cfu/g)	*E.coli* (cfu/g)	*Salmonella spp* (cfu/g)	*Shigella spp*(cfu/g)
1	Mohakhali	I1 (Vanilla, cup)	$7.5X10^2$	$3.2X10^1$	$5.2X10^2$	$1.7X10^1$	Nil	Nil
2	Dhanmondi	I1 (Orange, lolly)	$9X10^2$	Nil	$2.1X10^2$	Nil	Nil	Nil
3	Khilgaon	I1(Cornelli, cone)	$1.25X10^3$	Nil	$5.9X10^2$	Nil	Nil	Nil
4	Mohakhali	I2 (Vanilla, cup)	$8.75X10^2$	Nil	$3.5X10^2$	Nil	Nil	Nil
5	Khilgaon	I2 (Orange, lolly)	$1X10^3$	Nil	$6.1X10^2$	Nil	Nil	Nil
6	Dhanmondi	I2 (Chocodelight, cone)	$1.4X10^3$	$4.7X10^1$	$8.3X10^2$	$2.1X10^1$	Nil	Nil
7	Dhanmondi	I3 (Orange, lolly)	$1.15X10^3$	$3.1X10^1$	$5.6X10^2$	$1X10^1$	Nil	Nil
8	Khilgaon	I3 (Cornetto, cone)	$1.6X10^3$	$7.5X10^1$	$8.4X10^2$	$3.2X10^1$	Nil	Nil
9	Mohakhali	I4 (Mango, cup)	$6.3X10^2$	Nil	$1.4X10^2$	Nil	Nil	Nil
10	Mohakhali	I3 (Vanilla, cup)	$8.9X10^2$	Nil	$3.7X10^2$	Nil	Nil	Nil

4.1 Quantitative microbial analysis of ice cream samples

Quantitative microbial analysis of all ice cream samples are shown in the table 4.1. The cornetto cone ice cream of I3 showed the highest microbial count in nutrient agar media ($1.6X10^3$cfu/g). Chocodelight cone ice cream from I2 showed the second highest microbial growth on nutrient agar media ($1.4X10^3$cfu/g), however *Salmonella* and *Shigella* could not be observed in any sample. Table-4.1 shows that, mango and vanilla cup ice cream from I4 and I3 companies showed the lowest ($6.3X10^2$cfu/g) and second lowest microbial count ($8.9X10^2$cfu/g) respectively, though they did not show any growth on the MacConkey and XLD agar plate. Moreover, from the table, it can be seen that, the microbial quantity of the samples those were collected from Mohakhali area was significantly lower than the other two areas. But it does not imply that, the ice cream quality of Mohakhali area is better because same company supply ice cream in different places.

4.2 Quantitative microbial analysis of tok samples

The following 4.2 table of quantitative microbial analysis of tok samples represents that, the tok sample collected from the street food vendor of Shipahibag showed the highest microbial count in nutrient agar media ($1.5X10^4$cfu/ml); also on MAC ($7.05\ X10^2$cfu/ml), MSA ($8.3X10^3$cfu/ml) and XLD ($7X10^1$cfu/ml). Tok sample which was taken from Tilpapara local street food vendor shows the second highest microbial count on all the media ($1X10^4$cfu/ml). From the table-4.2, it can be seen that, tok collected from the roadside vendor of BRAC university contained the lowest microbial growth ($2.35X10^3$cfu/ml). Tok sample from Kolabagan showed the second lowest number of microbial count ($3.4X10^3$cfu/ml). Though presence of various microorganisms has seen on the agar plates, but no growth of *Salmonella spp* or *Shigella spp* was observed.

Table 4.2:Total viable count (TVC) and enumeration of coliforms, *E.coli*, *Staphylococci spp*, *Salmonella spp* and *Shigella spp* from different tok samples of Dhaka city

Sample No.	Location	TVC (cfu/mL)	Coliforms (cfu/mL)	*Staphylococci spp* (cfu/mL)	*E.coli* (cfu/mL)	*Salmonella spp* (cfu/mL)	*Shigella spp*(cfu/mL)
1	Tilpapara	$1X10^4$	$6X10^2$	$6.4\ X10^3$	$5.4X10^1$	Nil	Nil
2	Chowdhurypara	$7.5X10^3$	$3.03X10^2$	$4.07X10^3$	$4.2X10^1$	Nil	Nil
3	Taltola market	$8.5X10^3$	$2.7X10^2$	$5X10^3$	$2.9X10^1$	Nil	Nil
4	Shipahibag	$1.5X10^4$	$7.05\ X10^2$	$8.3X10^3$	$7X10^1$	Nil	Nil
5	Azimpur	$6.65X10^3$	$2.7\ X10^2$	$2.01X10^3$	$3.5X10^1$	Nil	Nil
6	Kolabagan	$3.4X10^3$	$1.9\ X10^2$	$1.4X10^3$	$2.25X10^1$	Nil	Nil
7	Nilkhet	$7.75X10^3$	$4\ X10^2$	$3.7X10^3$	$4.25X10^1$	Nil	Nil
8	BRAC university	$2.35X10^3$	$1.02\ X10^2$	$6.7X10^2$	$1X10^1$	Nil	Nil
9	TB gate	$6.05X10^3$	$2.05\ X10^2$	$3X10^3$	$2.01X10^1$	Nil	Nil
10	Titumir college	$4X10^3$	$3.4\ X10^2$	$4.1X10^3$	$3X10^1$	Nil	Nil

Table-4.3: Colony characteristics of the isolated microorganisms from ice cream and tok samples in different media

Isolate No.	Sample	Agar Media	Colony characteristics of the isolates				
			Size	Form	Color	Margin	Elevation
1	Tok	NA	Small	Circular	Orange	Entire	Convex
2		NA	Medium	Circular	Yellow	Entire	Flat
3		NA	Small	Circular	Cream	Entire	Raised
4		NA	Medium	Irregular	White	Entire	Convex
5		NA	Small	Circular	White	Entire	Raised
6		MSA	Large	Circular	Pink	Entire	Convex
7		MSA	Medium	Circular	Lemon	Entire	Raised
8		XLD	Small	Circular	Light yellow	Entire	Raised
9	Ice cream	MAC	Medium	Circular	Pink	Entire	Raised
10		MAC	Medium	Circular	Dark yellow	Entire	Raised
11		MSA	Large	Circular	Yellow	Entire	Raised
12		XLD	Small	Circular	Yellow	Entire	Raised

Table 4.4: Results of the biochemical tests for identification of selected isolates from ice cream and tok samples

Isolate No.	Gram strain	Nitrate reduction	Gas	Acid	H_2S Production	Catalase	Oxidase reaction	Dextrose	Sucrose	Lactose	Gelatin hydrolysis	Starch hydrolysis	Citrate utilization	Motility	Indole activity	Urease test	Methyl red test	Voges-Proskauer	Suspected organism
1	-ve	-	-	-	-	+	-	-	-	-	-	-	-	-	-	+	+	+	*Proteus mirabilis*
2	+ve	-	-	+	-	-	-	+	+	+	-	-	-	-	-	-	+	-	*Streptococcus sp*
3	-ve	-	-	+	-	+	+	-	-	-	-	-	+	+	-	-	-	-	*Alcalegenes sp*
4	+ve	-	-	-	-	+	+	+	+	-	+	+	+	+	-	-	-	+	*Bacillus sp*
5	-ve	+	+	+	-	+	-	+	+	+	-	-	+	-	-	-	-	+	*Klebshiella sp*
6	+ve	+	-	+	-	+	-	+	+	+	-	-	-	-	-	+	+	+	*Staphylococcus sp*
7	+ve	+	-	-	-	+	+	-	-	-	+	-	+	-	-	+	-	+	*Micrococcus luteus*
8	-ve	+	+	+	+	+	-	+	+	-	+	-	+/-	+	+	+	+	-	*Proteus vulgaris*
9	-ve	+	+	+	-	+	-	+	+	+	-	-	+	+	-	-	-	+	*Enterobacter spp*
10	+ve	+	-	+	-	+	-	+	+	+	+	-	+	-	-	+	+	+	*Staphylococcus aureus*
11	-ve	+	-	+	-	+	-	+	+	+	-	-	-	+	+	-	+	-	*E. coli*

