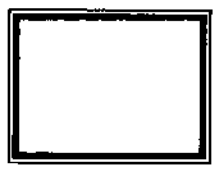
"A Study On The Quality Aspects Of Different Street-Foods To Determine The Fitness For Human Consumption"



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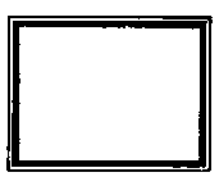

"INSTITUTE OF NUTRITION AND FOOD SCIENCE" (INFS)
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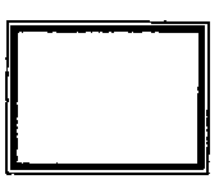
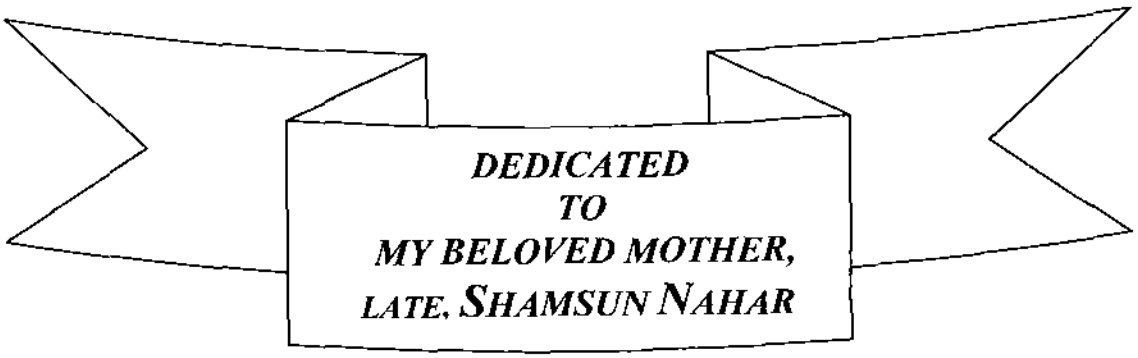


This Dissertation Is Submitted To the University Of Dhaka In Partial Fulfillment Of The Requirements For The Degree of Master of Philosophy (M. Phil) In 'Nutrition'



Approval Sheet

This is to certify that I have read the dissertation entitled **"A STUDY ON THE QUALITY ASPECTS OF DIFFERENT STREET-FOODS TO DETERMINE THE FITNESS FOR HUMAN CONSUMPTION"** submitted by **Kamrun Nahar Medora** in partial fulfillment of the requirements for the Degree of Master of Philosophy [M. Phil] in **'Nutrition'** in the Institute of Nutrition and Food Science [INFS], University of Dhaka, Dhaka - Bangladesh. And this is an original research carried out by her under my supervision and guidance.

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Science

Every great advance in science has issued from a new audacity of imagination.
---------- John Dewey.

True

The scientist is a lover of truth for the very love of truth itself, wherever it may load.
---------- Luther Burbank.

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Glossary

BNNS	= Bangladesh National Nutrition Survey
C/A	= Commercial Area
C	= Centigrade
CC	= Coli form Count
Cfu	= Colony Forming Unit
CHO	= Carbohydrate
et al	= Et allies
etc	= Et Citra
FAO	= Food Agriculture Organization
Fig	= Figure
gm	= Gram
HCL	= Hydro-Chloric Acid
H_2O	= Water
H_2S	= Hydrogen Sulphet
ICMSF	= International Commission on Microbiological Specification for Foods
ICDDR, B	=International Center for Diarrheal Diseases Research, Bangladesh
INFS	= Institute of Nutrition and Food Science
lb	= Pound
ml	= Milliliter
N	= Normal
NaCl	= Sodium Chloride
PAHO	= Pan American Health Organization
R/A	= Residential Area
SC	= Staphylococcus Count
SPC	= Standard Plate Count
TCC	= Total Coli form Count
TFC	= Total Fungal Count
TVBC	= Total Viable Bacterial Count
USA	= United States of America
WHO	= World Health Organization

ABSTRACT

Food borne illness is one of the major public health problems in Bangladesh. Safe food of adequate quality is one of important prerequisites for proper nutrition. But safety of food has tended to receive less attention and have been over looked. Safety of food is defined as all conditions that are necessary during production, processing, Storage, distribution and preparation of food to ensure that is microbiologically safe, sound and fit for human consumption.

The bakery food items like; cake, biscuits, breads, toasts and handmade rice-cakes, different types of sweetmeats, muree, moa, chanachur are randomly consumed food items in the streets of Dhaka city. These food items are easily available in the road side open shops, on the foot-path or in the pulling cart.

So far, there is no regular market monitoring system for qualitative and quantitative assessment of these street food items and no scientific report published yet. Considering the situation this study was undertaken to assess the general specifications, the qualitative and quantitative aspects of ingredients and microbial load of these food items.

The study was conducted in all the 10 places of Dhaka city. The sampling sites for this study are Gulshan, Banani, Dhaka Cantonment, Mohakhali, Uttara, Dhanmondi, Shahbagh, Nilkhet, Bakhshibazar, and Dhaka University area. A sampling frame of different biscuits, breads, cakes, rice-cakes, sweetmeats, muri and others were prepared. A total 60 different Bakery Products including 14 Biscuits, 06 Breads, 06 Buns, 04 Hot Dogs, 08 Pizzas, 08 Sandwiches, 06 cakes, 04 Pastries, 04 Beef Patties, 15 sweet-meats & Chocolates, 04 Moa, 02 Muree, 02 Tondur Rutee, 02 Bakorkhani, 01 Khaza, 01 Chanachur, 09 handmade Rice-cakes and 04 Egg Puddings were collected on payment for the microbial load, moisture & fat contents. All the tests were conducted following the common analytical methods for identification and quality aspects.

Different types of food samples were tested for total viable bacterial count (TVBC), total coli form count (TCC) and total fungal count (TFC). In bakery products the total viable bacterial count was 4.3×10^4 / gm, the total coli form count was 2.0×10^2 / gm and the total fungal count was 1.14×10^2 / gm. In between different Chocolates the total viable bacterial count was 1.7×10^5 / gm, the total coli form count was 1.48×10^2 / gm and the total fungal count was 0.56×10^2 / gm. Another between other Street- foods the total viable bacterial count was 2.48×10^3 / gm, the total coli form count was 1.16×10^2 / gm and similarly the total fungal count was 0.6×10^2 / gm.

On the basis of TVBC, 40 % of different Bakery Products, 6.66 % of different Chocolates, 48 % of other Street- foods were unsafe for consumption. The TCC showed that 26.66 % of Bakery products; 13.33 % of Chocolate & sweet-meets and 44 % of other Street-foods were contaminated (according to the Permissible Range recommended by **ICMFS** - 2000). Out of 24 strains 06 were Bacillus and 18 were Staphylococcus. From these street-food samples 20 % of different Bakery Products, 13.33 % of different Chocolates and 40 % of other Street-foods were unacceptable on the basis of TFC.

Anhydrous Chloroform Methanol mixture in the ratio of 2:1 was used to extract the fat from the dry sample. The examination of Moisture was done by constant wt methods. For proximate nutrient value were expressed as % in gm/100 gm for dry sample. To ensure the accuracy of analysis, triplicate of each sample were analyzed.

Out of the total Bakery products 48.33 % samples contained less then 05 % of moisture and 51.66 % contained equal or more than 05 %, Chocolates 87 % samples contained less then 05 % and 13 % contained equal or more than 05 % and the other Street-Food samples 48 % samples contained less than 05 % and 52 % contained equal or more than 05 % of moisture.

In total Bakery Products 38.33 % samples having fat content less than 05 % and 61.66 % content equal 05 % or more, out of total Chocolates 60 % samples contained less than 05 % and 40 % contained equal or more than 05 %. And out of other Street-food samples 44 % samples contained less than 05 % and 56 % contained equal or more than 05 % of fat.

After the analysis of bacteriological and chemical; finally the result of this study shows that the other street-foods were highly contaminated than the Bakery products and Chocolates. Microbial contamination incase of Bakery products were 73.34 %, in case of Chocolates were 86.67 % and only 56 % of the other Street-foods were fit for human consumption.

Indexing Key Words:

Street-food, Bakery Product, Chocolate.

Chapter-01

01. Introduction
(1.1 - 1.6)

01. Introduction:

Food is one of the basic needs of life for maintaining growth and development. So, food of proper nutritional value, hygienic in quality and in appropriate quantity is essential for good health and active life. Street-foods are recognized as having a very large role in urban food consumption, especially in developing countries for the poor and middle class people. Street-food sellers are attracted to this occupation because of the possibility of earning relatively high incomes.

It is now widely accepted. Street-foods are mainly sold in urban areas, but they are also prepared in confectionary and sol by shop-keepers or vendors in rural area. Not strictly on the street exactly. It is now setting up simple facilities to prepared and sells ready to eat foods to school children and other passers by the same advantages and risks as urban Street-foods. Those are also convenience for travelers, students and busy urban people, like factory workers, who can't attend lunch at home or can't carry from home. Many poor people don't have proper cooking facilities everywhere. They can take Street-foods any time, especially in the middle of the day or break-fast or dinner as a main food also.

The demand of Street-foods increases rapidly with rapid urbanization. Street foods are most popular among the teenagers to aged person in all over the country. As the street food gets popularity, street-food shops are rooming across the city and also extended to other cities and urban areas in Bangladesh. Dhaka, the capital of Bangladesh, is the most densely populated city in South East Asia. The city already contains a huge number of street-food selling shops and restaurants for local people and tourist. And its number is increasing rapidly. However, in most cases their sanitation and hygienic quality is not maintained or improved. This may cause the risk of infection with the contaminated food. The disease agents spread by foods not only incapacitate large groups of people but also sometimes result in serious disability and death. The traditional restaurants of Dhaka city also sell Street foods to various consumers, city dwellers or outsiders. The number of restaurants and variety of street foods are also increasing day-by-day without concerning the minimum sanitation and hygienic condition. As a result, the foods are contaminated with food borne or food associated microorganism. For good health safe food, free of pathogens and not causes any illnesses after ingestion are essential. Food should have low bacterial count. Foods suspected of causing food poisoning give higher count ranging from one million to ten million per gram of food (Sami and Bari, 1986; Hobbs, 1953; Sami and Lindam et al, 1995). The Centers for Disease Control and Prevention (USA) estimate that each year more than 5,000 Americans die from food borne illness, more than 300,000 are hospitalized and another 76 million get sick.

Food consumption patterns are matters of choice for many people, although determined partially by factors such as cost and availability. The sensory pleasures provided by eating foods with brilliant colors, tantalizing aromas, intriguing textures, and appealing flavors are bonuses enjoyed along with nutrition. These pleasurable aspects of food play a key role in determining food choices and eating patterns.

A huge number of different Bakery products, Chocolates and other Street-foods are available in the developed and developing countries. While convenient and economical for a busy lifestyle, majority of those foods are typically high in calories, fat, saturated fat, sugar and salt. Especially different Bakery products and Chocolates have been modified to reflect consumers' concern about the fat content of their food. Many restaurants have switched from beef tallow or lard to hydrogenated vegetable oils for frying. Their nature and preparation procedures vary with the food habit of consumers and countries.

Operational definition:

Street-food: FAO has defined **"Street-food"** as follows:

"Street-foods are ready to eat foods and beverages prepared and or sold by vendors especially in streets and other similar public places." (A. Allain, 1998).

Relevant definition:

Food Hygiene: **"Food hygiene"** implies, 'hygiene in the production, handling, distribution and serving of all types of food.'

Food Adulteration: Extraction of valuable component from food or addition of something of lower price which is absent in the food normally with an evil motive, which lower the food value and has injurious effect to health is called **"food adulteration".**

Food Additives: **"Food additives"** are 'non-nutritious substances which are added intentionally to food, generally in small quantity to improve its appearance, flavor, texture or storage properties.'

Street-foods in Bangladesh:

Most of the Bangladeshi People spend more than half of their income on food. Dhaka is the biggest urban center and capital city of Bangladesh. Street vending is an essential part of the city's life. The problems commonly associated with street vending related to cleanliness of the city environs and to the orderliness of the city activities. Some popular Bangladeshi Street-foods are as follows:

1. Moa (Cheera Moa & Muree Moa)
2. Muree
3. Handmade rice-cakes
4. Chanachur
5. Khaza
6. Bakorkhani
7. Biscuits
8. Breads
9. Cakes
10. Beef Patties
11. Chocolates

Though some research programme have been taken in the field of Street-foods quality in some neighboring countries like India and Nepal with the collaboration of FAO, But there was no research programmed about overall (nutritional & hygienic) quality aspects of this selected Street-foods in Bangladesh, both in national and international level. A study was conducted in 1990 on Street-foods named, "Survey report on Street vended and weaning foods from Dhaka Municipal area under WHO Project BANCWS-001 (Siddique A. B et al). So, this is now important to know about the Street-foods quality at Dhaka City Zones. Dhaka is a Mega City today. Above 1.5 million people live and increasing day by day here. A great number of people are taking foods from the Street. It is very essential to justify such kinds of people nutritional and hygienic condition in Bangladeshi point of view. That's why I was interested about the matter and tried to do some thing in this important field.

1.1 The Ingredients of all food samples are analyzed:

1.1.1 The Ingredients of the Bakery Products:

a. Biscuits = Wheat Flour + Sugar + Edible Vegetable Oil / Dalda / Ghee / Butter + Baking Soda + Liquid Glucose + Leavening Agents + Salt + Edible Starches + Permitted Emulsifiers + Antioxidants + Flavors
+ Yeast Powder + Milk + Egg [only for Toast Biscuits]
+ Milk + Milk Cream [only for Milk Cream Biscuits]
+ Cheese Powder [only for Cheese Flavored Biscuits]
+ Nut [only for Nut mixed Biscuits]
+ Egg + Essence [only for Dry Cake]

b. Breads / Bun = Wheat Flour + Vegetable Oil / Dalda / Ghee / Butter + Yeast Powder + Sugar (little) + Milk + Salt

c. Hot Dog / Pizza / Sandwich = Breads +
Beef / Chicken / Sausage + Onion + Tomato + Capsicum + Cucumber + Lettus + Other Vegetable + Cream Cheese / Dressing + Ketchup + Spices + Green Chili + Vegetable Oil [Feeling]

d. Cakes / Pastry = Wheat Flour + Egg + Sugar + Vegetable Oil / Dalda / Ghee / Butter + Baking Soda + Milk + Essence + Cream

d. Beef Patties = Wheat Flour + Egg + Vegetable Oil / Dalda / Ghee / Butter + Baking Soda + Salt [Dough] +
Beef + Onion + Spices + Green Chili + Vegetable Oil [Feeling]

1.1.2 The Ingredients of the Chocolates:

a. Candy = Liquid Glucose + Cane Sugar + Citric Acid + Candy syrup + Food Color
+ Chili + Salt [only for Jhal Candy]
+ Tamarind Extract [only for Tamarind Candy]
+ Fresh Milk + Milk cream + Butter oil + Corn syrup + Cocoa Powder + Vanilla Cream + Titanium dioxide (E-171) + Lecithin (E-322) + Sodium Chloride + Hydrogenated Palm Kernel Oil + Natural identical Milk Flavor [only for Milk Candy]

b. Chocolate Biscuits = Cocoa Powder + Cocoa Solids + Wheat Flour + Sugar + Hydrogenated Vegetable Fat + Edible Oil + Liquid Glucose + Full Cream Milk Powder / Milk solid + Permitted Emulsifiers + Soya Lecithin + Salt + Edible Starches + Leavening Agents + Food Flavor (Swiss) + Natural Color
+ Rice Crisps + Raisins + Peanuts [only for Wafers]

c. Mimi Bar = Cocoa powder + Cocoa mass + Milk solid + Cocoa butter substitute + Sugar + Lecithin

d. Chocolate Pops = Sugar + Glucose Syrup + Citric Acid + Permitted Synthetic Food Color + Natural Flavors

e. Gems = Sugar + Coated Milk + Cocoa powder + Permitted Food Grade Color + Flavors

f. Mini fruit jelly = Sugar + Coconut Chunk + Starch + Citric Acid + Lychee Flavor

1.1.3 The Ingredients of the other Street-Foods:

a. Moa = Cheera / Muree + Molasses + Vegetable Oil / Dalda (little)

b. Muree = Rice

c. Tondur Rutee = Wheat Flour + Yeast Powder + Vegetable Oil / Dalda / Ghee / Butter + Salt + Sugar (little)

d. Bakorkhani = Wheat Flour + Yeast Powder + Vegetable Oil / Dalda (little) + Salt + Sugar

e. Khaza = Wheat Flour + Vegetable Oil / Dalda + Sugar Syrup + Yeast Powder + Black Sesame / Teel + Salt

f. Chanachur = Chickpea flour + Peanuts + Green beans + Lentils + Split chickpeas + Rice flakes + Vegetable Oil + Spices + Salt + Citric acid + Red chili powder + Turmeric powder

g. Rice-cakes = Rice powder / Wheat Flour + Sugar / Molasses + Coconut crumbs + Egg + Condensed Milk + Salt

h. Egg Pudding = Egg + Milk + Sugar + Essence

1.2 The Nutritional Value of all food samples is analyzed:

1.2.1 The Nutritional Value of the Bakery Products:

Bakery products are made of mainly wheat flour and baking soda or yeast powder. Wheat flour contains mainly carbohydrate. Intake of 1 gm of carbohydrate gives us 4 Kcal energy value.