+ = Positive reaction; - = Negative reaction

The isolates from the ice cream and tok samples were identified on the basis of their biochemical test results. The biochemical test results were analyzed using ABIS software to identify the isolates and the following bacteria were identified - *Proteus mirabilis, Streptococcus spp, Alcalegenes spp, Bacillus spp, Klebshiella spp, Staphylococcus spp, Micrococcus luteus, Proteus vulgaris, Enterobacter spp, Staphylococcus aureus* and *E. coli*.

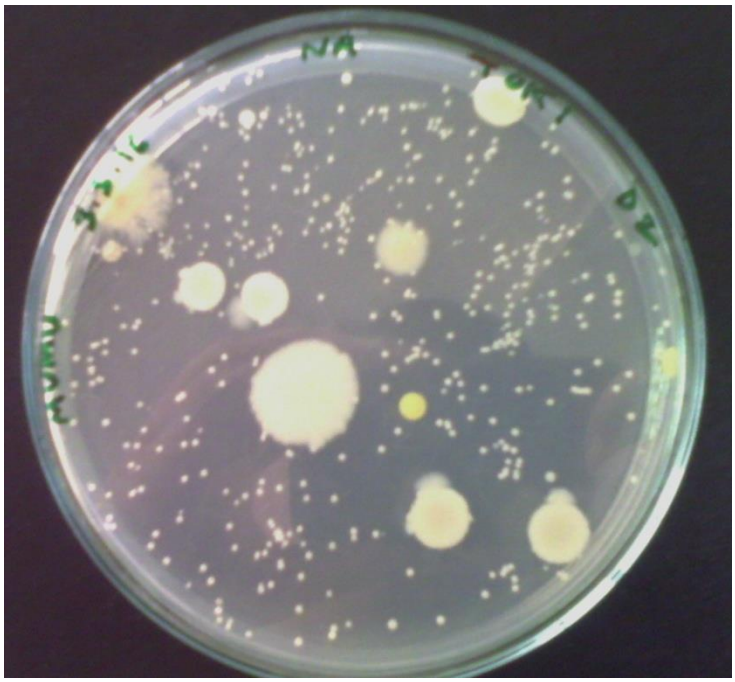
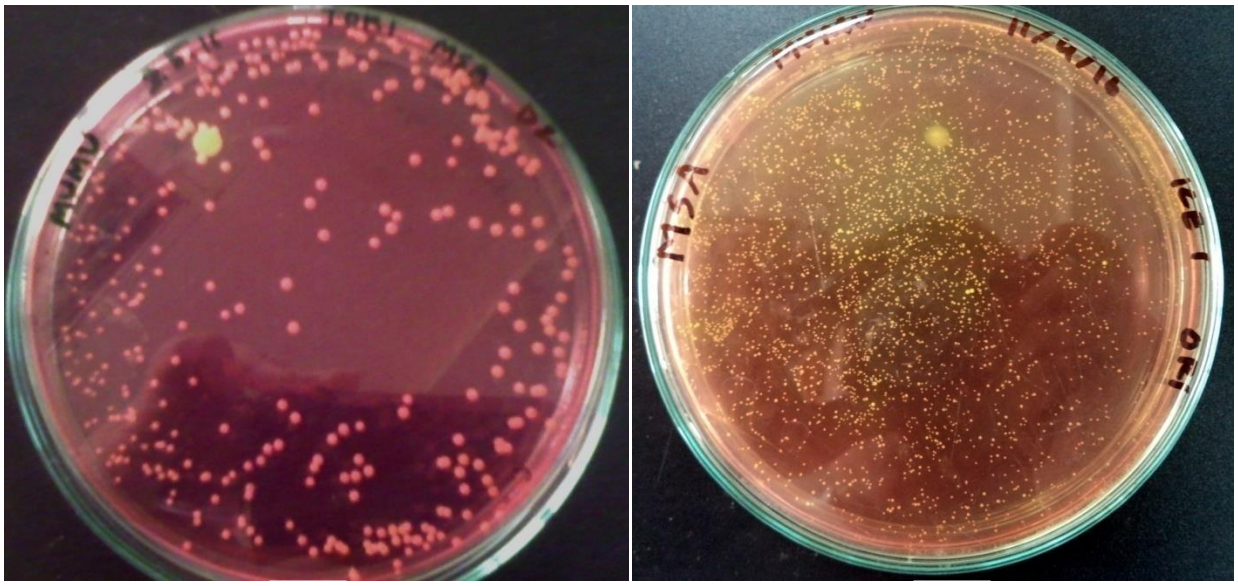


Figure 4.1: Ice cream (I4, cup) sample on Nutrient Agar



A B

Figure 4.2 and 4.3: Ice cream (I1, lolly) sample on MacConkey Agar (A) and Tok (Tilpapara) sample on Mannitol Salt Agar (B) respectively

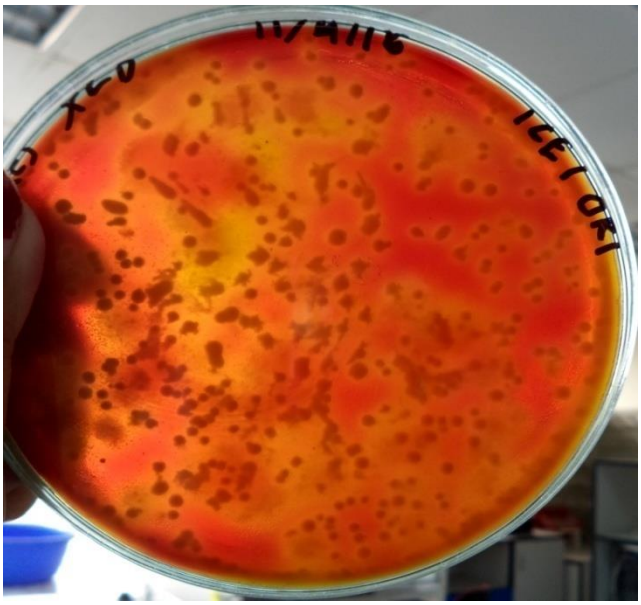


Figure 4.4: Tok (Taltola) sample on Xylose Lysine Deoxycholate Agar

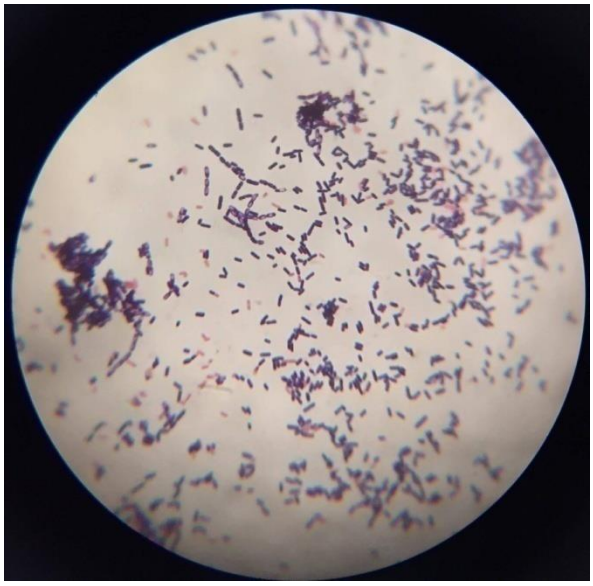


Figure 4.5: Gram Straining (gram +ve)