1.2.2 The Nutritional Value of Chocolates:

Chocolates are made of mainly glucose syrup and sugar. Those are present in the form of concentrated solution and contain mainly carbohydrate. Intake of 1 gm of carbohydrate gives us 4 Kcal energy value. The art of chocolate preparation make it taste very pleasant.

1.2.3 The Nutritional Value of other Street-Foods:

The other street foods are the combination of verities of food products here. Some of them as moa, muree, tondur rutee, bakorkhani, khaza and rice cake are basically carbohydrate type food products. Solely chanachur is the protein based food here and egg pudding is the mixture of carbohydrate and protein. Intake of 1 gm of carbohydrate or protein gives us 4 Kcal energy value.

1.3 Food and Food Groups:

Foods are the chemical substances which an individual takes digest to and assimilate. It provides the nutritive requirements to maintain growth and physical well being.

A good diet helps to assure more vitality and health. It prevents illness as well as essential in recovery from illness.

Nutrient is any substance nourishing the body. It classified into following six groups:

1. Protein
2. Fat
3. Carbohydrate
4. Vitamin
5. Mineral &
6. Water or Moisture.

In the event of inadequacy of these nutrients failing health and specific nutrient disorders are bound to occur (BNNS, 1998).

1.4 Fundamental Properties and Nutritional Significance of Fat and Moisture:

1.4.1 Fat:

A group of naturally occurring organic compounds called triglycerides comprised of three molecules of fatty acids and one molecules of glycerol. They are slightly soluble in alcohol and are readily dissolve in ether and other organic solvents. Fat is an important component of diet and serves a number of functions in the body. Presence of fat in the diet is important for the absorption of fat soluble vitamins like vitamin A. and carotene present in the diet.

Some fats particularly those derived from vegetables source provide essential fatty-acid. These essential fatty acids are also important for the structure and function of cells. Fat is a concentrated source of energy, yielding 9 kcal, from 1 gm of fat, which is then twice the energy supplied by CHO per unit wt.

The requirement of fat is a about 50-60 gm for a normal man that is to provide 30% of the total calories from dietary fat. For children 15 to 20% of the total calories should be supplied by dietary fat (Swamminathan, 1985).

1.4.2 Moisture:

Water or moisture form the greatest component of the human body, making up 50% to 70% of the body's weight and distributes itself over the body. Among lean and other issues (in both intra cellular and extra cellular fluids. Lean muscle contains about 73% water. Adipose tissue is about 20% H_2O. Thus as fats content increase (and the % of lean tissue decreases) in the body; total body H_2O content drifts towards 50%.

Depending an how much fat has been stored, an adult can survive for about 8 weeks without eating food but only a few days without drinking water. Became the body can neither readily store nor entirely conserve H_2O. We can survive only a few days without it.

1.5 Review of Literature:

A number of studies in developing countries have shown the potential for serious food poisoning out breaks due to microbial contamination and use of non-permitted food additives, food colors and presence of many other adulterants (WHO, 1992).

A consultation noted that the Street-food sellers are mostly between 20-50 years of age though country variation exist (FAO Food and Nutrition Paper 46, 1990).

Most of the foods were at temperature at which bacterial growth occurs. Over 50 % of the foods were at an optimum temperature, for the growth of Pathogenic food borne bacteria. Most of the shop-keepers and vendors did not reheat their foods and some reheat 6-8 hours often cooking (WHO, 1992).

Street-foods are also available in front of the markets, schools and at any busy places. Where low income generating people and the students take foods as these are readily available and cheaper (Siddique A. B et al).

Result from study revealed that 30 % of house holds food expenditures were devoted to prepared food purchased outside of the house hold. Approximately two-third of these expenditures was made at Street-food establishments (FAO Food and Nutrition Paper 46, 1990).

In addition, it was reported that the higher educational level of the Street-food handler or vendor, the better were their personal hygiene and the food handling practices. In general, women were found pursuing better general hygiene practices (personal and on food) than men (FAO and PAHO).

It was found that because of the difficulties in obtaining clean potable water, many vendors simply re-use their water, especially for cleaning utensils equipment and dishes (FAO and PAHO, 1985).

Microbial contamination of Street-food is an indicator of poor sanitary practices in the preparation and storage of the food. Bacteria may be introduced into food by raw materials, unclean cooking materials, environmental contamination and by the people handling the food in its preparation and sale (R. J. Dawson et al).

A FAO representative pointed out the importance of Street-foods especially regarding their socio-economic impact in the context of intrusive urbanization. The safety and quality of Street-foods is a major concern and action is necessary to insure proper consumer protection (R. J. Dawson).

Different studies have shown that in some countries street foods provides a very significant proportion of total food intake for many people. It is surprising that the nutritional health social and economic impact of street foods has not been studied or appreciated until relatively recently. FAO has a leading role in drawing attention to the importance of street foods. The organization has held conference on the topic and provided advice to make these foods safer for the consumer. Because of its expertise in this area, they can provide very useful advice and assistance to member countries.

FAO has had a number of activities in the region of Asia to evaluate problems associated with street-foods and to work Governments to develop programmers to improve and insure the quality and safety of these foods, of particular interest also is the socio-economic impact of street-foods, including creation of employment, provision of affordable foods at or near place of work in rapidly growing urban areas foods and nutrition aspects of street foods have also been studied. In addition, a number of studies have recently been carried by various national institutions. In the first FAO expert consultation of street foods, the Experts said, "Street foods are an important source of economic and nutritious food, particularly for the urban poor in developing countries." It identified and recommended measures to improve the quality and safety of street foods.

At a global level, over the past 20 years FAO has working in the evaluation of street food quality safety and socio-economics and in implementing recommendation for improvement. For this purpose the FAO technical meeting on street foods was held in Kolkata from 6 to 9 November, 1995.

1.6 Background of the Study:

Street foods emerge as an integral part of the urban life style. It can be considered as nutritious and often superior in quality to their industrially manufactured counter parts (Cohen. M, 1986). Urban school children have been identified as significant purchasers of street foods around the world (EPOC, 1984).

Street food vending and selling is at times perceived as an employment source of the last report for the urban poor and particularly women (Cohen. M, 1986).

Street food vending is an important source of income particularly for women (IDRC, 1993).

Street food accounts for a part of the daily diet and so contributes towards meeting nutritional requirements, although the contribution varies and is rarely quantified. Food contamination is a major contributor to illness.

There is no epidemiological data about the risks of food-borne disease resulting from street foods in Bangladesh, but information about the risk of street foods in order developing countries has been published. Laboratory evidence showed that the risk of spreading agents of food-borne disease through street foods can be high or that such foods frequently have high microbial counts.

Inadequately washed glasses, plates and utensils by food sellers is one of the most important source of breakout cholera (Reiff F. M, 1992).

As micronutrient deficiencies are major public health concerns in developing countries and street foods are widely consumed by millions of people in the country. So fortification with micronutrient could improve the nutrient profile of these foods and serve as a means of introducing micronutrient rich foods to the consumers (Draper A, 1998). The street food trade provides a means of livelihood and an affordable source of food to millions of people in developing countries. However, the potentials of street foods to improve the food security and nutritional status of urban populations have received little official attention (Draper A, 1998).

Chapter-02

02. Aims of the Research
(2.1 – 2.2)

02. Aims of the Research:

2.1 Justification of the Study:

Study and research works in the field of overall quality aspects of Street-foods for human consumption at several zones of Dhaka City in an under develop country like Bangladesh are of nutritional importance. Because we all, especially our kids consume a huge quantity of Bakery products, Chocolates and other Street-foods and also very fond of these foods. The contaminated and adulterated Street-foods has got health hazards .Thus the justification of this study is,

- To have a clear idea to know; means types of contaminations, also its effect and Moisture and Fat contents of Bakery products, Chocolates and other Street-foods.
- To develop a paper for recommendations and action model on community awareness regarding health hazards on the quality aspects of Street-foods is available in the Dhaka City zones.
- To help further research on the contaminations and adulterations of Street-foods.

2.2 Objectives of the Study:

a. General objective:

The main objective of the study is qualitative and quantitative assessment of street-foods with respect to public health.

b. Specific objectives:

- To isolate and identify the microbial loads of different Bakery Products, Chocolates and other Street-foods.
- To see the growth of E Coli in the different Bakery Products, Chocolates and other Street-foods.
- To see the growth of Fungi in the different Bakery Products, Chocolates and other Street-foods.
- To see the effect of temperature, P^{H} and salt concentration on the growth of different Bakery Products, Chocolates and other Street-foods.
- To estimates the proximate Moisture and Fat content of Bakery Products, Chocolates and other Street-foods.
- And to survey and compare the hygienic condition with Moisture & Fat value in between the Bakery products, Chocolates and other Street-foods.

Chapter-03

03. Materials and Methods

(3.1 – 6.2)
(Table: 01 - 27)
(Fig: 01 - 02)
(Plate: 01 - 02)

03. Materials and Methods:

In this study identification of microbial loads, fungal growth and proximate Moisture and Fat content of different Bakery products, Chocolates and other Street-foods were analyzed. Those procedures are described as follows:

Microbiological and Bacterial Analysis:

3.1 Selection of the Study Area:

Different zones of Dhaka City were selected as sampling sites for this study, such as: Gulshan, Banani, Dhaka Cantonment, Mohakhali, Uttara, Dhanmondi, Shahbagh, Nilkhet, Bakhshibazar and Dhaka University area.

3.2 Period of Sample Collection:

Total Street-food samples were collected for all analysis from June, 2005 to December, 2005.

3.3 Sterilization Procedures:

a. Equipments: All the sampling equipments e.g. Test tubes, Pipettes, Petri dishes, Beakers, Motor and pestle and other glass wares which were used for bacteriological analysis were washed, rinsed, dried and sterilized. Sterilization is done by using oven dry heat at 200°C for 1 hour.

b. Medias and Aqueous Solutions: All the culture media and aqueous solutions were sterilized by using an autoclave machine following a moist heat sterilization method by autoclaving at 120°C at 15 lb. per square inch pressure for 15 minutes.

3.3.1 Operational Categories of the Street-food Establishment:

Based on the packing and storage standard as offers the bakery products, chocolates and other street-food establishments were categories into four grades here. Depending also on those street foods pricing, preservation and service quality to the clients, departmental stores, hotels and road side shops can be classified into different categories. All foods kept in a glass and net protection to protect from flies and dust. Foods served with tongue are a non-touch technique, which prevents contamination. The using of uniforms during duty hour is considered as hygienic practice and maintains a high slandered. The absence of eating arrangement reduces the probability of contamination. In this study following four grades are chosen for all street-food products depending cost of the product mainly for the food collection shops.

01. Standard-I (Std-I): Foods are very costly [e.g.: 5 star hotel (Hotel Sheraton)].
02. Standard-II (Std-II): Glass and net protection, using uniform by the food providers, foods are costly [e.g.: Cooper's].
03. Standard-III (Std-III): Glass and net protection, not using uniform by the food providers, foods are not so costly [e.g.: Dhaka University TSC canteen].
04. Standard-IV (Std-IV): Open road-side hawkers and vendors, foods are very cheep.

3.4 Selection and Collection of Street-foods:

The locally available and popular Bakery products, Chocolates and other Street-foods were again categorized before collection and testing for the study. Four types of bakery products (Table-01), six types of chocolates (Table-02) and five types of other street-food (Table-03) samples were selected from those mentioned shops.

Names, categories and types of the Bakery products are shown on the following Table-01:

Table-01

Names, categories and types of the Bakery Products

Names of the products	Categories & Types (sample code)	Numbers	Total
Biscuits	01. Toast		14
	a. Open (BT-1)	01	
	b. Packed (BT-2)	01	
	02. Salted		
	a. Open (BS-1)	01	
	b. Packed (BS-2)	01	
	03. Diabetic		
	a. Open (BD-1)	01	
	b. Packed (BD-2)	01	
	04. Sweet		
	a. Open (BSW-1)	01	
	b. Packed (BSW-2)	01	
	05. Cream (BC)	01	
	06. Cheese Flavored (BCF)	01	
	07. Glucose (BG)	01	
	08. Nut mixed (BN)	01	
	09. Dry Cake (BDC)	01	
	10. Bela (BB)	01	
Breads	01. Open:		06
	Collecting from		
	a. Std-I shops (Br-1)	01	
	b. Std-II shops (Br-2)	01	
	c. Std-III shops (Br-3)	01	
	d. Std-IV shops (Br-4)	01	
	02. Packed:		
	a. Branded(BrB)	01	
	b. Own bakery product (BrO)	01	

Names of the products	Categories & Types (sample code)	Numbers	Total
Bun	01. Open: Collecting from a. Std-I shops (Bu-1) b. Std-II shops (Bu-2) c. Std-III shops (Bu-3) d. Std-IV shops (Bu-4) 02. Packed: a. Branded(BuB) b. Own bakery product (BuO)	 01 01 01 01 01 01	**06**
Hot Dog	Collecting from a. Std-I shops (HD-1) b. Std-II shops (HD-2) c. Std-III shops (HD-3) d. Std-IV shops (HD-4)	 01 01 01 01	**04**
Pizza	01. Beef: Collecting from a. Std-I shops (PB-1) b. Std-II shops (PB-2) c. Std-III shops (PB-3) d. Std-IV shops (PB-4) 02. Chicken: Collecting from a. Std-I shops (PC-1) b. Std-II shops (PC-2) c. Std-III shops (PC-3) d. Std-IV shops (PC-4)	 01 01 01 01 01 01 01 01	**08**
Sandwich	01. Beef: Collecting from a. Std-I shops (SB-1) b. Std-II shops (SB-2) c. Std-III shops (SB-3) d. Std-IV shops (SB-4) 02. Chicken: Collecting from a. Std-I shops (SC-1) b. Std-II shops (SC-2) c. Std-III shops (SC-3) d. Std-IV shops (SC-4)	 01 01 01 01 01 01 01 01	**08**
Cakes	01. Open: Collecting from a. Std-I shops (C-1) b. Std-II shops (C-2) c. Std-III shops (C-3) d. Std-IV shops (C-4) 02. Packed: a. Branded (CB) b. Own bakery product (CO)	 01 01 01 01 01 01	**06**
Pastry	Collecting from a. Std-I shops (P-1) b. Std-II shops (P-2) c. Std-III shops (P-3) d. Std-IV shops (P-4)	 01 01 01 01	**04**
Beef Patties	Collecting from 01. Std-I shops (BP-1) 02. Std-II shops (BP-2) 03. Std-III shops (BP-3) 04. Std-IV shops (BP-4)	 01 01 01 01	**04**
		Grand Total	**60**

Names, categories and types of the Chocolates are shown on the following Table-02:

Table-02

Names, categories and types of the Chocolates

Types of the chocolates	Categories & Types (sample code)	Numbers	Total
Candy	01. Milk: a. Rich milky caramel (CaR) b. Toffee milky (CaTM) c. Only milky (CaM) 02. Other flavored a. Orange (CaO) b. Tamarind (CaT)	 01 01 01 01 01	05
Chocolates Biscuits	01. Chocolate coated fingers (CF) 02. Chocolate coated peanut wafers (CC) 03. Cream wafers (CW)	01 01 01	03
Mimi Bar	01. Orange (MBO) 02. Milk (MBM)	01 01	02
Chocolate Pops	01. Lolly Pops (LP) 02. Foot Pops (FP)	01 01	02
Gems	01. Haque (GH) 02. Cocola (GC)	01 01	02
	Grand Total		15

Names, categories and types of the other Street-foods are shown on the following Table-03:

Table-03

Names, categories and types of the Other Street-foods

Names of the products	Categories & Types (sample code)	Numbers	Total
Moa	01. Cheera: a. Open (CMO) b. Packed (CMP) 02. Muree: a. Open (MMO) b. Packed (MMP)	 01 01 01 01	04
Muree	01. Open (MO) 02. Packed (MP)	01 01	02
Tondur Rutee	Collecting from a. Std-I shops (TR-1) b. Std-II shops (TR-2)	 01 01	02
Bakorkhani	Collecting from 01. Std-III shops (B-3) 02. Std-IV shops (B-4)	 01 01	02
Khaza	Collecting from 01. Std-IV shops (K-4)	 01	01
Chanachur	Collecting from 01. Std-IV shops (Ch-4)	 01	01

Name of the products	Categories & Types (sample code)	Numbers	Total
Rice-cake	01. Patishapta: Collecting from a. Std-I shops (RP-1) b. Std-II shops (RP-2) c. Std-III shops (RP-3) d. Std-IV shops (RP-4) 02. Bhapa: Collecting from a. Std-I shops (RB-1) b. Std-II shops (RB-2) c. Std-III shops (RB-3) d. Std-IV shops (RB-4) 03. Chitui: Collecting from a. Std-IV shops (RC-4)	 01 01 01 01 01 01 01 01 01	09
Egg Pudding	Collecting from 01. Std-I shops (EP-1) 02. Std-II shops (EP-2) 03. Std-III shops (EP-3) 04. Std-IV shops (EP-4)	 01 01 01 01	04
		Grand Total	25

3.5 Sample Investigation:

Different bakery products, chocolates and other street food samples were randomly collected in hygienic condition from different bakery and road side shops of Dhaka City Zones. After collection all samples were immediately transported to the laboratory for biochemical or chemical and microbiological examination.

3.5.1 Sample Container:

Samples of different bakery products, chocolates and other street foods were collected in sterile jars or polythene.

3.5.2 Number of Field Samples:

A total number of 55 street-food samples were selected for the study. Here 30 bakery products, 15 Chocolates and 10 other street- foods were collected and immediately shifted to the "Microbiology and Food Science Laboratory" at "Institute of Nutrition and Food Science" (INFS) at Dhaka University.

The following Table-04 shows the total number of field samples:

Table-04			
Names, Categories and Numbers of the samples			
Names of the products	**Categories & Types**	**Numbers**	**Total**
Bakery Products	Biscuit	14	**60**
	Bread	06	
	Bun	06	
	Hot Dog	04	
	Pizza	08	
	Sandwich	08	
	Cake	06	
	Pastry	04	
	Beef Patties	04	
Chocolates	Candy	05	**15**
	Chocolate Biscuits	03	
	Mimi Bar	02	
	Chocolate Pops	02	
	Gems	02	
	Mini fruit jelly	01	
Other Street-foods	Moa	04	**25**
	Muree	02	
	Tondur Rutee	02	
	Bakorkhani	02	
	Khaza	01	
	Chanachur	01	
	Rice-cake	09	
	Egg Pudding	04	
		Grand Total	**100**

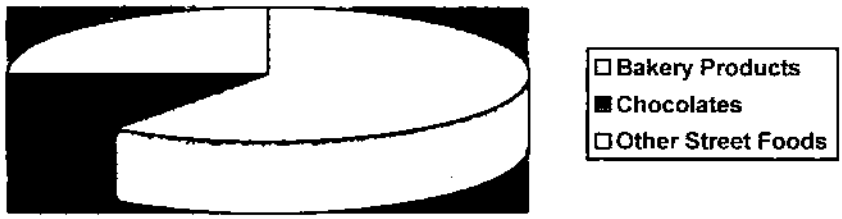


Fig-01: Categories and Numbers of the samples

3.5.3 Size of Samples:

About 20 gm of each sample was collected in each separate container. From there 10 gm of each sample was used for the first dilution and the successive testing in the laboratory.

3.6 Preparation of Samples:

Different bakery products such as Bread, Biscuit, Cake, Beef Patties; Chocolates and some other street-foods such as Moa, Khaza, Chanachur etc were examined by taking deep samples as well as surface samples. So, homogenate of samples and weighed on a sterile weighing paper using sterile scalpel or forceps. And these were mashed in sterile motor and pestle, which was sterilized by autoclaving and swabbing with alcohol. These mashed samples were inserted aseptically into sterile cotton plugged conical flask by using sterile forceps. Then these were mixed thoroughly by shaking 20 times and the solution was allowed to stand for 5-10 minutes. Thus, the samples and the diluents were homogenized; that is, 90 ml contains 10 gm of bakery product, chocolate and other street food samples. Further dilutions were prepared from this initial dilution of homogenate.

3.7 Serial Dilutions of the Samples:

Dilutions are usually made in multiples of ten. A single dilution is calculated as follows:

$$\text{Dilution} = \frac{\text{Volume of the sample}}{\text{Total volume of the sample and the dilution}}$$

For example, the dilution of 1 milliliter into 9 milliliters equals,

$$= \frac{1}{1+9} \quad \text{which is,} \quad \frac{1}{10} \quad \text{and is written, 1:10}$$

For serial dilutions 9 ml amount of the 9% sodium chloride solution was transferred into test tubes within suitable caps and sterilized by autoclave at 121°C at 15 lb per square inch pressure for 15 minutes. These were the dilution blanks.1 ml of the sample solution was pipette out by using sterile pipettes and was delivered into the first dilution blank, about half an inch above the level of the liquid and the test tube was shaken 10 minutes with the elbow resting on the table. After waiting for 3 seconds the rest of the fluid of the pipette was discarded. A fresh and sterilized pipette was dipped half an inch into the liquid and 1 ml of liquid was removed and transferred to the next dilution blank and the pipette was discarded. The process was continued for the required number of dilutions and care was taken to see that the pipette was discarded each time after delivering its contents.

Ten fold serial dilution of an initial 1 gm material + 10 ml 0.9% NaCl solution in water indicate the values shown on the following Table-05:

Table-05

Serial Dilution Values

Dilution	1st	2nd	3rd	4th
	1:10 (10^{-1})	1:100 (10^{-2})	1:1000 (10^{-3})	1:10000 (10^{-4})
10 ml contains (gm)	1	0.1	0.01	0.001
1 ml contains (gm)	1	0.01	0.001	0.0001

Inoculation of media was carried out within 30 minutes of the preparation of the dilutions.

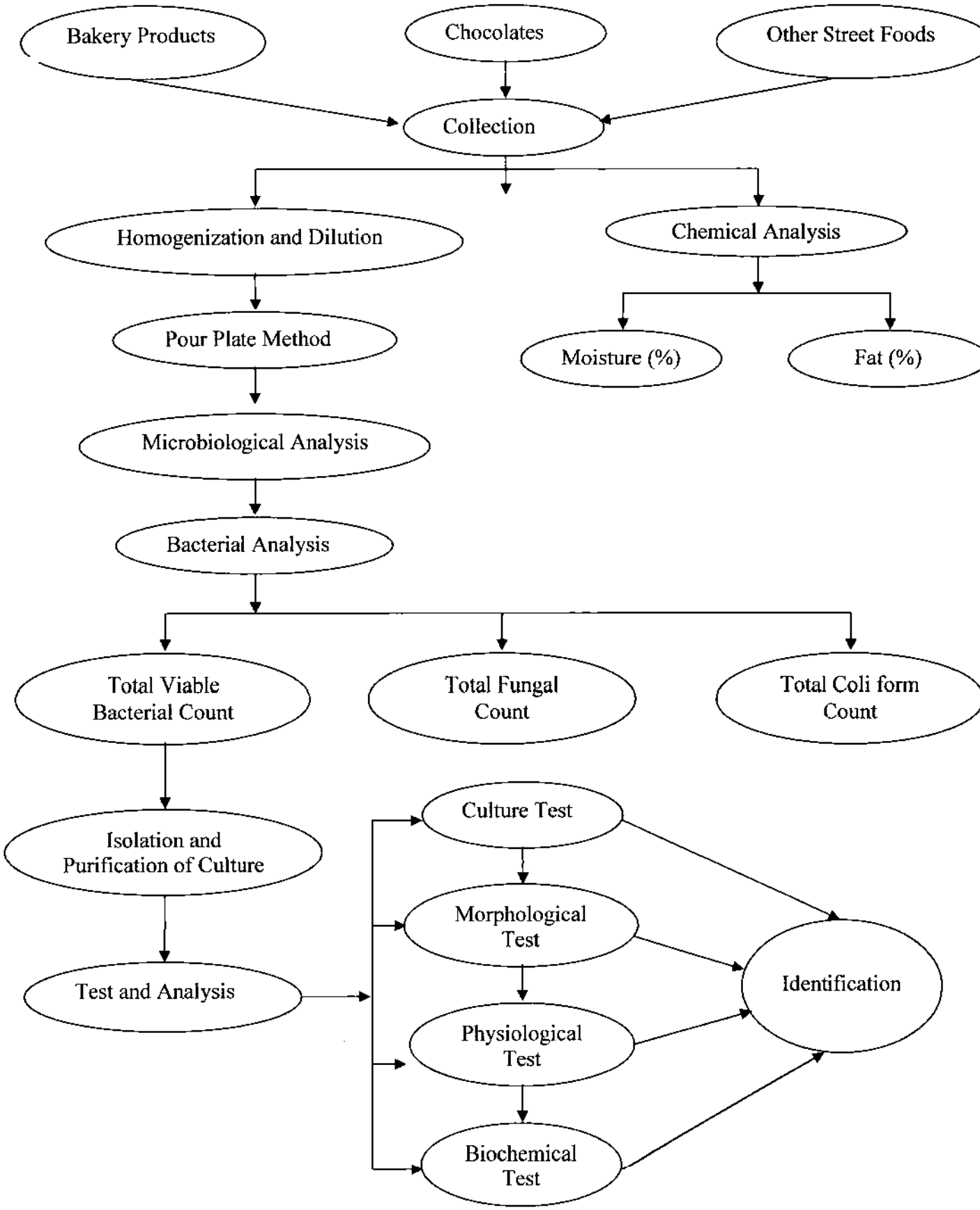


Fig-02: Flow Diagram for Microbiological and Chemical Analysis and Identification of Street Foods collecting from different types of Food Shops.

3.7.1 Composition and Preparation of Saline Water:

a. Composition: In this study we need 0.9 % NaCl for research. The following Table-06 shows the composition of saline water which used for serial dilution.

Table-06	
Composition of Saline Water	
Ingredients	**Quantity**
NaCl (dry)	0.9 gm
Distilled Water	100 ml

b. Preparation: Weight out NaCl and collect it into a conical flask. Mix distilled water. Shake very well. After smoothly mixing filter it.

Distribute 9 ml of saline water into each test-tube by a pipette. Fill up all those test-tubes need for serial dilution. Cotton plugged all and autoclave at 121^0 C at 15 lb. per Square inch pressure for 15 minutes.

3.8 Microbiological Analysis:

After several trials of different Bakery products, Chocolates and other Street-foods with the following four Medias were found most suitable for total count and used for these purpose. Those are shown on Table: 07, 08, 09 & 10.

Table-07	
Medium-01	
Ingredients	**Quantity**
Peptone	10 gm.
Meat Extract	10 gm.
Sodium Chloride (NaCl)	05 gm.
Agar	15 gm.
Distilled Water	1000 ml.
$P^H = 7.2$	

Table-08	
Medium-02	
Ingredients	**Quantity**
Peptone	05 gm.
Yeast Extract	03 gm.
Agar	15 gm.
Distilled Water	1000 ml.
$P^H = 7.4$	

Table-09	
Medium-03	
Ingredients	**Quantity**
Peptone	10 gm.
Meat Extract	10 gm.
Yeast Extract	05 gm.
D.Glucose	20 gm.
Sodium Acetate	05gm.
Tri Ammonium Citrate	02gm.
$MgSO_4.\ 7H_2O$	200gm.
$MnSO_4.\ 4H_2O$	50gm.
Agar	15gm.
Distilled Water	1000ml.
$P^H = 7.6$	

Table-10	
Medium-04	
Potato Dextrose Agar (PDA)	
Ingredients	**Quantity**
Peptone	10 gm.
Meat Extract	10 gm.
Yeast Extract	05 gm.
D.Glucose	20 gm.
Sodium Acetate	05gm.
Tri Ammonium Citrate	02gm.
$MgSO_4.\ 7H_2O$	200gm.
$MnSO_4.\ 4H_2O$	50gm.
Agar	15gm.
Distilled Water	1000ml.
$P^H = 7.6$	

Medium-01, 02 & 03, these three different types of media as recommended by Aiso et al (1965) were compared in this experiment for the growth of the bacteria. The colonies developed on the plates were also compared and counted after incubation for 18 - 48 hours at 37^0 C.

a. Preparation of Media:

For each media first had to weight out every ingredient and mixed in a conical flask. Shake very well. Cotton plugged that flask and heat in a steamer. Heat with 100^0 C temperatures until dissolved every ingredients smoothly. After cooling filter and adjust P^H. Autoclave at 121^0 C at 15 lb. per Square inch pressure for 15 minutes.

b. Adjust the P^H of a Medium using the Comparator Method:

After cooling and before autoclaving adjust the P^H of those Medias are very important. If the P^H of any media was less than the exact P^H requirement than need to add 0.1N (normal) NaOH and if it was more than the exact P^H requirement than need to add 0.1N (normal) HCl very slowly. After adding one or two drops by using a graduated pipette slowly than again measure the P^H level by P^H indicator papers. If had to remember that this P^H adjustment is very important, because if P^H level was not set as requirement than the growth of bacteria will not found exactly and also media will not solidified. For this calculate the amount of N NaOH (or N HCL) require to adjust the P^H of 1 liter of culture medium.

For example,0.6 ml of 0.1 N NaOH were required to adjust P^H of 10 ml of medium to 7.2,so 6 ml of N NaOH are required to adjust the P^H of 1 liter of medium to 7.2.Add the required amount of N NaOH (or N HCL) to the bulk medium as indicated by the calculation. Mix well. Check that the P^H is satisfactory and readjust if necessary.

3.8.1. Total Viable Bacterial Count (TVBC):

The number of living bacteria or viable bacteria in a liquid culture or suspension was counted by a culture method such as the 'pour-plate method'. A measured amount of the suspension is mixed with molten agar medium in a Petri dish. After setting, the inoculated plate was incubated at 37^0 C for 18-48 hours to facilitate total viable bacterial growth and then the number of colonies was counted.

3.8.2. Total Coli form Count (TCC):

Mac Conkey agar plates were used to determine the total number of Coli form. The Mac Conkey Agar is a useful medium for the cultivation of entire bacteria. To prepare the desired volume of the media at first the chemical ingredients were weighed. Then the peptone bile salt and the sodium chloride were dissolved in distilled water by steaming. After cooling the media P^H was adjusted to 7.4. Then the agar was added and dissolved by autoclaving. At last the lactose and neutral red were added and steamed until dissolved and then the media was autoclaved at 121^0 C for 15 minutes at 15 lb per square inch pressure.

Inoculated plates were incubated for 24 hours at 37^0 C to facilitate the coli form growth and then the number of colonies was counted.