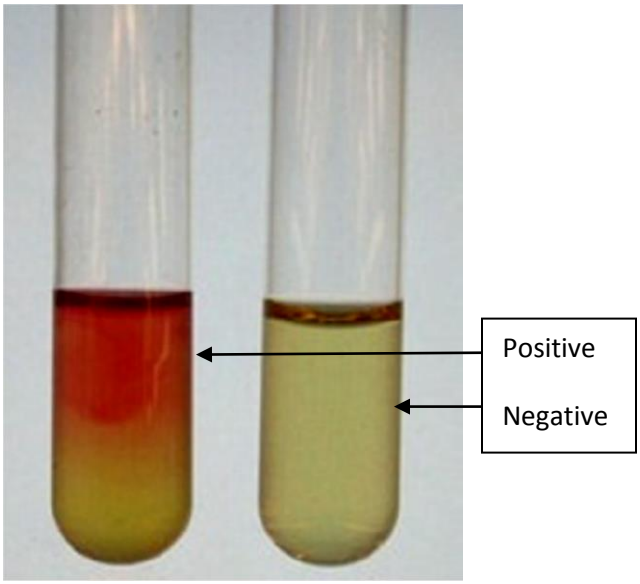


Figure 4.6: Nitrate reduction

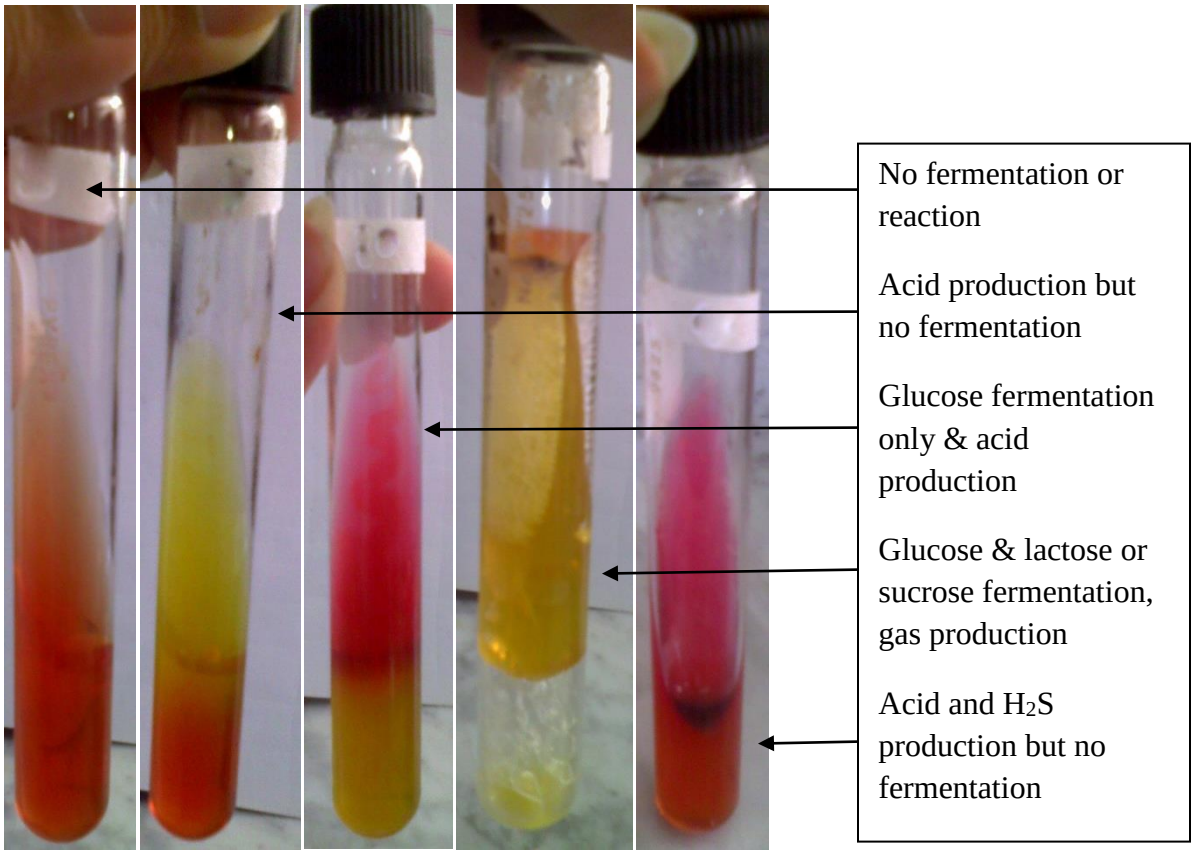


Figure 4.7: Triple sugar iron test

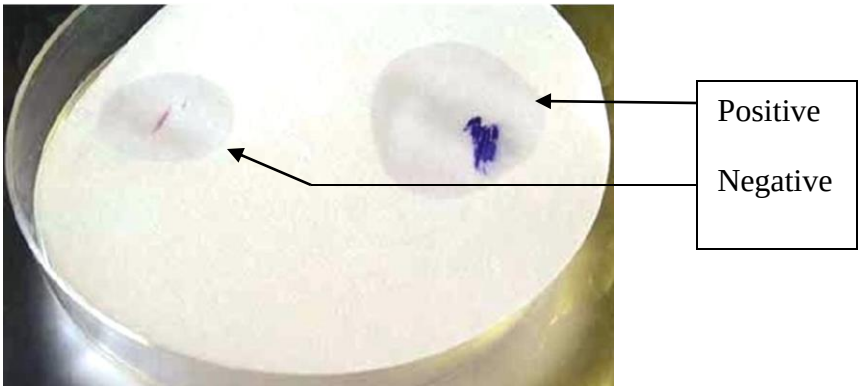


Figure 4.8: Oxidase test

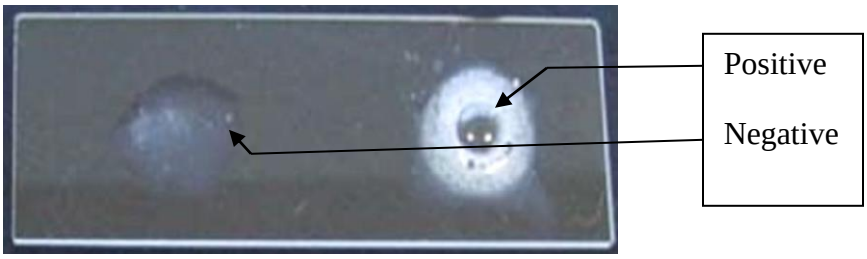


Figure 4.9: Catalase test

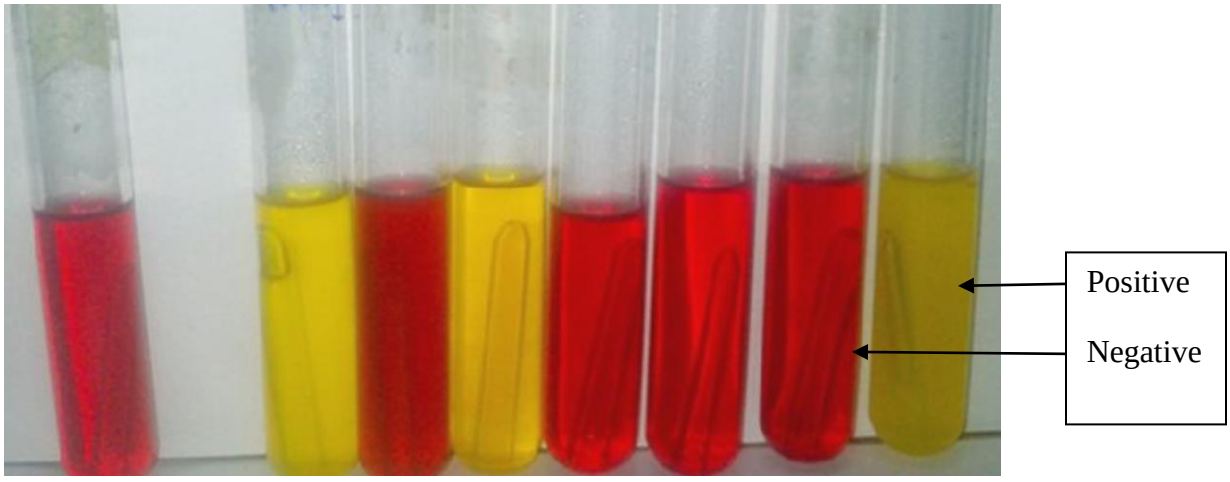


Figure 4.10: Carbohydrate fermentation (Dextrose, Sucrose, Lactose) test

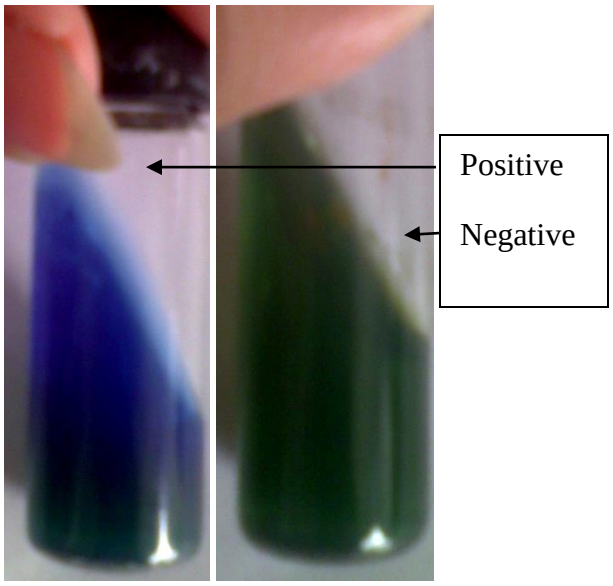


Figure 4.11: Citrate utilization test

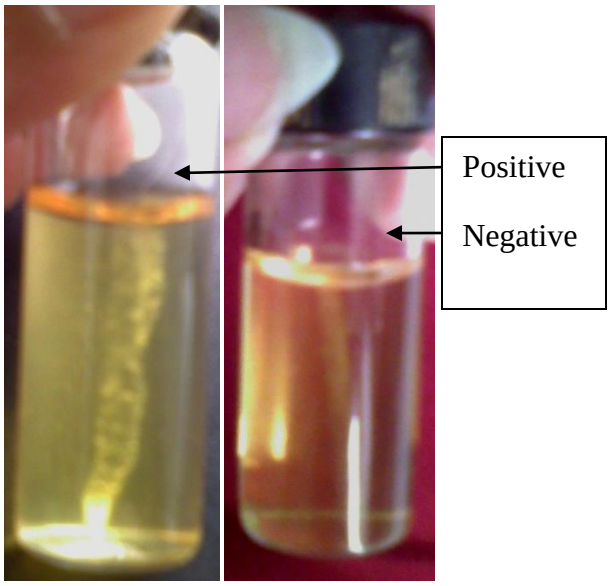


Figure 4.12: Motility test

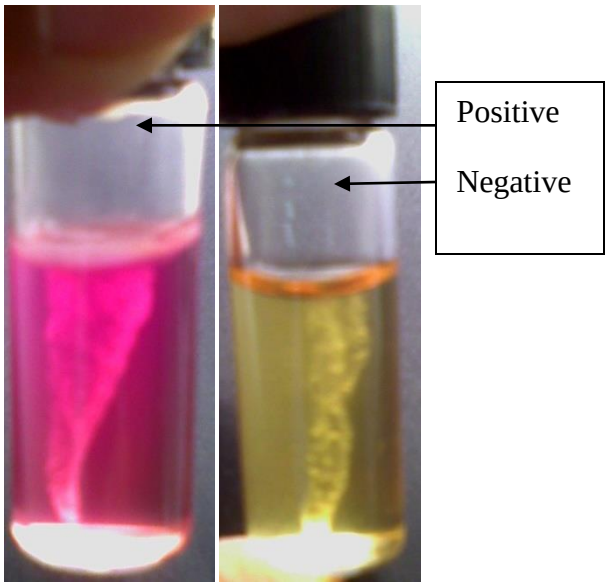


Figure 4.13: Urease test

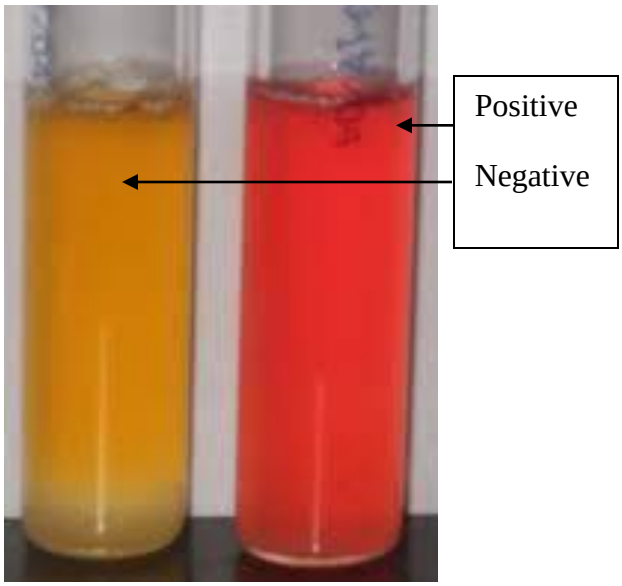


Figure 4.14: Methyl red test

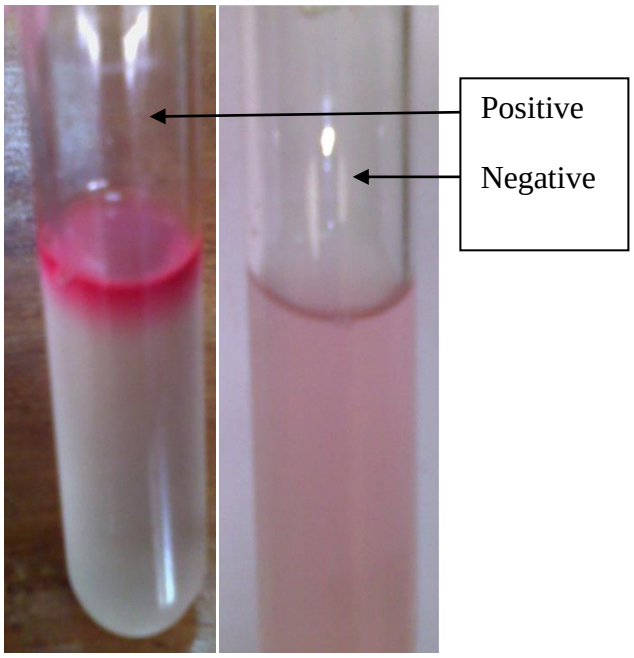


Figure 4.15: Indole test

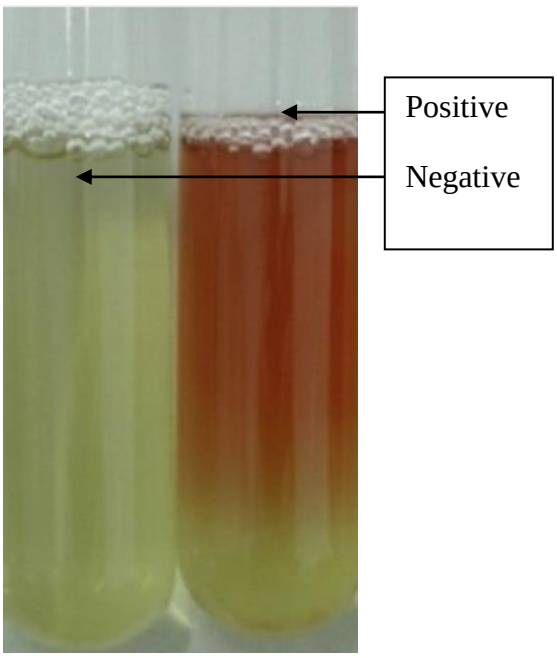


Figure 4.16: Voges - Proskauer test

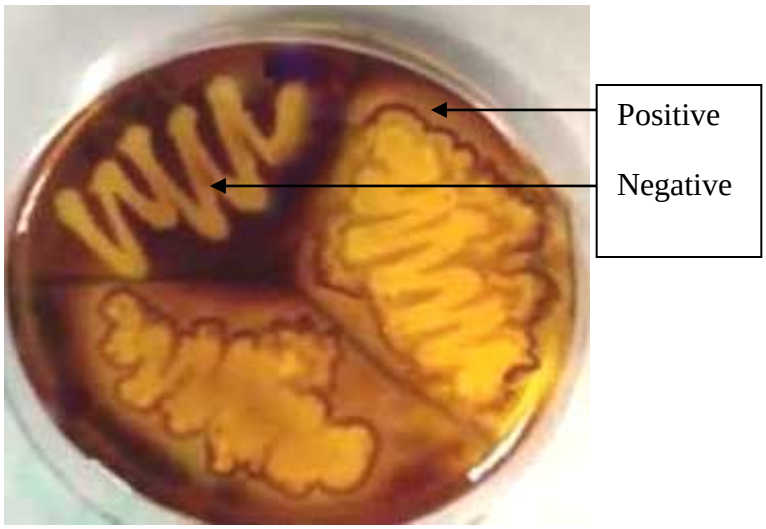


Figure 4.17: Starch hydrolysis test

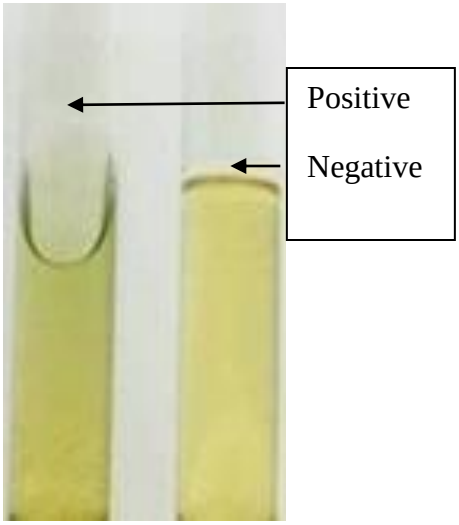


Figure 4.18: Gelatin hydrolysis test