Composition of **"Mac Conkey Agar"** is shown on the following Table-11:

Table-11	
Medium-05	
Mac Conkey Agar	
Ingredients	**Quantity**
Peptone	20 gm.
Bile Salts	05 gm.
NaCl	05 gm.
Lactose	10 gm.
Neutral Red	07 gm.
Agar	20 gm.
Distilled Water	2000 ml.
$P^H = 7.4$	

3.8.3 Preparation of Agar for Plate Counts:

Heating in boiling water melted prepared agar for count. The medium did not allow remaining at their high temperature beyond the time necessary to melt it. Prepared agar was melted once only.

Melted agar was placed in a tempering water bath maintained at a temperature of 44-46^0 C. This temperature was maintained not for more than three hours to avoid formation of precipitate, which confuse the counting of colonies. A thermometer was immersed in a separate flask in the water bath to monitor the temperature.

3.8.4 Preparation of Plating:

Duplicate plates were used for each sample or dilution tested. The Petri dishes was arranged and marked in a reasonable order for use. An aliquot was aseptically pipette from the appropriate dilution into to the bottom of each Petri dish. After delivery the tip of the pipette was touched once to a dry spot in the dish. A separate sterile pipette was used to transfer an aliquot to each set of Petri dish, for each sample dilution used. The initial dilution of homogenate and dilution tubes was vigorously shaken before each transfer was made.

3.8.5 Pouring Agar Plates:

After the agar deep had thoroughly melted, it was cooled to approximately 45^{0}C. Then the agar medium was added to each Petri dish containing and aliquot of the sample or its dilution. The inoculated medium was mixed carefully by rotating the Petri dishes gently. After the agar had cooled and solidified the plates were incubated at 37^{0}C for 18-48 hours. A control plate should be kept to check the sterility of pipettes, agar, dilution water and Petri dishes.

3.8.6 Counting Method:

The dilution count provides an estimate of the number of living organisms in a sample. In this study the standard plate count was used to determine the total number of viable bacteria in sweets. The number of colony forming units (c.f.u) were determined by this method, which is calculated by the following formula-

$$\text{Organism /gm / milliliter of sample} = \frac{\text{Number of Colonies}}{\text{Amount plated X Dilution factor}}$$

The following rules were maintained to report the standard plate counts:

a) Plates with between 30-300 colonies are suitable for counting. When replicate plates from are dilution were countable, counts of colonies on all plates were added and divided by the volume tested (in ml.) as follows-

$$\frac{\text{Sum of colonies}}{\text{Sum of volumes tested (ml)}} = \text{Standard Plate Count /gm}$$

b) When two or more consecutive dilution were countable then the mean of these Counts /ml. were calculated for the reported value.

c) When all plates were shown less then 30 colonies, the actual numbers of colonies on the lowest dilution plate were recorded.

d) When all plates were show greater then 300 colonies, the count was computed by multiplying the mean count by the dilution used and reported as a greater than (>), standard plate count per milliliter.

3.8.7. Preparation of Stock Culture:

a. Preparation of Agar Slants:

The nutrients agar was also used for the preservation and maintenance of stock culture. Aliquots of 5ml of the media were dispensed and distributed in test tubes, plugged with cotton wool and were autoclaved. After autoclaving sterilized tubes were kept in sloping for solidifying.

b. Pure Culture:

Clear, discrete bacterial colonies were picked up by inoculating loop and streaked on the fresh and dried agar plates. Plates were incubated at 37^0C for 24 hours. Individual & isolated single colony of each type was transferred to agar slopes and incubated. After 24 hours bacterial growths were preserved as stock cultures for the further tests.

c. Preservation and Maintenance:

Nutrient agars were used for the maintenance of the isolates for this purpose agar slopes that contained the individual pure bacterial cultures were kept in refrigerator at 5^0C. For maintenance these slants cultures were transferred to freshly prepared slants after 15 days. Duplicate slopes for each pure isolate were kept at refrigerator as stock culture for further use.

3.8.8. Cultural of the Strains:

a. Coding of Isolates:

The pure culture of the isolates was coded according to Table-01, 02 & 03 shown on page: 17 - 20. If one strain was isolated from the same sample of the same grade then, those were coded with numerical.

b. Cultural Characterization:

i) Cultural Characteristics of the Strains:

The shape, size, color, elevation, texture and pigment production were observed visually and recorded.

ii) Morphological Characteristics:

For the determination of morphological character gram stained slide were viewed under an oil immersion objective.

Gram Stains Solution:

a) 0.5 % Crystal Violet (CV) Solution.

b) Lugol's Iodine Solution as mordant (1 % Iodine in 2 % Potassium Iodine)

c) 95 % Ethanol (Ethyl Alcohol)

d) Safranin Solution (0.1 % Safranin in 20 % Acetic acid)

3.8.9. Physiological Characteristics of the strain:

i) Growth at Anaerobic condition (Anaerobic Test):

To test the anaerobic characters of the isolates, the nutrient agar plates were streaked with the fresh cultures by a sterile inoculating loop. The plates were then enclosed in an anaerobic jar. The air in the anaerobic jar was replaced by the placing a burning candle inside the jar. Then the jar is incubated at 37^0 C for 24 hours. Growth of the isolates on the plates indicated positive results.

ii) Growth at different concentrations of salt:

Considering the importance of Sodium Chloride concentration on the growth of bacteria, Sodium Chloride tolerance tests were carried out.

Nutrient Broth was used for this test. Nutrient Broth containing 5 % and 9 % Sodium Chloride were prepared and 5 ml of which was distributed in test tubes and autoclaved under 15 lb pressure at 121^0 C for 15 minutes. After autoclaving the medium was inoculated from fresh cultures and incubated at 37^0 C for 24 hours. Control tubes without inoculums were maintained in the same manner. After 24 hours the turbidity in the media due to growth of the bacteria was observed at first visually and then turbidity was measured by using a spectrophotometer at 540 nm.

Nutrient Broth: An empirical medium of general use for the cultivation of most bacteria.

Composition of **"Nutrient Broth"** is shown on the following Table-12:

Table-12

Medium-06

Nutrient Broth

Ingredients	Quantity
Peptone	10 gm.
Meat Extract (Lab-Lemco)	10 gm.
NaCl	05 gm.
Distilled Water	1000 ml.
$P^H = 7.2$	

iii) Growth at different temperatures:

Microbial growth and activities are affected by temperature differences. Each strain maintains a temperature for its optimum growth.

Nutrient broth was used for determining this optimum growth temperature. The medium was prepared and 5 ml of which was distributed in test tubes, plugged with cotton wool and autoclaved under 15 lb pressure at 121^0 C for 15 minutes.

The tubes were inoculated from fresh cultures and incubated at 55^0 C and in refrigerator [4^0 - 6^0 C] for 24 hours. Control tubes without inoculums were maintained in the same manner.

After 24 hours the turbidity (growth) was observed visually and then the turbidity was measured by using a spectra-photometer at 540 nm.

iv) Growth at different P^H :

Microbial growth and activities were strongly effected by the P^H of the medium. But there were wide differences between the P^H requirements of the various species. Each species can grow only within a certain P^H range and most rapid growth occurs in a narrow optimum P^H zone.

Nutrient broth was used for this test. The medium was prepared and P^H was adjusted to 9.5 and 2.5 by using "MERCK" P^H paper strip (Germany) and 5 ml of which was dispensed in test tubes, plugged with cotton wool and autoclaved under 15 lb pressure at 121^0 C for 15 minutes. The tubes were inoculated from fresh culture and incubated at 37^0 C for 24 hours. Control tubes without inoculums were maintained in the same manner. After 24 hours the turbidity was observed at first visually and after this by using a spectra-photometer the turbidity was measured at 540 nm.

04. Biochemical Characteristics of the Strain:

4.1. Biochemical Test:

A group of tests were done for the identification of a particular isolate according to the criteria described in Bergey's Manual of Determinative Bacteriology, 8th edition, 1974, Manual of Clinical Microbiology (Albert Balows et al, 1991) and Applied and Environmental Microbiology 40 (Mark et al, 1980). By following the methods of Collins and Lyne (1989), AOAC (1980), Harrigan and McCance (1966) and Difco Manual of Dehydrated Cultures (1953), the following tests were done.

A) Tests for metabolism of Carbohydrates and related compounds:

1. Hydrolysis of Starch
2. Fermentation Test
3. Methyl Red (MR) Test
4. Voges-Proskouer Test
5. Citrate Utilization Test
6. Carbohydrate Utilization Test

B) Tests for metabolism of Protein:

1. Hydrolysis of Gelatin
2. Hydrolysis of casein
3. Indole Test
4. Production of Ammonia from Peptone
5. Production of Hydrozen Sulphid

C) Tests for Enzymes:

1. Catalase Test
2. Oxidase Test
3. Urease Test
4. Nitrate Reduction Test

D) Miscellaneous Tests:

1. Motility Test

A) Tests for metabolism of Carbohydrates and related compounds:

i) Hydrolysis of Starch:

Nutrient Agar plates with starch were prepared. This medium consisted of nutrient agar in which soluble starch (0.2 % - 1.0 %) was added. These plates were prepared by pouring of 10 ml nutrient agar into each plate, allowing it to set and then overlaying this with 5 ml of starch agar.

Starch Agar: For the detection of starch-hydrolyzing ability.

Composition of **"Starch Agar"** is shown on the following Table-13:

Table-13	
Medium-07	
Starch Agar	
Ingredients	**Quantity**
Soluble Starch	0.2 gm.
Yeast Extract Agar or Nutrient Agar	100 ml.

A poured dried plate of the medium was inoculated by streaking once across the surface and incubated at 37^0 C for 2-14 days.

Test Reagent: Gram's Iodine Solution as used for gram's stain.

Composition of **"Gram's Iodine"** is shown on the following Table-14:

Table-14	
Gram's Iodine	
Ingredients	**Quantity**
Iodine	1.0 gm.
Potassium Iodine	2.0 gm.
Distilled Water	300 ml.

Recording Result:

The plates were flooded with 5-10 ml of iodine solution. Unhydrolysed starch formed a blue color with the iodine. Clear zones around the growth indicated hydrolysis.

ii) Fermentation Test:

Hugh and Leifson's Medium (Hugh and Leifson, 1953) was used for fermentation test. Composition of **"Gram's Iodine"** is shown on the following Table-15:

Table-15	
Medium-08	
Hugh and Leifson's Medium	
Ingredients	**Quantity**
Peptone	2.0 gm.
Sodium Chloride	5.0 gm.
Dipotassium Hydrogen Phosphate	0.3 gm.
Bromthymol blue, 01 % aqueous solution Agar	3.0 ml
Distilled Water	3.0 gm.
	1 liter.
$P^H = 7.1$	

iii) Methyl Red (MR) Test:

For this test the medium used is Glucose Phosphate (GP) Broth.

Composition of **"Glucose Phosphate (GP) Broth"** is shown on the following Table-16:

Table-16	
Medium-09	
Glucose Phosphate (GP) Broth	
Ingredients	**Quantity**
D-glucose	0.5 gm.
K_2HOP_4	0.5 gm.
Peptone	0.5 gm.
Distilled Water	100 ml
$P^H = 7.5$	

The above ingredients were taken in desired amount of distilled water and were allowed to boil until they dissolved completely. 05 ml of the media in tubes were sterilized in the autoclave for 15 minutes under 15 lb per square inch pressure at 121^0. Inoculums from the fresh culture of the isolated strains were taken by a sterile inoculating loop and inoculated separately and were kept in the incubator at 37^0 C for 2-7 days depending on the rate of growth of organisms in question.

Test Reagent:

Composition of **"Methyl Red Solution"** is shown on the following Table-17:

Table-17	
Methyl Red Solution	
Ingredients	**Quantity**
Methyl Red	0.1 gm.
95 % ethanol	300 ml.
Distilled Water	100 ml.

Recording Result:

Five drops of the indicator were added to 05 ml of culture. A red color, denoting aph of 4.5 or less, was described as positive. A yellow coloration was recorded as negative.

iv) Acetone Production [Voges Proshauer (VP) Test] from Glucose:

Production of acetone by the bacteria was tested with **"Glucose Phosphate Broth"**, as a methyl red test.

The media was prepared following the preparation procedure of methyl red test. The medium was inoculated with inoculum from fresh culture and incubated at 37^0 C for 2-7 days.

Voges Proskauer (VP) Test is dependent on the production of acetyl methyl carbinol (acetone) from glucose.

Test Reagent:

Composition of **"Baritt's Modification"** (Baritt, 1936) is shown on the following Table-18:

Table-18

Baritt's Modification

Ingredients	Quantity
Ethanolic Solution of Alpha-napthol	06 %
Solution of Potassium Hydroxide (KOH)	16 %

Recording Result:

To do Baritt's modification take 1 ml of culture in a test tube, 0.5 ml of 6 % Alpha-napthol solution and 0.5 ml of 16 % Potassium Hydroxide was added. The tubes were shaken for about 05 second after adding of the reagents. A positive reaction was indicated by the development of pink coloration within 2-5 minutes and becoming crimsoned within 30 minutes.

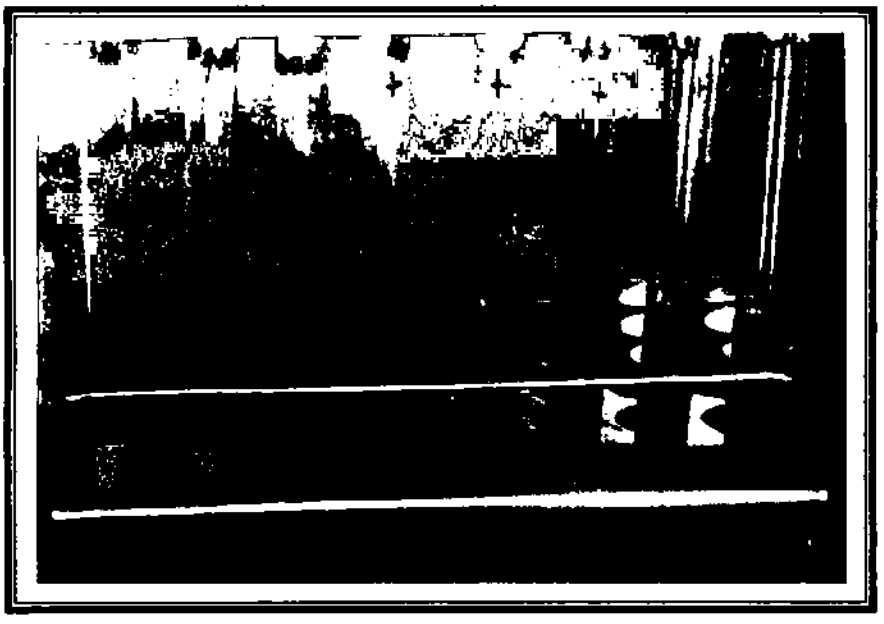


Plate-01: Voges - Proskauer Test.

v) Citrate Utilization Test:

Simmon's Citrate Medium:

Simmon's Citrate Medium is a modification of Koser's medium with agar and an indicator added. Here, Agar = 20 gm and Bromothymol Blue = 0.08 gm. The indicator is added in the form of a 0.002 % solution.

Composition of **"Koser's Citate Medium"** of 1 liter is shown on the following Table-19:

Table-19	
Medium-10	
Koser's Citate Medium	
Ingredients	**Quantity**
Sodium Ammonium Hydrogen Phosphate	1.5 gm.
Potassium Di-hydrogen Phosphate	1.0 gm.
Magnesium Sulphate	0.2 gm.
Sodium Citrate	2.0 gm.
Distilled Water	1000 ml.
$P^H = 7.0$	

The above ingredients were added except the indicator solution to the distilled water, Distributed in the test tubes for use as slopes, with a 1 inch butt. The slope culture was inoculated by streaking over the surface with a loop full of peptone water culture or preferably with a wire needle of saline suspension and incubated at the optimum temperature (37^0 C) for up to 7 days.

Recording Result:

Utilization of citrate and growth on the citrate agar results in an alkaline reaction so that the bromothymol blue indicator in the medium change from green to bright blue, when no growth occur and citrate not utilized, the color of the medium remain unchanged.

vi) Carbohydrate Utilization Test:

Production of acid or gas or both from glucose, to test production of acid:
Agar slants with Fermentation Basal Medium were prepared.