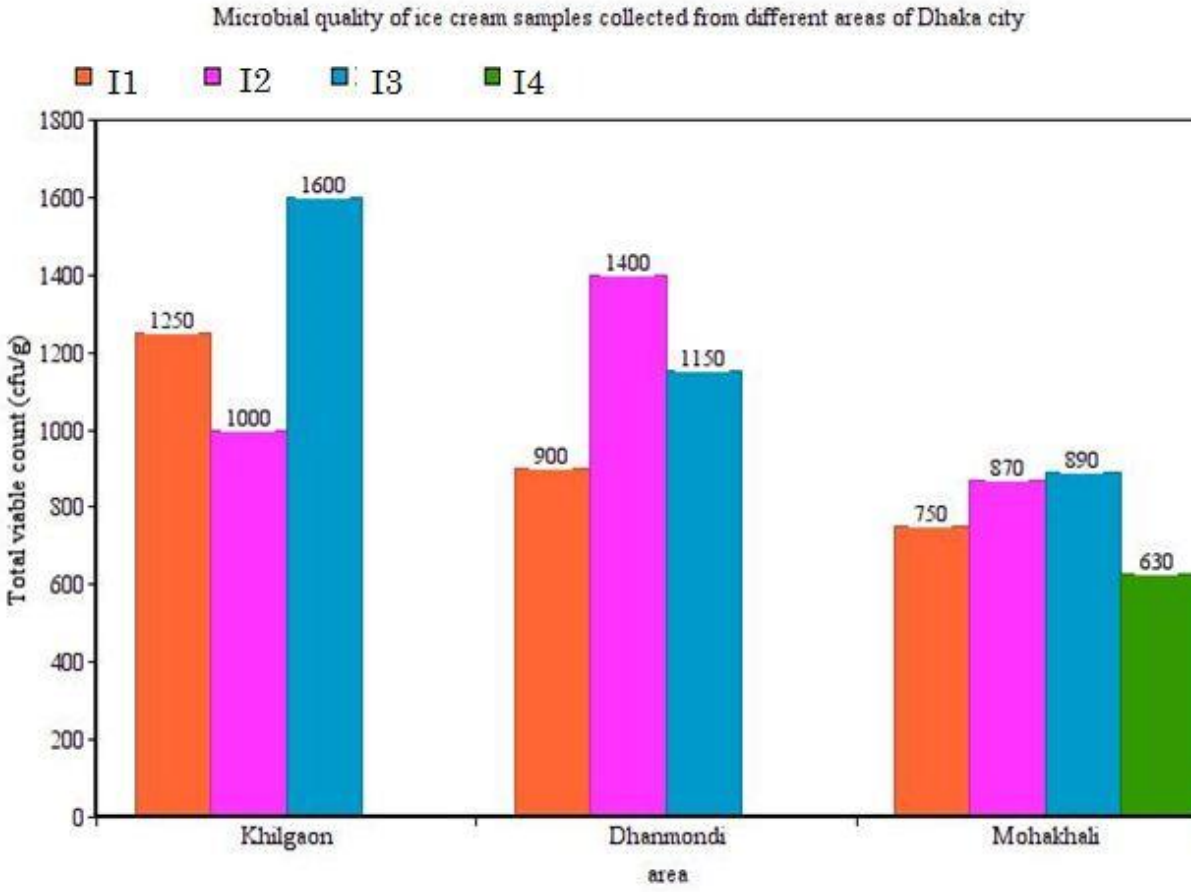


Figure 4.19: Microbial quality (total viable count) of the ice cream samples collected from different areas of Dhaka city. Total viable count of microorganisms from the samples taken from Khilgaon area was higher than the samples that were taken from Dhanmondi and Mohakhali areas. The samples taken from Dhanmondi area showed medium level microorganisms and the ice creams collected from Mohakhali showed the lowest number of microorganisms.