Composition of **"Fermentation Basal Medium"** is shown on the following Table-20:

Table-20	
Medium-11	
Fermentation Basal Medium	
Ingredients	**Quantity**
$(NH_4)_2 HPO_4$	1.0
KCL	0.2
$MgSO_4,7 H_2O$	0.2
Yeast Extract	0.2
Bromocresol Purple	0.008
Glucose (added after sterilization)	0.5
Agar	15.0
$P^H = 2.0$	

For the preparation, desired volume of the above ingredients except glucose was taken and the media was prepared. The media was sterilized in autoclave for 15 minutes under 15 lb/sq inch pressure at 121^0 C. An amount of 5 gm of glucose was taken in 100 ml distilled water and was autoclaved for 5 minutes under lb/sq inch pressure. The sterile glucose solution was then added to the media. 5 ml of the prepared media was then taken in each sterile test tube and slants were made. The tubes were incubated with inoculums from the fresh culture in nutrient agar by a sterile inoculating loop. The inoculated slants were kept in the incubator for 37^0 C for 24 hours. Two un-inoculated tubes were kept for controls. Change of color to yellow as shown by the indicator (Bromocreasol purple) was indicated as acid positive.

B. Tests for Metabolism of Protein:

i) Hydrolysis of Gelatin:

Frazier's gelatin agar (modified) was used.

Medium:

Nutrient agar + 0.4 percent gelatin, final $P^H = 7.2$
The medium was sterilized by autoclaving for 20 minutes at 115^0C.

A poured dried plate of the medium was inoculated by streaking once across the surface and incubated at 37^0C for 2-14 days.

Composition of **"Mercuric Chloride Solution"** is shown on the following Table-21:

Table-21

Test Reagent	
Mercuric Chloride Solution	
Ingredients	**Quantity**
Mercuric Chloride	15 gm
Concentrated Hydrochloric Acid	20 ml
Distilled Water	1000 ml

Recording result:

The plates were flooded with 8-10 ml of test reagent. Un-hydrolyzed gelatin formed a white opaque precipitate with the reagent. Hydrolyzed gelatin appeared as a clear zone.

ii) Casein Hydrolysis:

Skim milk agar was used to observe clearing around colonies of casein-hydrolyzing organisms. Mild agar, which consisted of nutrient agar with the addition of 10 % skim milk, was sterilized by autoclaving for 20 minutes at 115^0C at 15 lb/sq inch pressure.

A poured dried plate of the medium was inoculated by streaking once across the surface and incubated at 37^0C for 2-14 days.

Test reagent:

The reagent was prepared by mixing of 10 % Mercuric Chloride solution in 20 % Hydrochloric Acid.

Recording results:

Clear zones, which were visible after incubation of the plates, were presumptive evidence of casein hydrolysis. To confirm that clearing was the results of casein hydrolysis the plates were flooded with the above test reagent. If the cleared area disappeared, casein was not hydrolyzed.

iii) Production of Indole from Tryptophan:

Peptone water (tryptone 1-2 %, Sodium Chloride 0.5 %, final P^H 7.2) was dispensed in test-tubes and sterilized by autoclaving for 15 minutes at 121^0C. Tryptone was chosen for this test, since it is a tryptophan – rich peptone. Peptone water was inoculated as for broth culture from young agar slope cultures, and incubated at the optimum 37^0C growth temperature for 2-7 days.

Test reagent:

Composition of **"Kovac's Indole Reagent"** is shown on the following Table-22:

Table-22	
Test Reagent	
Kovac's Indole Reagent	
Ingredients	**Quantity**
Pure amyl or alcohol	50 ml
Para-Dimethylamino Benzaldehyde	10 gm
Concentrated pure hydrochloric Acid	50 ml

The reagent was prepared by dissolving the aldehyde in alcohol and then the acid was added slowly.

Recording result:

5 ml Kovac's Indole Reagent was added and the tube was shaken gently and then allowed to stand. A deep red color indicated the presence of indole, which separated out in the alcohol layer.

Plate-02: The Indole Production Test.

iv) Ammonia from Peptone:

Inoculate the tubes of peptone water [Peptone = 1 % and Sodium Chloride (NaCl) = 0.5 %] and incubate with a sterile control tube at 37^0C for 2-7 days.

Test reagent:

The name of the reagent is "Nessler's Solution".

Recording result:

Add 1 ml culture to 1 ml Nessler's reagent in a clean tube. The development of an orange to brown color indicates the presence of ammonia. The control tube should turn pale yellow or show no color reaction.

v) Kligler's Iron Agar:

Kligler's Iron Agar is a complex medium containing 0.03 % ferric citrate was sterilized by autoclaving.

Composition of medium:

Composition of **"medium"** is shown on the following Table-23:

Table-23	
Medium-12	
Ingredients	**Quantity**
Kligler's Iron Agar	55 gm
Distilled water	1000 ml

After sterilization the medium was slanted with a deep butt (1 - inch butt, 1 – ½ inch slant). The medium was inoculated by stabbing the butt and streaking the slant. The incubated medium was inoculated at optimum temperature for up to 7 days. Blackening of the medium was showed production of H_2S.

C) Test for Enzymes:

i) Catalase test:

In this test with the help of sterilized glass rod or capillary tubes fresh bacterial cultures were taken from slants individually and placed on a clean slide. A small drop of 30 5 hydrogen peroxide was emulsified with the culture. Liberation of free oxygen, as gas bubbles due to enzyme activity of isolates was indicative of positive results.

ii) Oxidize Test:

Teat reagent: 1 % aqueous solution of tetra methyl - p - phenylenediamine hydrochloride.

Method: The reagent was poured over the surface of the agar growth in a Petridis.

Recording result:

Oxidize positive colonies developed a pink color, which became successively dark red, purple and black in 10-30 minutes.

iii) Urease Activity:

Christensen's Urea Agar was used for this test.

Composition of **"Christensen's Urea Agar"** is shown on the following Table-24:

Table-24

Christensen's Urea Agar

Ingredients	Quantity
Peptone	1.0 gm
NaCl	5.0 gm
KH_2PO_4	2.0 gm
D-Glucose	1.0 gm
Agar	20 gm
Phenol Red	6 ml (0.2 % solution)
Distilled water	1000 ml
Final P^H = 6.8 - 7.0	

The basal medium was distributed in test tubes, heat sterilized and cooled to 50^0C. Sufficient 20 % urea solution, previously sterilized by filtration, was then added to give a final concentration of 2 %. The medium was slanted, allowed to set and was then ready to use.

The medium was inoculated for a slope culture. A control of basal medium containing no added urea was also inoculated at the same time to check that ammonia was produced from urea and not from peptone although the inclusion of glucose in the medium tends to counteract the slight alkaline reaction which may be obtained with the breakdown of the peptone. The inoculated medium was incubated at 37^0C for 1-7 days.

Recording result:

Urease production and hydrolysis of urea resulted in the production of ammonia. The resulting increase in P^H was shown by a change in color of the medium, from yellow to pink.

iv) Reduction of Nitrate:

This is a test for the presence of the enzyme nitrate reductase (or nitratase) which causes the reduction of nitrite in the presence of a suitable electron donor. This nitrite can be tested for by an appropriate colorimetric reagent. Almost all entero bacteriaceae reduce nitrate to nitrite.

Nitrate peptone water was the medium employed, consisting of peptone water with the addition of 0.02 - 0.2 % potassium nitrate (analytical reagent grade). 5 ml amount of the medium was distributed in tubes, each with an inverted Durham tube and sterilized by autoclaving for 15 minutes at 121^0C at 15 lb per square inch pressure.

Composition of **"Peptone Water"** is shown on the following Table-25:

Table-25	
Peptone Water	
Ingredients	**Quantity**
Tryptone	10 gm
NaCl	5 gm
Distilled water	1 Liter
$P^H = 7.2$	

The nitrate peptone water was inoculated as for broth culture and incubated together with a sterile control tube at 37^0C for 2-7 days.

Test reagent:

"Griess-Ilosvay's" reagents

1) 8 gm sulphanilic acid in 1000 ml of 5 N acetic acid.
2) 5 gm alpha-Napthylamine in 1000 ml of acetic acid.

Recording result:

1 ml of the test reagents was added to the test culture and to the control tube. Presence of nitrite was indicated by the development of red color within a few minutes and hence the ability of the organism to reduce nitrate to nitrite. The control tube showed no coloration.

A negative result was confirmed by the addition of small quantity of Zinc dust to the tube. Red color developed which indicated the presence of nitrate and thus that no reduction had taken place. The tube which shown no development of color with the addition of Zinc indicate that no nitrate remained or the nitrate has been completely reduced by the culture beyond the nitrite stage. The presence of gas in the Durham tube indicate that the formation of gaseous nitrogen and therefore complete reduction of nitrate.

D) Miscellaneous tests:

i) Motility test: To test the motility of bacteria the motility test media was used.

Composition of **"Motility Test Medium"** is shown on the following Table-26:

Table-26	
Motility Test Medium	
Ingredients	**Quantity**
Tryptone	10 gm
NaCl	5 gm
Agar	5 gm
Distilled water	1000 ml

The medium was dissolved completely by heating to boiling temperature. The media was distributed in test tubes and sterilized by autoclaving for 15 minutes at 15 lb pressure at 121^0C. The medium was allowed to cool in the test tubes in an upright position. The sterile medium was inoculated with a needle by stabbing through the center of the medium and was incubated at 37^0C and examined at the end of 8, 24 and 48 hours. Motility was manifested macroscopically by a diffuse zone of growth spreading from the line of inoculation.

05. Mycological Analysis:

Fungi were isolated from different Bakery products, Chocolates and other Street-foods and the Total Fungal Count (TFC) was determined.

5.1. Total Fungal Count (TFC):

To determine Total Fungal Count (TFC) "Sabouraud's Glucose Agar" plates were used. P^H of the media was adjusted to 5.0. Inoculated plates were incubated at room temperature for 72 hours to facilitate fungal growth.

Composition of **"Sabouraud's Glucose Agar"** is shown on the following Table-27:

Table-27	
Medium-06	
Sabouraud's Glucose Agar	
Ingredients	**Quantity**
Glucose	40 gm.
Peptone	10 gm.
Agar	20 gm.
Distilled Water	1000 ml.
$P^H = 5.0$	

06. Biochemical Analysis:

6.1. Size of Samples:

About 10 gm of each sample was collected in each separate container. From there 05 gm of each sample was used for the first oven dry.

6.2. Preparation of Samples:

Homogenate of all samples and mashed in motor-pestle first. Weigh on weighing papers using clean scalpel or forceps. Collect each sample in crucibles. Weight empty crucible and record those. Mark all crucibles with the sample codes. Take sample in those and weight again. Put them all in pre-heated oven for oven dry at 150°C.

a. Estimation of Moisture content:

Procedure:

The estimation of moisture was done by constant weight methods (Gopalan, 1971) here. The edible part of the samples (10-11 gm) was taken in constant weight, pre-washed and dried at 105^0 C crucibles. Those were then kept 105^0 C temperature in an oven for 04 hours and cooled in a desiccators for ½ an hour and weight again. Heating, cooling and weighing were continued until a constant weight was obtained.

Calculation:

$$\text{Percentage of Moisture (\%)} = \frac{\text{Initial weight} - \text{Final weight}}{\text{Sample weight}} \times 100$$

Here,
Initial weight = Sample weight + crucible weight (before heating)
Final weight = Sample weight + crucible weight (after heating).

b. Estimation of Fat content:

Crude Fat was analyzed by solvent extraction and was determined according to the modified method described by Folch et al (1957) here. Anhydrous chloroform-methanol mixture in the ratio of 2:1 was used to extract the fat from the dry samples.

Reagents:

1. Chloroform-methanol mixture 2:1
   [Chloroform was mixed with methanol in the ratio of 2:1]

2. 0.58 % of Sodium Chloride (NaCl).
   [0.58 of NaCl was dissolved in distilled water and final volume was made up to 100 ml]

Procedure:

05 grams of dry samples were taken in a conical-flask and to it sodium 20-30 ml Chloroform-methanol mixture (2:1) was added. The sample was allowed to stand for overnight and filtered. Filter paper was washed repeatedly (03 times) with Chloroform-methanol solution (2:1). The filtrate was taken in a separating funnel and to it 0.58 % NaCl sodium (20 ml) was added. The separating funnel was vigorously shaken for proper mixing and allowed to stand for 4-6 hours. The lower phase was then collected and washed with Sodium-Chloride solution repeatedly till the lower phase was clear. Finally the lower phase was collected in a conical-flask. Total volume of extract was recorded. Then all of the extract was taken into a 50 ml vile or beaker and allowed to air dry and the dried in an oven 105^0 C for the determination of total fat. Fat content was determination of total fot. Fat content was calculated by the following formula.

Calculation:

$$\text{Gm \% of Fat} = \frac{\text{Weight of extract (gm)}}{\text{Sample weight (gm)}} \times 100$$

Chapter-04

07. Results

(7.1 – 7.6)

(Table: 28 - 65)

(Fig: 03 - 35)

(Plate: 03 - 34)

07. Results:

The microbiological and chemical (Moisture & Fat) analyses of locally available street-foods were carried out in this research work collected from different shops and restaurants situated in Dhaka City. Different types of Bakery products, Chocolates and other Street-foods were analyzed in this research work. For analysis, each type of sample collected from different classes of street food shops. These tests included Total Viable Bacterial Counts (TVBC), Total Coli form Counts (TCC), Total Fungal Counts (TFC) and Moisture and Fat content.

7.1 Bacteriological load of different types of Bakery products, Chocolates and other Street-foods:

A total of 100 street food samples were collected from Dhaka city zones to analyze here TVBC, TCC, & TFC.

7.1.1 Total Viable Bacterial Counts (TVBC) of Bakery Products, Chocolates and other Street-foods:

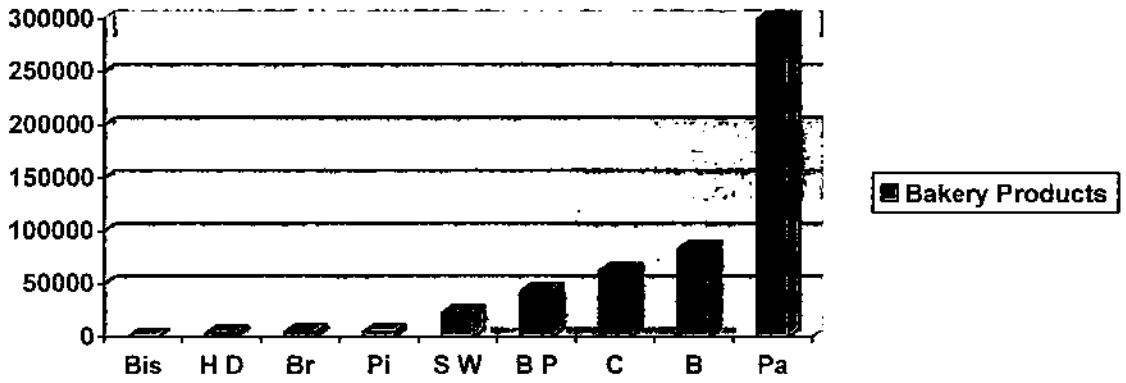
The total number of Viable Bacteria in different Bakery Products detected on standard plate count (SPC) agar as above is shown in Table-28 and Figure-03. Among the samples, 85 % (51 samples) contained mesophilic bacterial cells >10 gm^{-1} range while the rest samples contained bacterial cells <10 gm^{-1}. The highest count was 3.0×10^5 cfu/gm in Pastry and the lowest count was 2.0×10^2 cfu/gm in Biscuits. And the mean count was 4.3×10^4.

Total Viable Counts of different Bakery Products are shown on the following Table-28:

Table-28

Total Viable Counts of different Bakery Products

Name of the samples	Total number of the samples	Number of the sample showing growth<10 gm^{-1}	Number of the sample showing growth>10 gm^{-1}	Mean SPC/gm of samples incubated at 37^0
Biscuit (Bis)	14	03	11	2.0×10^2
Hot Dog (H D)	04	01	03	3.3×10^3
Bread (Br)	06	0	06	4.5×10^3
Pizza (Pi)	08	01	07	4.7×10^3
Sandwich (S W)	08	01	07	2.3×10^4
Beef Patties (B P)	04	01	03	4.3×10^4
Cake (C)	06	01	05	6.2×10^4
Bun (B)	06	0	06	8.3×10^4
Pastry (Pa)	04	04	0	3.0×10^5
Grand Total	**60 (100 %)**	**09 (15 %)**	**51 (85 %)**	**4.3×10^4**



(Name of the samples)

Fig-03: Total Viable Counts of different Bakery Products.

The SPC range wise distributions of the bacterial cell in different Bakery Products are also shown on Table-29 and Figure-04. The height 61.66 % (37 samples) showed the acceptable range (01^2 - $<10^4$ gm^{-1}) of total viable bacterial count. This was followed by 23.34 % poor range (01^4 - $<10^5$ gm^{-1}), 5 % safe (01^2 gm^{-1}) and 10 % dangerous (01^5 – above gm^{-1}).

Total Viable Count Range of different Bakery Products is shown on the following Table-29:

Table-29

Total Viable Count Range of different Bakery Products

SPC range/gm	Bis (n=14)	Br (n=06)	B (n=06)	H D (n=04)	Pi (n=08)	S W (n=08)	C (n=06)	P (n=04)	B P (n=04)	Total (N=60)	%
01 - $<10^2$	02	0	0	01	0	0	0	0	0	**03**	**5**
01^2 - $<10^4$	10	04	03	03	06	01	05	02	03	**37**	**61.66**
01^4 - $<10^5$	02	01	02	0	02	03	01	02	01	**14**	**23.34**
01^5 - above	0	01	01	0	0	04	0	0	0	**06**	**10**

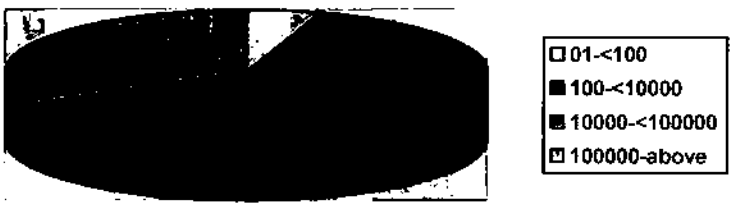


Fig-04: Total Viable Count Range of different Bakery Products

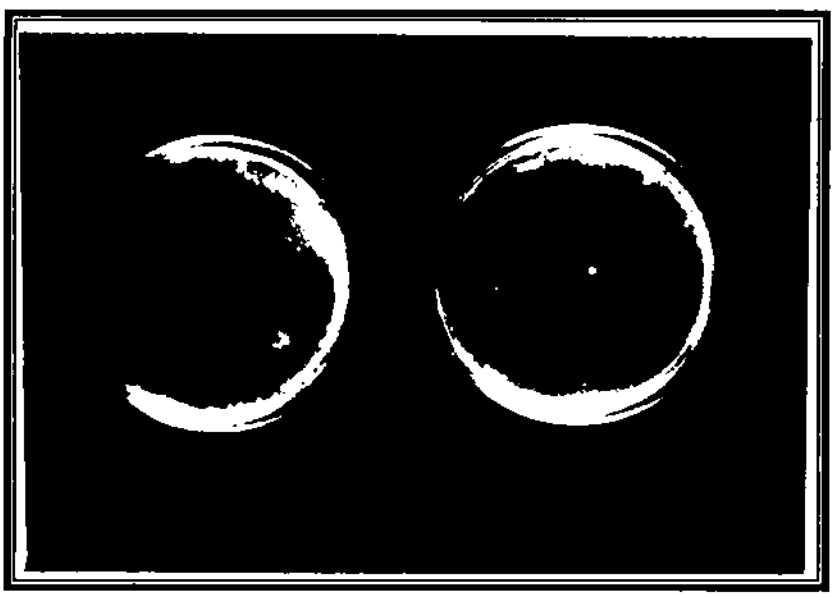


Plate-03: Growth of various bacterial colonies on Nutrient Agar plate from Biscuit (left: 10^{-1} & right: 10^{-2}) of different Bakery Products.

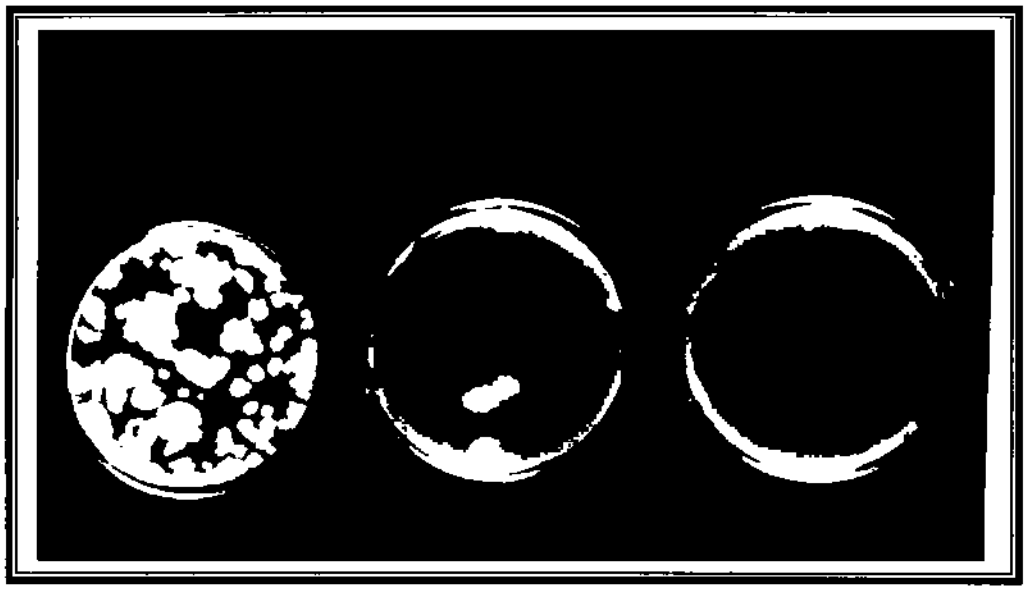


Plate-04: Growth of various bacterial colonies on Nutrient Agar plate from Pastry (left: 10^{-3}, center: 10^{-4} & right: 10^{-5}) of different Bakery Products.

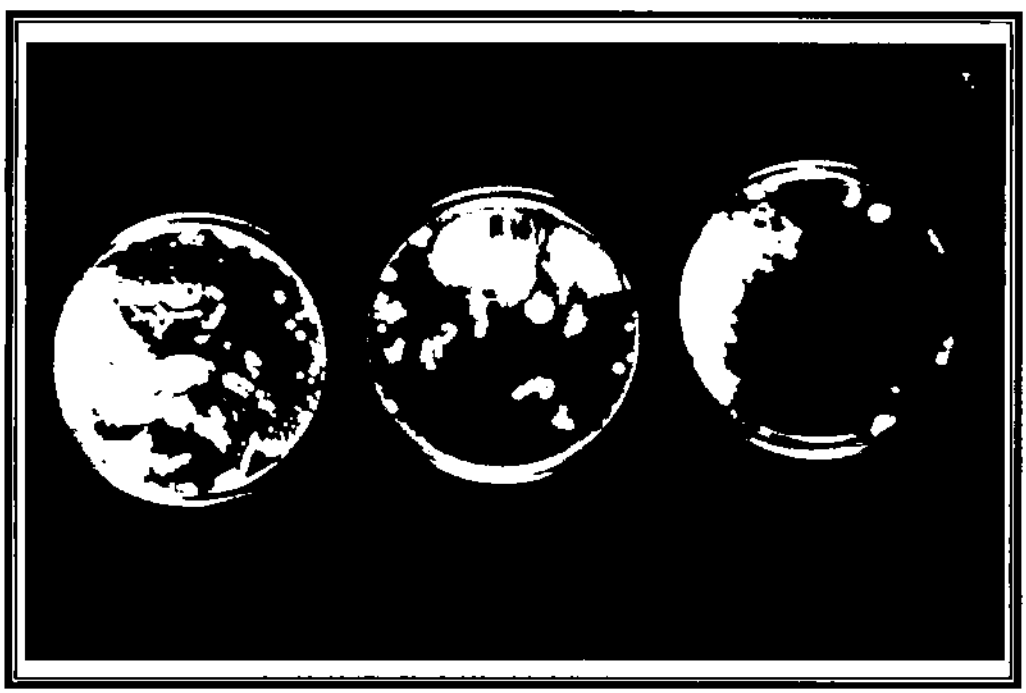


Plate-05: Growth of various bacterial colonies on Nutrient Agar plate from Bun (left: 10^{-3}, center: 10^{-4} & right: 10^{-5}) of different Bakery Products.

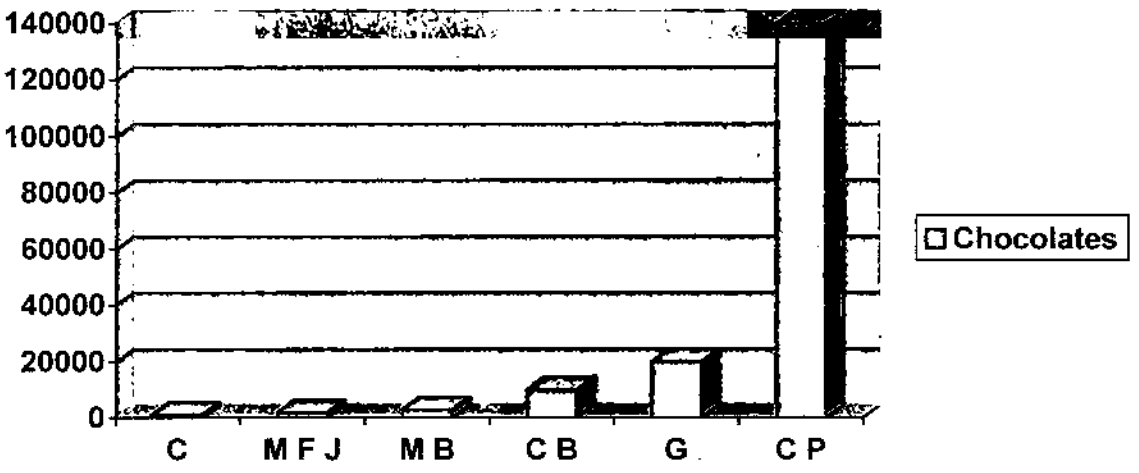
The total number of Viable Bacteria in different Chocolates detected on standard plate count (SPC) agar as above is shown in Table-30 and Figure-05. Among the samples, 93.34 % (14 samples) contained mesophilic bacterial cells >10 gm^{-1} range while the rest samples contained bacterial cells <10 gm^{-1}. The highest count was 1.4×10^5 cfu/gm in Chocolate Pops and the lowest count was 1.3×10^3 cfu/gm in Candy. And the mean count was 1.7×10^5.

Total Viable Counts of different Chocolates are shown on the following Table-30:

Table-30

Total Viable Counts of different Chocolates

Name of the samples	Total number of the samples	Number of the sample showing growth<10 gm^{-1}	Number of the sample showing growth>10 gm^{-1}	Mean SPC/gm of samples incubated at 37^0
Candy	05	0	05	1.3×10^3
Mini fruit jelly	01	0	01	2.0×10^3
Mimi Bar	02	0	02	3.0×10^3
Chocolate Biscuits	03	01	02	1.0×10^4
Gems	02	0	02	2.0×10^4
Chocolate Pops	02	0	02	1.4×10^5
Grand Total	**15 (100 %)**	**01 (6.66 %)**	**14 (93.34 %)**	**1.7×10^5**



(Name of the samples)

Fig-05: Total Viable Counts of different Chocolates.

The SPC range wise distributions of the bacterial cell in different Chocolates are also shown on Table-31 and Figure-06. The height 46.66 % (07 samples) showed the acceptable range (01^2 - $<10^4$ gm^{-1}) of total viable bacterial count. This was followed by 33.34 % poor range (01^4 - $<10^5$ gm^{-1}), 6.66 % safe (01^2 gm^{-1}) and 13.34 % dangerous (01^5 – above gm^{-1}).

Total Viable Count Range of different Chocolates is shown on the following Table-31:

Table-31

Total Viable Count Range of different Chocolates

SPC range/gm	C (n=05)	C B (n=03)	M B (n=02)	C P (n=02)	G (n=02)	M F J (n=01)	Total (N=15)	%
01 - $<10^2$	0	01	0	0	0	0	**01**	**6.66**
01^2 - $<10^4$	03	01	01	0	01	01	**07**	**46.66**
01^4 - $<10^5$	01	01	01	01	01	0	**05**	**33.34**
01^5 - above	01	0	0	01	0	0	**02**	**13.34**

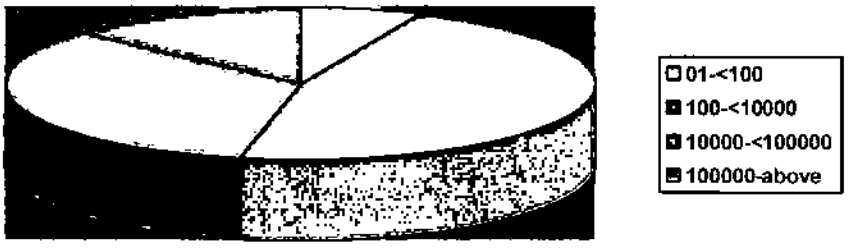


Fig-06: Total Viable Count Range of different Chocolates.

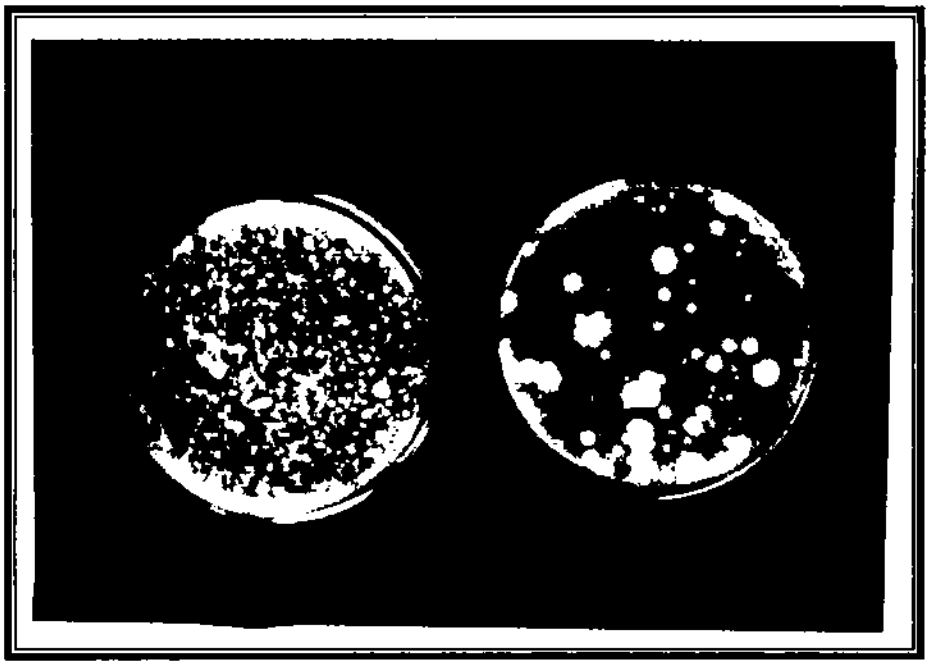


Plate-06: Growth of various bacterial colonies on Nutrient Agar plate from Mini Fruit Jelly (left: 10^{-1} & right: 10^{-2}) of different Chocolates.