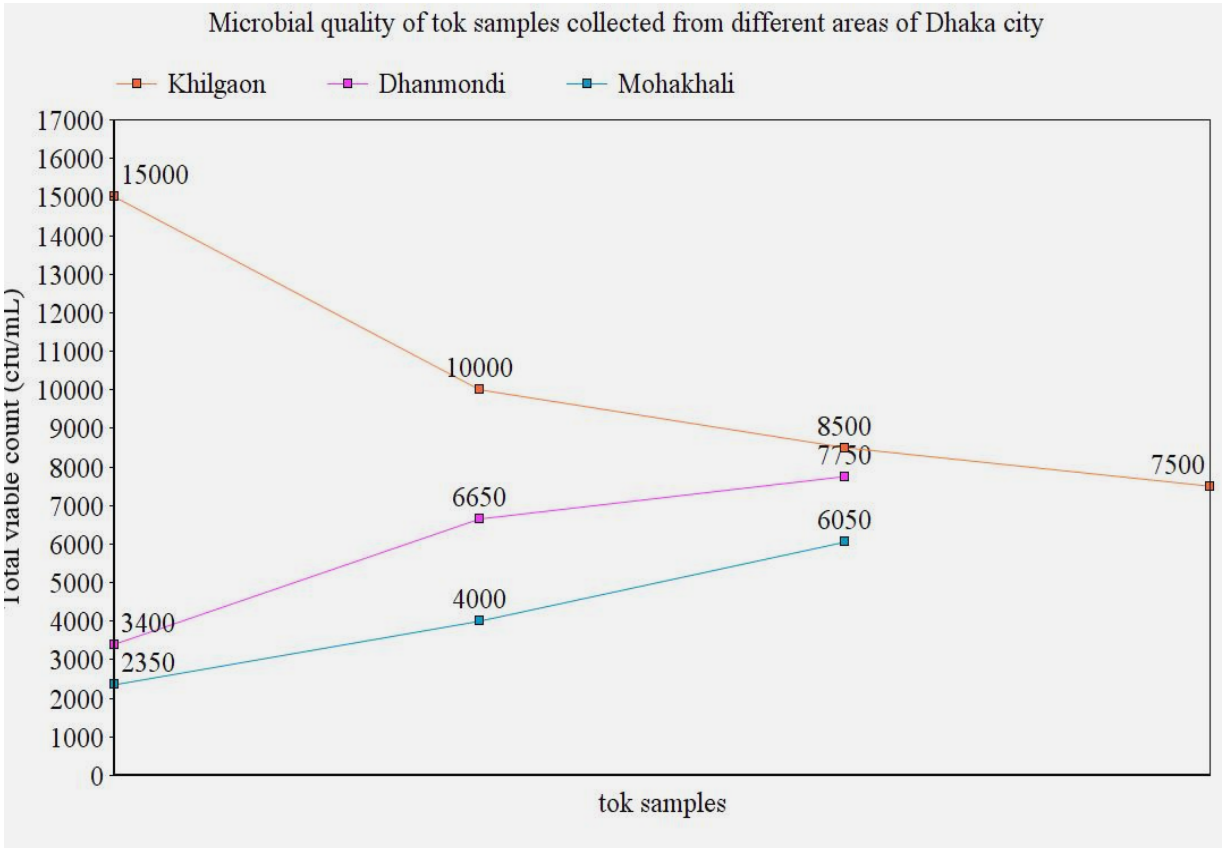


Figure 4.20: Microbial quality (total viable count) of the tok samples collected from different areas of Dhaka city. The total microbial quality of the samples taken from Khilgaon area was higher than the other two areas. The samples taken from Dhanmondi and its adjacent locations showed average number of microorganisms; like the ice cream samples. The tok samples collected from different locations of Mohakhali showed lowest number of microorganisms compared to the other two areas.

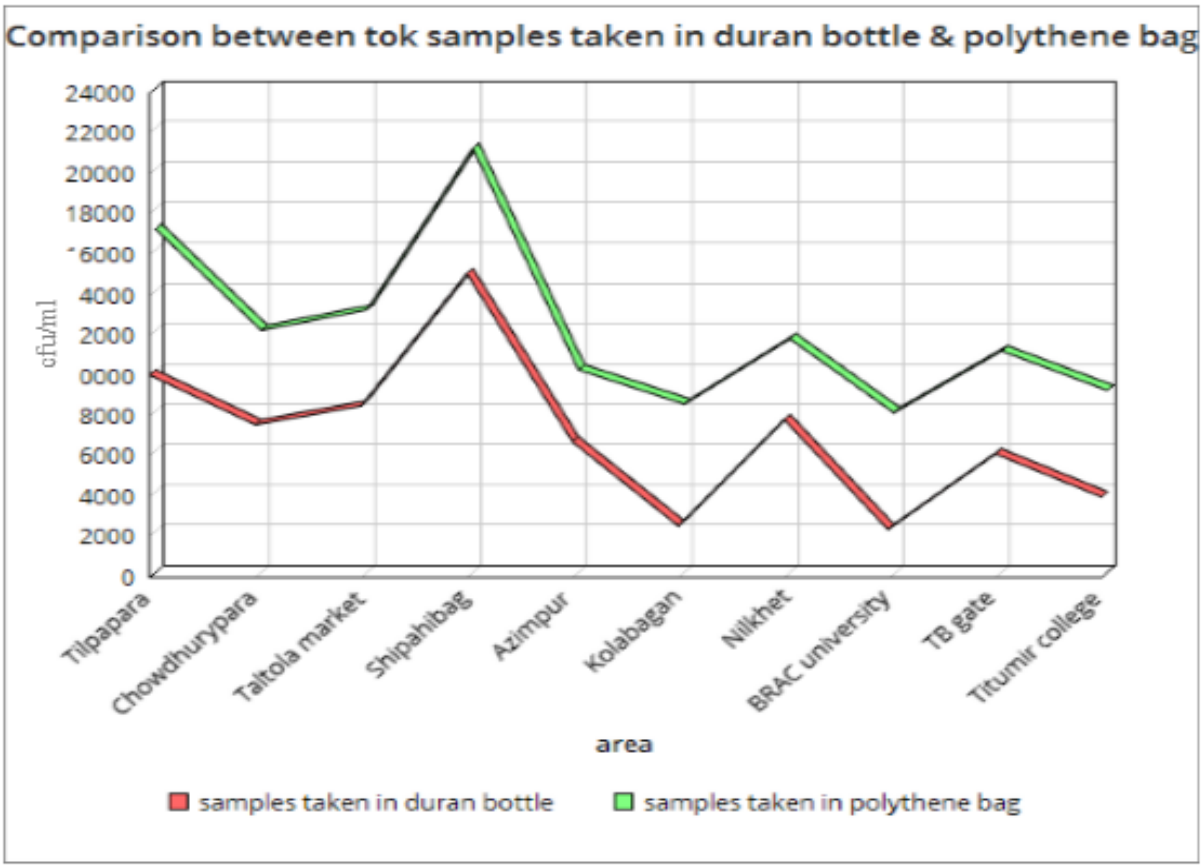


Figure 4.21: Comparison of microbial load between the tok samples collected in duran bottle and polythene bag. The level of microorganisms in the samples collected in the polythene bag was higher than the samples collected in duran bottle.