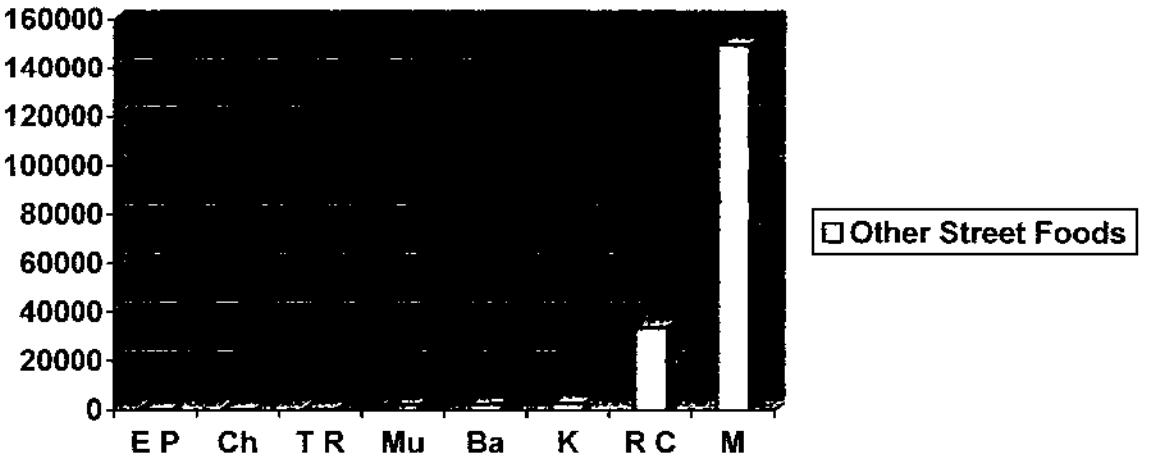
The total number of Viable Bacteria in other Street-foods detected on standard plate count (SPC) agar as above is shown in Table-32 and Figure-07. Among the samples, 84 % (21 samples) contained mesophilic bacterial cells >10 gm^{-1} range while the rest samples contained bacterial cells <10 gm^{-1}. The highest count was 1.5×10^5 cfu/gm in Moa and the lowest count was 1.2×10^2 cfu/gm in Egg Pudding. And the mean count was 2.48×10^3.

Total Viable Counts of other Street-foods are shown on the following Table-32:

Table-32

Total Viable Counts of other Street-foods

Name of the samples	Total number of the samples	Number of the sample showing growth<10 gm^{-1}	Number of the sample showing growth>10 gm^{-1}	Mean SPC/gm of samples incubated at 37^0
Egg Pudding	04	01	03	1.2×10^2
Chanachur	01	0	01	1.3×10^2
Tondur Rutee	02	0	02	4.7×10^2
Muree	02	0	02	2.2×10^3
Bakorkhani	02	0	02	2.6×10^3
Khaza	01	01	0	3.0×10^3
Rice-cake	09	01	08	3.4×10^4
Moa	04	01	03	1.5×10^5
Grand Total	**25 (100 %)**	**04 (16 %)**	**21 (84 %)**	**2.48×10^3**



(Name of the samples)

Fig-07: Total Viable Counts of other Street-foods.

The SPC range wise distributions of the bacterial cell in other Street-foods are also shown on Table-33 and Figure-08. The height 48 % (12 samples) showed the acceptable range (01^2 - $<10^4$ gm^{-1}) of total viable bacterial count. This was followed by 28 % poor range (01^4 - $<10^5$ gm^{-1}), 08 % safe (01^2 gm^{-1}) and 16 % dangerous (01^5 – above gm^{-1}).

Total Viable Count Range of other Street-foods is shown on the following Table-33:

Table-33

Total Viable Count Range of other Street-foods

SPC range/gm	Moa (n=04)	Mu (n=02)	T R (n=02)	Ba (n=02)	K (n=01)	Ch (n=01)	R C (n=09)	E P (n=04)	Total (N=25)	%
$01 - <10^2$	01	0	0	0	0	0	01	0	02	08
$01^2 - <10^4$	02	01	0	0	0	01	05	03	12	48
$01^4 - <10^5$	01	01	01	01	0	0	02	01	07	28
01^5 - above	0	0	01	01	01	0	01	0	04	16

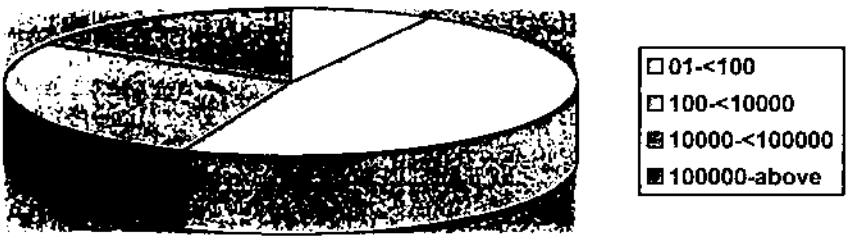


Fig-08: Total Viable Count Range of other Street-foods.

Plate-07: Growth of various bacterial colonies on Nutrient Agar plate from Moa (left: 10^{-1} & right: 10^{-2}) of other Street Foods.

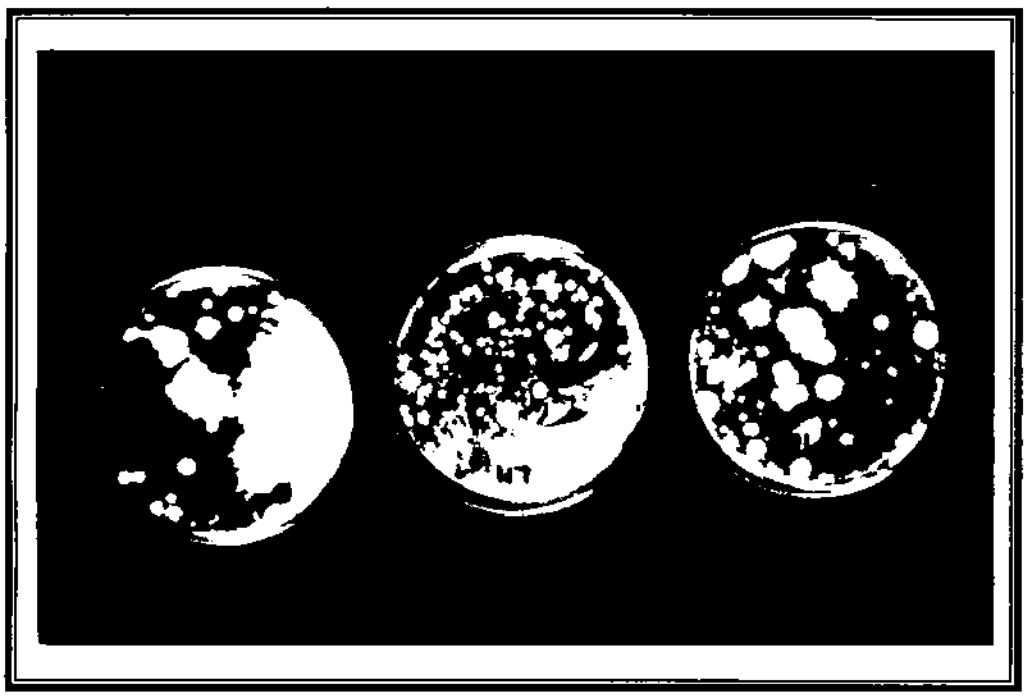


Plate-08: Growth of various bacterial colonies on Nutrient Agar plate from Rice-cake (left: 10^{-3}, center: 10^{-4} & right: 10^{-5}) of other Street Foods.

7.1.2 The Microbial Contamination with comparison of Bakery products, Chocolates and other Street-foods:

The percentages of unacceptable samples of different Bakery products are shown on Table-34 and Figure-09, on the basis of cfu/gm. Of the 60 samples 40 % (24 samples) were found seriously contaminated. In comparison to the various samples, the highest sample contamination 75 % was obtained in Hot Dog. A few samples of Biscuit, Bread, Bun, Sandwich, Cake and Pastry were also found to be contaminated. On the other hand Pizza was found to be safe to intake.

The Microbial Contamination of different Bakery products is shown on the following Table-34:

Table-34

Occurrence of Microbial Contamination in different Bakery products

Name of the samples	Total number of the sample analyzed	Seriously contaminated food samples (%)
Biscuit	14	06 (43 %)
Bread	06	02 (33 %)
Bun	06	04 (67 %)
Hot Dog	04	03 (75 %)
Pizza	08	Nil
Sandwich	08	03 (37.50 %)
Cake	06	02 (33.33 %)
Pastry	04	01 (25 %)
Beef Patties	04	02 (50 %)
Grand Total	**60 (100 %)**	**24 (40%)**

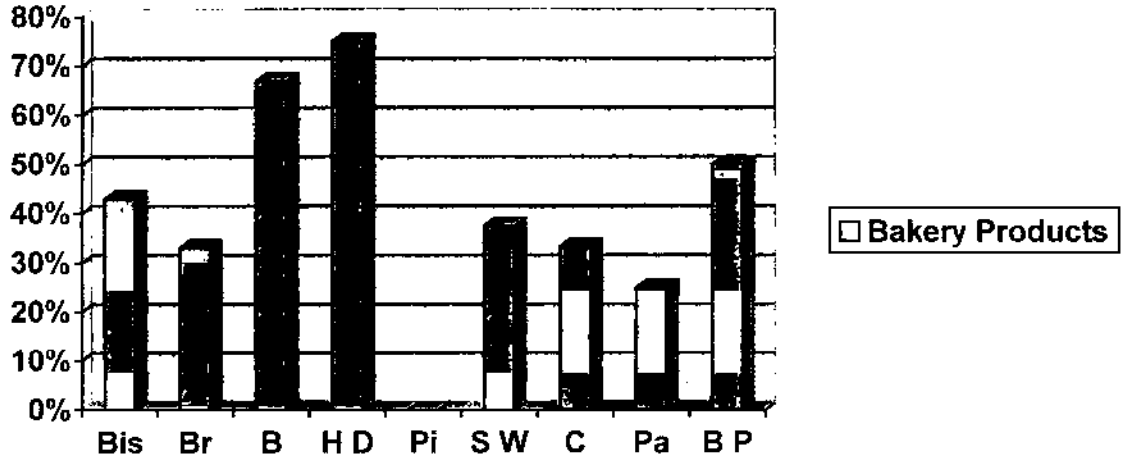


Fig-09: Microbial Contamination of different Bakery Products.

Plate-09: Gram stained cellular morphology of Bacillus isolated from Biscuit.

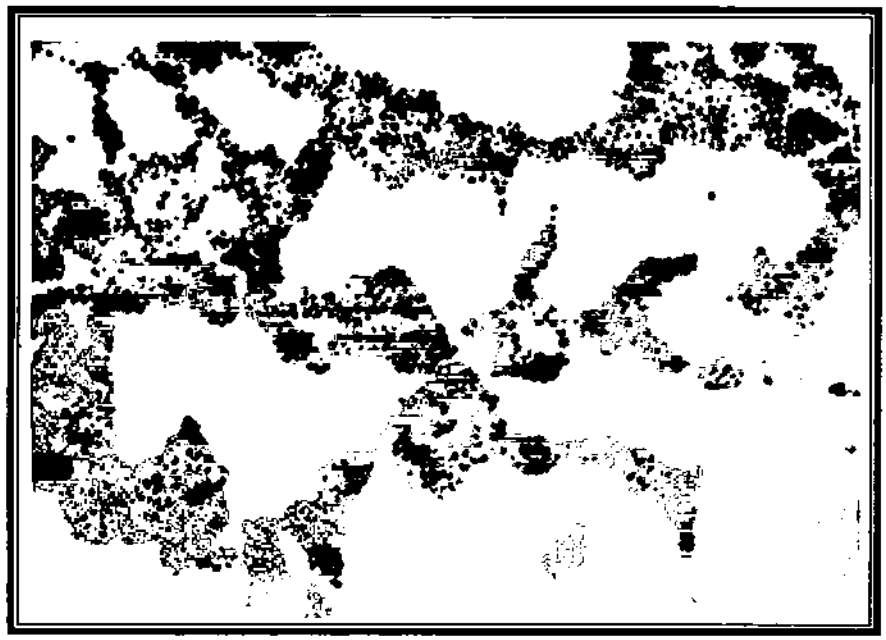


Plate-10: Gram stained cellular morphology of Staphylococcus isolated from Bread.

Plate-11: Gram stained cellular morphology of Staphylococcus isolated from Hot Dog of different Bakery Products.

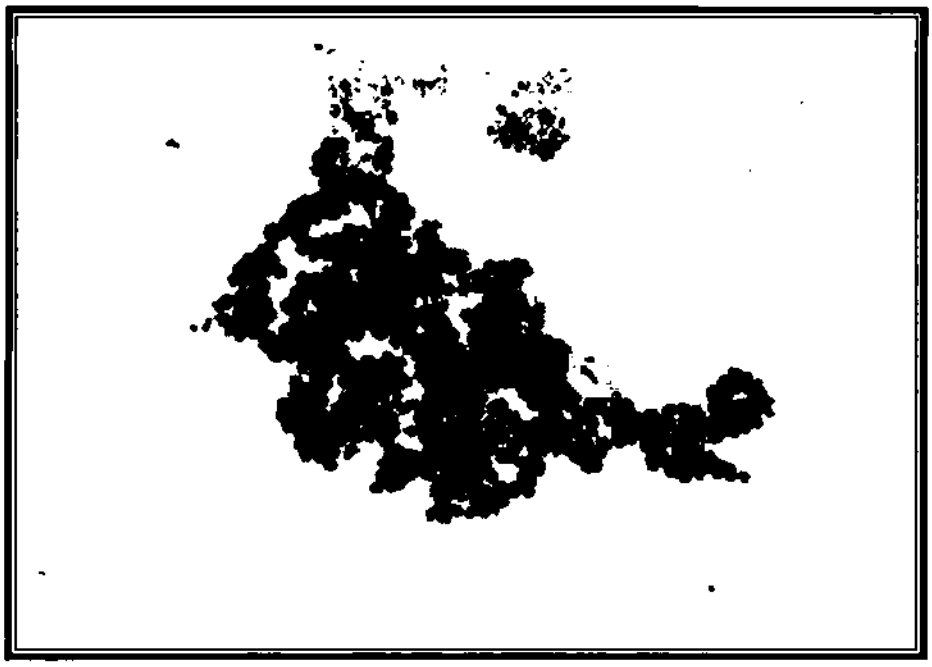


Plate-12: Gram stained cellular morphology of Staphylococcus isolated from Chicken Sandwich of different Bakery Products.

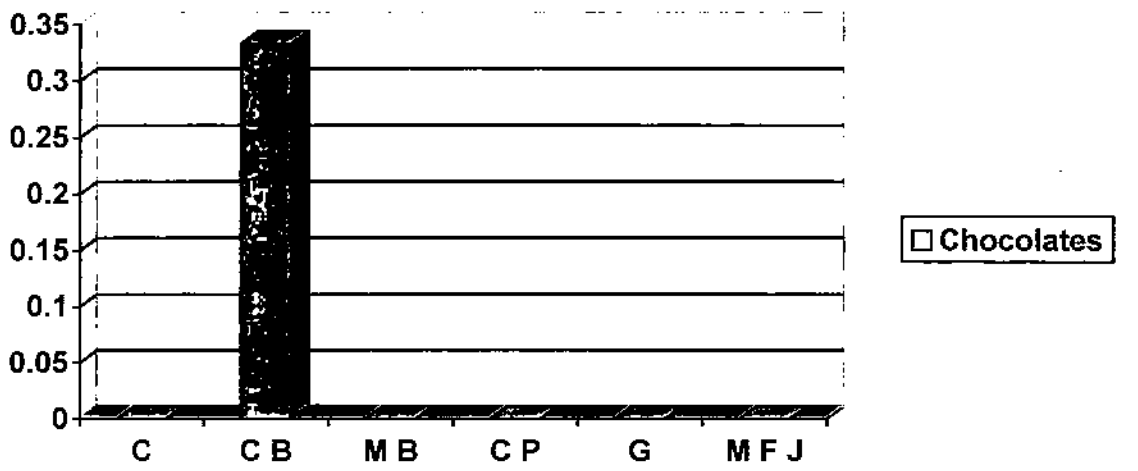
The percentages of unacceptable samples of different Chocolates are shown on Table-35 and Figure-10, on the basis of cfu/gm. Of the 15 samples only 6.66 % (01 sample) were found seriously contaminated. In comparison to the various samples, the solely sample contamination was 33.33 % obtained in Chocolate Biscuits. On the other hand, a few samples of Candy, Mimi Bar, Chocolate Pops, Gems and Mini fruit jelly were found to be safe to intake.