CHAPTER 5: DISCUSSION

5. Discussion

Food is one of the basic needs in our life. It plays very important role in our health and life. The microbial quality of ice cream and tok samples were studied in this research project. Low quality food can cause various types of diseases like: food poisoning, diarrhea, cholera, typhoid etc in the human body. If proper hygiene is not maintained during the preparation of food, then that food may easily cause any of the foodborne diseases. Not only that, addition of excess preservatives can also decrease the real quality of food items.

In this study, ten ice cream and ten tok samples were tested for detecting the microbial quality of those packaged and street food items respectively. The microbial quality of the cornetto cone ice cream of I3 showed the highest count in all the agar media. In the table-4.1, the number is $1.6X10^3$cfu/g. Chocodelight cone ice cream from I2 shows the second highest microbial growth on all the media. Mango and vanilla cup ice cream from I4 and I3 companies showed the least and second least microbial growth respectively.

On the other hand, in case of quantitative microbial analysis of tok, the sample collected from the street food vendor of Shipahibag showed the highest microbial count in all the agar media; the number was $1.5X10^4$cfu/ml. Tok sample which was taken from Tilpapara local street food vendor showed the second highest microbial growth on all the media. Table-4.2also shows that, the tok collected from the roadside vendor of BRAC University contained the least microbial growth ($2.35X10^3$cfu/ml). Tok sample from Kolabagan shows second least number of microbial growths ($3.4X10^3$cfu/ml). Presence of various microorganisms was observed on the agar plates, but no growth of *Salmonella spp* and *Shigella spp* was observed.

From the results of standard biochemical test for identification of all the eleven isolates from both ice cream and tok samples, it was observed that the isolates are: *Proteus mirabilis, Streptococcus spp, Alcalegenes spp, Bacillus spp, Klebshiella spp, Staphylococcus spp, Micrococcus luteus, Proteus vulgaris, Enterobacter spp, Staphylococcus aureus* and *E. coli* respectively. These microorganisms may easily cause different types of foodborne diseases in the human body e.g. diarrhoea, cholera, food poisoning etc.

Proteus mirabilis might have come from the water used to prepare ice cream or tok samples. The *Alcaligenes spp* might have come from the skin of the street vendors or from the dairy products used in the tok and ice cream samples respectively. It could have also come from water used for food preparation similarly like the *Bacillus spp.*

Staphylococcus spp and *E. coli* were also found in the food samples. These strains are the resident and transient bacteria, respectively on hands and are associated with poor hygiene practice (Department of Health, 2000). Toxin producing strain of *Staphylococcus* is the leading cause of gastroenteretis following handling of food by the person who carry the microorganism in their nose and skin. These bacteria are present in about 60% of a given human population and can also survive on hand knives, chopping board and dish clothes. These suggest that the food contamination in the Dhaka city is mainly due to poor water quality and hygiene, and because the vendors are very crowded and poorly maintained.

A study was conducted by taking one hundred twenty ice-cream samples (cups) those were collected randomly from four different companies available at Sylhet region in Bangladesh. From that study, the researchers found that coliform counts of the ice cream samples were higher than the standard limits, which reflects the lack of standard hygiene and sanitation measure during manufacture ice cream (Sudeb et al., 2012). The serving utensils used at the

vending site are often contaminated with *Staphylococcus spp* and *Micrococcus spp*. which might have originated from the vendors hands when they touch the food preparation area, dish, cloth or water during dish washing or hand washing indicates cross contamination between dishwasher, food preparation surface and food itself. In this study, *Staphylococcus spp* was observed mostly in all the samples. Not only that, the presence of *E.coli* also indicated the poor practice of sanitary conditions during handling and transportation of food items. The organisms gaining access to the food items were not only the cause of deterioration and spoilage but also responsible for giving warning signal of indication of the presence of many food borne disease outbreaks. Absence of *Shigella spp and Salmonella spp*. in the food items must secure better quality of the ice cream and tok samples, as these organisms are responsible for causing hazard.

Figure 4.19 shows that, the overall microbial quality of the ice cream samples taken from Khilgaon area was higher rather than the samples that were taken from Dhanmondi and Mohakhali areas. Samples from Dhanmondi and Mohakhali area contained medium level and least level of microorganisms respectively.

Similarly, Figure 4.20 shows that, the total microbial quality of the tok samples taken from Khilgaon area was higher than the other two areas. The tok samples of Dhanmondi area also had mid-level microorganisms and the tok samples of Mohakhali area contained the lowest amount of microorganisms. These figures suggest that, the environment of an area or a shop may be responsible for the microbial contamination of food items besides the other factors of contamination. The difference between the microbial qualities of the samples from each area might have occurred due to the usage of preservatives in the food items. The hygiene practice of the street vendors or the shop keepers might have also influence this difference.

Moreover, from the graph of the comparison between the tok samples collected in duran bottle and polythene bag (figure 4.21), it was observed that, the level of microorganisms in the samples collected in the polythene bag was higher than the samples collected in duran bottle from each location. This proved the necessity of the hygiene practice to secure us from the various foodborne diseases.

CHAPTER 6: CONCLUSION

6. Conclusion

The microbial contamination in the food items may come from various sources including our environment. In the present study, we observed the presence of *Proteus mirabilis, Streptococcus spp, Alcalegenes spp, Bacillus spp, Klebshiella spp, Staphylococcus spp, Micrococcus luteus, Proteus vulgaris, Enterobacter spp, Staphylococcus aureus* and *E. coli*, which indicates that the sample food items (ice cream and tok) were not prepared in hygienic environment. Presence of *Staphylococcus spp* and *E. coli* from same samples also suggests that personal hygiene was not maintained during the food preparation. Total viable counts were not so high (ranged from $6.3X10^2$cfu to $1.5X10^4$cfu). A good sign is that neither *Salmonella spp* nor *Shigella spp* was present in any sample. Though *Salmonella spp* or *Shigella spp* was not found in this research work, it does not mean that these food items are safe. Lack of awareness in proper hygiene practice during food preparation can provide the non-pathogenic microorganisms the opportunity to turn into the pathogenic one. Therefore, by increasing awareness between the workers, customers, consumers and street vendors about food contamination can further improve the current situation.

CHAPTER 7: BIBLIOGRAPHY

7. Bibliography

Addo, K. K., et al. (2007). Food and its preparation conditions in hotels in Accra, Ghana: A concern for food safety. African Journal of Food, Agriculture, Nutrition and Development; pp. 5.

Argaw, S. and Addis, M. (2015). A Review on Staphylococcal Food Poisoning. pp. 1-3.

Armstrong, P., Pupillo, M. (2012). Vanilla Top Ice Cream Flavor with Americans; Frozen Yogurt Regaining Fans. Retrieved 5 August, 2016, from http://www.idfa.org/news-views/news-releases/article/2012/06/21/vanilla-top-ice-cream-flavor-with-americans-frozen-yogurt regaining-fans-

Fellows, P and Hilmi, M. (2012). Selling street and snack foods. Rome. FAO. pp. 15.

Fun Facts in Honor of July.'National Ice Cream Month'. (2011). Retrieved fromhttps://historyfan2.wordpress.com/2011/07/05/fun-facts-in-honor-of-july-%e2%80%98national-ice-cream-month%e2%80%99/

Hanes, P. 1985. If you're curious, over-55 set eats the most ice cream. Retrieved 2016, from http://www.csmonitor.com/1985/1224/hfice.html.
International Dairy Food Association. Ice Cream Sales & Trends. (n.d.). Retrieved 2016, from http://www.idfa.org/news-views/media-kits/ice-cream/ice-cream-sales-trends.

Kadariya, J., Smith, T. C. and Thapaliya, D. (2014). "*Staphylococcus aureus* and Staphylococcal Food-Borne Disease: An Ongoing Challenge in Public Health". BioMed Research International, Article ID 827965, 9 pages, 2014. doi:10.1155/2014/827965.

Laurie, M. (2013). The Street food Revolution: The Social and Economic arguments in support of the UK's fledgling Street food industry. Nationwide Caterers Association. pp. 4-5.

Malik, K. (2015). Shocking News: How Unhygienic is the Street Food We Love To Eat?. Smart Cooky. Retrieved from http://food.ndtv.com/food-drinks/how-unhygienic-is-the-street-food-we love-767051.

Nicolas, B, et al. (2007). Street-Vended Foods Improvement: Contamination Mechanisms and Application of Food Safety Objective Strategy: Critical Review. Pakistan Journal of Nutrition 6 (1): 1-10/ Asian Network for Scientific Information. pp. 1-2.

Physician, A. M. (2000). Foodborne Disease and Shiga Toxin-Producing *E.coli*. American Academy of Family Physicians. 61(7):2194.

Rahman, A., Shafinaz, S, et al. (2005). Bangladesh Health and Injury Survey Report on Children. Directorate General of Health Services (DGHS)/ Institute of Child and Mother Health (ICMH)/ United Nations Children's Fund (UNICEF).

Staffcorrespondent. Hotel News ME. "Driving a Taste Revolution". (2015). Retrieved 5 August, 2016, from http://www.hotelnewsme.com/catering-news-me/driving-a-taste-revolution/.

Sudeb, S., et al. (2012). Quality of Commercially Produced Ice Cream Sold in Sylhet City, Bangladesh. pp. 1-4.

WHO, (2010). Food safety. Provisional agenda item 11.8. World Health Organization (WHO), Geneva.

WHO, (2015). Food safety. Fact sheet N-399. World Health Organization (WHO), Geneva.

WHO, (2015). WHO's first ever global estimates of foodborne diseases find children under 5 account for almost one third of deaths. World Health Organization (WHO), Geneva.

Winarno, F.G and Allain, A. (2012). Street foods in developing countries: lessons from Asia. Rome. 1991. FAO. Web.

Yang, C. S. (2008). Sampling and Testing for Sewage and fecal contamination. pp. 1-2.

CHAPTER 8: APPENDICES

Appendices

APPENDIX-I

Media composition

The composition of the media used in this study has been given below. Unless otherwise mentioned, all the media were autoclaved at 121^0C for 15 min.

1) Nutrient agar (Himedia, India)

Ingredients	Amount (g/L)
Peptic digest of animal tissue	5.0
Beef extract	1.50
Sodium chloride	5.0
Yeast extract	1.50
Agar	15.0

2) MacConkey agar (Oxoid, England)

Ingredients	Amount (g/L)
Peptone	20.0
Lactose	10.0
Bile salts	5.0
Sodium chloride	5.0
Neutral red	0.075
Agar	12.0

3) Mannitol Salt agar (Oxoid, England)

Ingredients	Amount (g/L)
Peptone	10.0
Manitol	10.0
Lab-lemco powder	1.0
Sodium chloride	75.0
Phenol red	0.025
Agar	15.0

4) Xylose Lysine Deoxycholate agar (Himedia, India)

Ingredients	Amount (g/L)
L- lysine	5.0
Lactose	7.50
Sucrose	7.50
Xylose	3.50
Sodium chloride	5.0
Sodium deoxycholate	2.50
Yeast extract	3.0

5) Nutrient Broth (Oxoid, England)

Ingredients	Amount (g/L)
Lab-lemco powder	1.0
Yeast extract	2.0
Peptone	5.0
Sodium chloride	5.0

6) Simmon's citrate agar (Oxoid, England)

Ingredients	Amount (g/L)
Magnesium sulfate	0.2
Ammonium dihydrogen phosphate	0.2
Ammonium phosphate	0.8
Sodium citrate	2.0
Sodium chloride	5.0
Agar	15.0
Bactobromthymol blue	0.08

7) Peptone Water

Ingredients	Amount (g/L)
Peptone	10.0
Sodium chloride	5.0

8) MR-VP broth

Ingredients	Amount (g/L)
Peptone	7 g
Dextrose	5 g
Potassium phosphate	5 g

9) Triple sugar iron agar (Himedia, India)

Ingredients	Amount (g/L)
Peptic digest of animal tissue	10.0
Sodium chloride	5.0
Lactose	10.0
Sucrose	10.0
Dextrose	1.0
Ferrous sulfate	0.20
Sodium thiosulfate	0.30
Casein enzymatic hydrolysate	10.0
Yeast extract	3.0
Beef extract	3.0

10) Phenol red (Lactose, Dextrose, Sucrose) Broth

Ingredients	Amount (g/L)
Trypticase	0.4
Lactose	0.2
Sucrose	0.2
Dextrose	0.2
Sodium chloride	0.2
Phenol red	0.00072

APPENDIX-II

Buffers and reagents

1. Phosphate buffered saline (PBS)

PBS was prepared by dissolving 8.0 g of NaCl, 0.2 g of KCl, 1.44 g of Na_2HPO4 and 2.0 g of KH_2PO4 in 800 ml of distilled water. The pH was adjusted to 7.4 with HCl. The final volume was adjusted to 1 liter by distilled water. The solution was sterilized by autoclaving and was stored at room temperature.

2. Kovac's reagent

5 g of para-dimethyl aminobenzaldehyde was dissolved in 75 ml of amyl alcohol. Then concentrated HCl was added to make the final volume 25 ml. This reagent was covered with aluminum foil and stored at 4 ^{0}C.

3. Methyl red reagent

0.1 g of methyl red was dissolved in 300 ml of 95% ethyl alcohol. Then distilled water was added to make the final volume 500 ml. This reagent was covered with aluminum foil and stored at 4 ^{0}C.

4. Barritt's reagent

Solution A

5 g of alpha-naphthol was dissolved in 95% ethanol. This solution was covered with aluminum foil and stored at 4 ^{0}C.

Solution B

40 g of KOH was dissolved in distilled water. The solution became warm. After cooling to room temperature, creatine was dissolved by stirring. Distilled water was added. This solution was covered with aluminum foil and stored at 4 ^{0}C.

5. Oxidase reagent

100 mg of N,N,N1,N1-tetramethyl-p-phenyldiamine-dihydrochloride was dissolved in 10 ml of distilled water and covered with aluminum foil. Then the solution was stored at 4 ^{0}C.

APPENDIX-III

Instruments

The important equipment used through the study are listed below:

Autoclave ---------- SAARC
Freeze (-20°C) ---------- Siemens
Incubator ---------- SAARC
Micropipette (10-100µl) ---------- Eppendorf, Germany
Micropipette (20-200µl) ---------- Eppendorf, Germany
Oven, Model:MH6548SR ---------- LG, China
pH meter, Model: E-201-C ---------- Shanghai Ruosuaa Technology company, China
Refrigerator (4oC), Model: 0636 ---------- Samsung
Safety cabinet Class II Microbiological------- SAARC
Shaking Incubator, Model: WIS-20R --------- Daihan Scientific, Korea
Vortex Mixture ---------- VWR International
Water bath ---------- Korea
Weighing balance ---------- ADAM EQUI