The Microbial Contamination of different Chocolates is shown on the following Table-35:

Table-35

Occurrence of Microbial Contamination in different Chocolates

Name of the samples	Total number of the sample analyzed	Seriously contaminated food samples (%)
Candy	05	Nil
Chocolate Biscuits	03	01 (33.33 %)
Mimi Bar	02	Nil
Chocolate Pops	02	Nil
Gems	02	Nil
Mini fruit jelly	01	Nil
Grand Total	**15 (100 %)**	**01 (6.66 %)**



(Name of the samples)

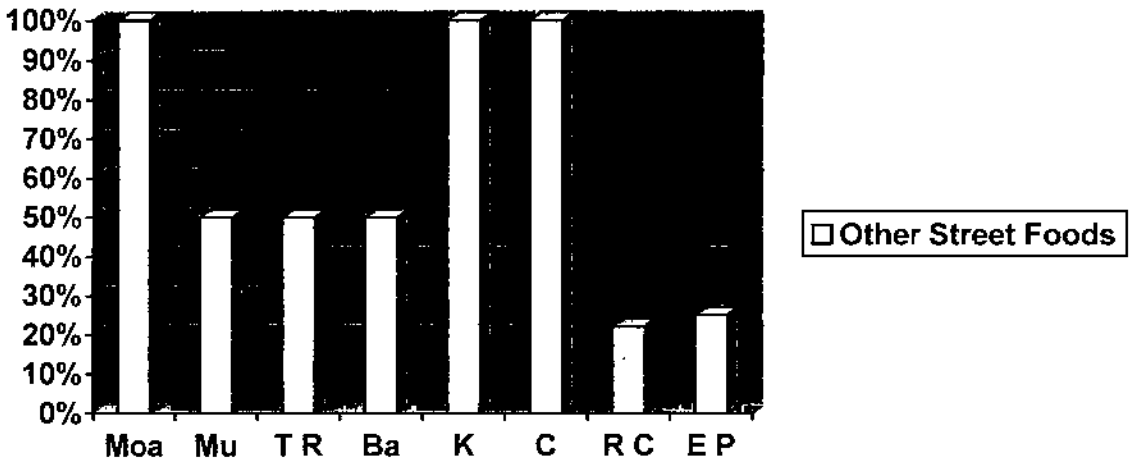
Fig-10: Microbial Contamination of different Chocolates.

Plate-13: Gram stained cellular morphology of Staphylococcus isolated from Chocolate Biscuits.

The percentages of unacceptable samples of other Street-foods are shown on Table-36 and Figure-11, on the basis of cfu/gm. Of the 25 samples 48 % (12 samples) were found seriously contaminated. In comparison to the various samples, the highest sample contamination 100 % were obtained in Moa, Khaza and Chanachur. On the other hand, other all samples of street foods as Muree, Tondur Rutee, Bakorkhani, Rice-cake and Egg Pudding were also found to be contaminated.

The Microbial Contamination of different other Street-foods are shown on the following Table-36:

Table-36		
Occurrence of Microbial Contamination in other Street-foods		
Name of the samples	**Total number of the sample analyzed**	**Seriously contaminated food samples (%)**
Moa	04	04 (100 %)
Muree	02	01 (50 %)
Tondur Rutee	02	01 (50 %)
Bakorkhani	02	01 (50 %)
Khaza	01	01 (100 %)
Chanachur	01	01 (100 %)
Rice-cake	09	02 (22.22 %)
Egg Pudding	04	01 (25 %)
Grand Total	**25 (100 %)**	**12 (48 %)**



(Name of the samples)

Fig-11: Microbial Contamination of other Street-foods.

Plate-14: Gram stained cellular morphology of Bacillus isolated from Muree.

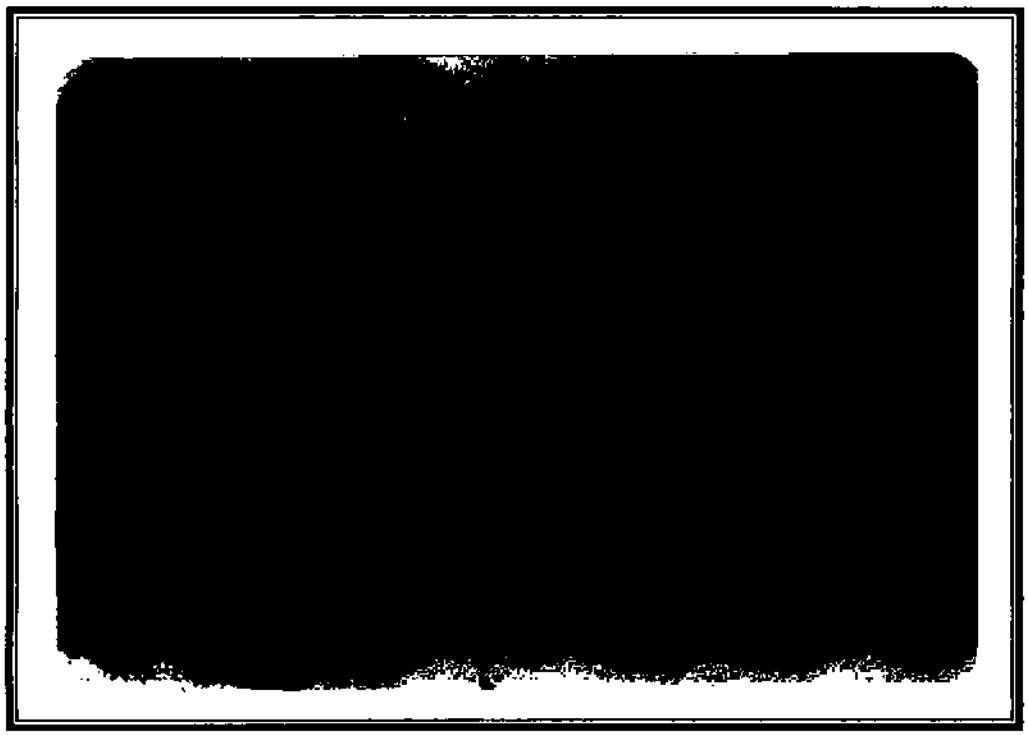


Plate-15: Gram stained cellular morphology of Staphylococcus isolated from Moa.

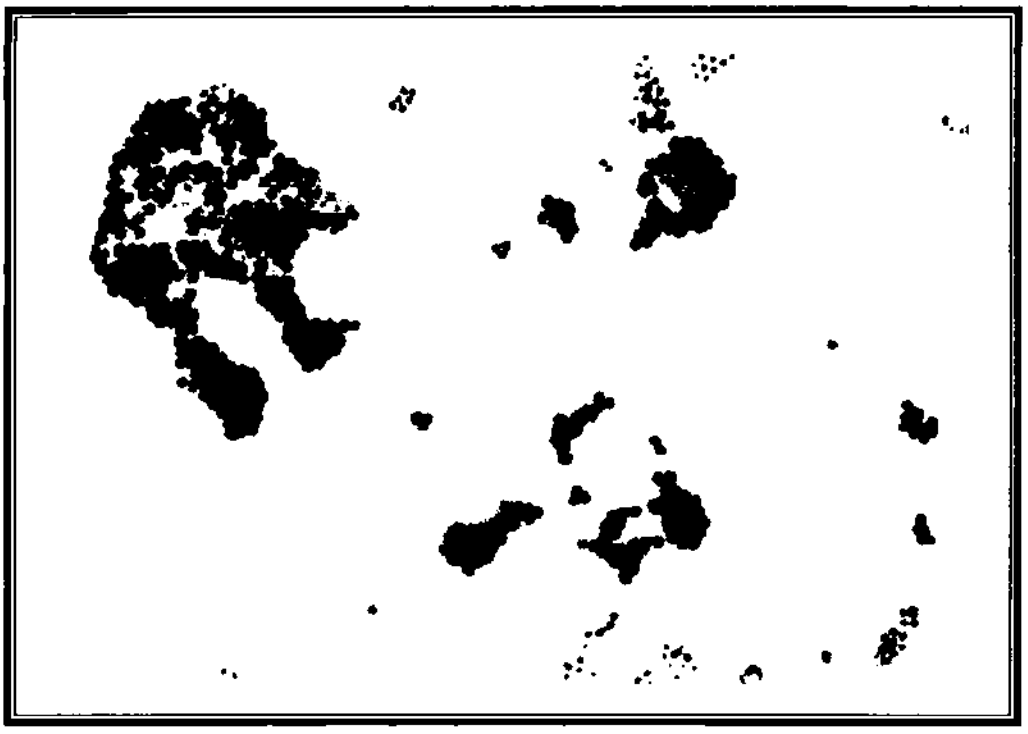


Plate-16: Gram stained cellular morphology of Staphylococcus isolated from Bakorkhani of other Street Foods.

Table-37 and Figure-12 are showing the comparison of different Bakery Products, Chocolates and other Street-foods. 40 % of Bakery products, 48 % of Other Street-foods and only 6.66 % of Chocolates were contaminated here.

Comparison of Microbial Contamination of different Bakery Products, Chocolates and other Street-foods are shown on the following Table-37:

Table-37

Comparison of Microbial Contamination of different Bakery Products, Chocolates and other Street-foods

Types of the samples	Total number of the samples	Number of contaminated foods	% of contaminated foods
Bakery Products	60	24	40 %
Chocolates	15	01	6.66 %
Other Street-foods	25	12	48 %

Fig-12: Comparison of Total Microbial Contamination of different Bakery Products, Chocolates and other Street-foods.

7.1.3 Total Coli form Count (TCC) of Bakery Products, Chocolates and other Street-foods:

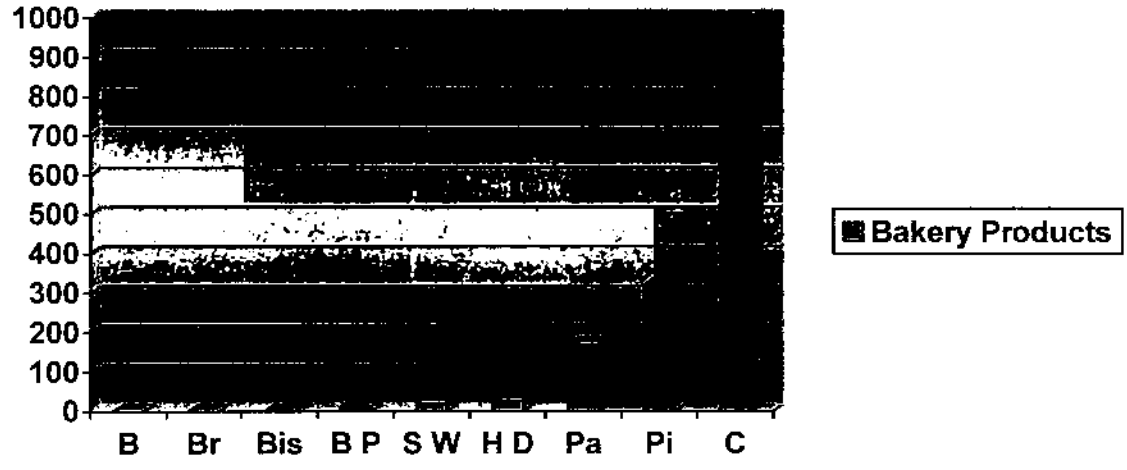
By using Mac-Conkey Agar media, Table-38 and Figure-13 is showing as above the total coli forms of Bakery Products were detected. About 40 % (24 samples) showed positive result for coli form. The mean total coli form was 2.0×10^2 cfu/gm. The highest coli form count 1.0×10^3 cfu/gm in Cake and the lowest count were only 1.3×10 cfu/gm in Beef Patties. About 60 % did not show growth of coli form. On the other hand Bun, Bread and Biscuits are completely safe from coli form contamination.

Total Coli form Count of different Bakery Products is shown on the following Table-38:

Table-38

Total Coli form Count of different Bakery Products

Name of the samples	Total number of sample analyzed	Number of sample showing no growth	Number of sample showing growth	Mean total Coli form/gm
Bun	06	06	0	0
Bread	06	06	0	0
Biscuit	14	14	0	0
Beef Patties	04	0	04	1.3×10
Sandwich	08	03	05	2.1×10
Hot Dog	04	01	03	2.7×10
Pastry	04	01	03	1.7×10^2
Pizza	08	05	03	3.2×10^2
Cake	06	0	06	1.0×10^3
Grand Total	**60**	**36 (60 %)**	**24 (40 %)**	**2.0×10^2**



(Name of the samples)

Fig-13: Total Coli form Count of different Bakery Products.

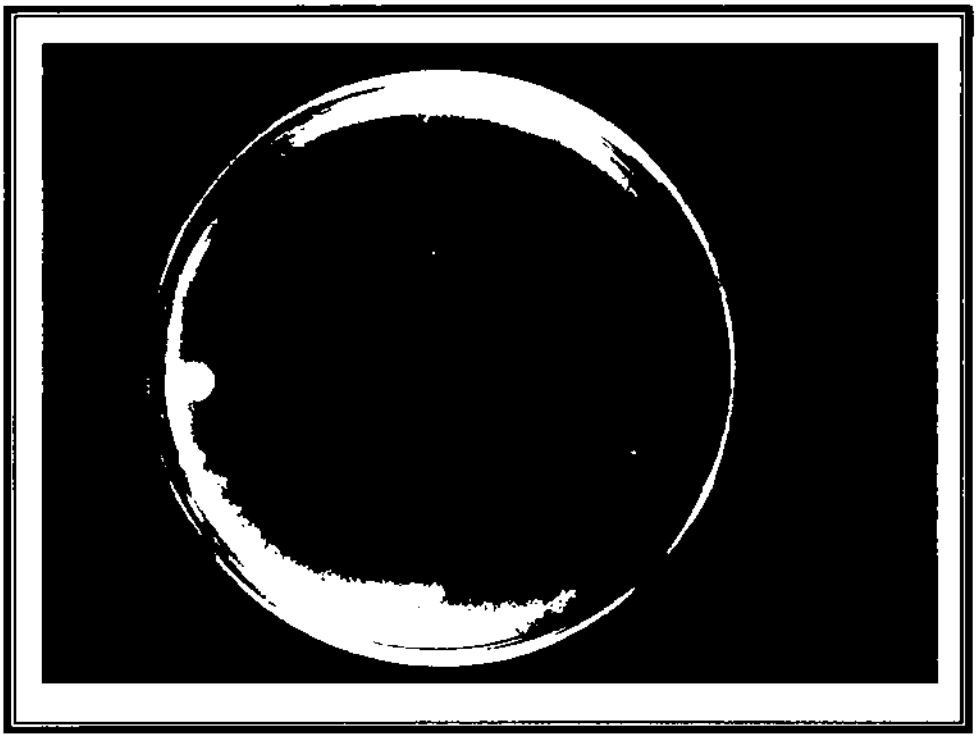


Plate-17: Growth of various coli forms bacterial colonies on Mac Conkey Agar plate from Cake (10^{-1}) of different Bakery Products.

By using Mac-Conkey Agar media, Table-39 and Figure-14 is showing as above the total coli forms of Chocolates were detected. About 46.66 % (07 samples) showed positive result for coli form. The mean total coli form was 1.48×10^2 cfu/gm. The highest coli form count 1.3×10^3 cfu/gm in Mini fruit jelly and the lowest count were only 1.2×10 cfu/gm in Chocolate Biscuits. About 53.34 % did not show growth of coli form. On the other hand Gems and Mimi Bar are completely free from coli form contamination.

Total Coli form Count of different Chocolates is shown on the following Table-39:

Table-39

Total Coli form Count of different Chocolates

Name of the samples	Total number of sample analyzed	Number of sample showing no growth	Number of sample showing growth	Mean total Coli form/gm
Gems	02	02	0	0
Mimi Bar	02	02	0	0
Chocolate Biscuits	03	02	01	1.2×10
Chocolate Pops	02	01	01	1.4×10^2
Candy	05	01	04	2.0×10^2
Mini fruit jelly	01	0	01	1.3×10^3
Grand Total	**15**	**08 (53.34 %)**	**07 (46.66 %)**	**1.48×10^2**

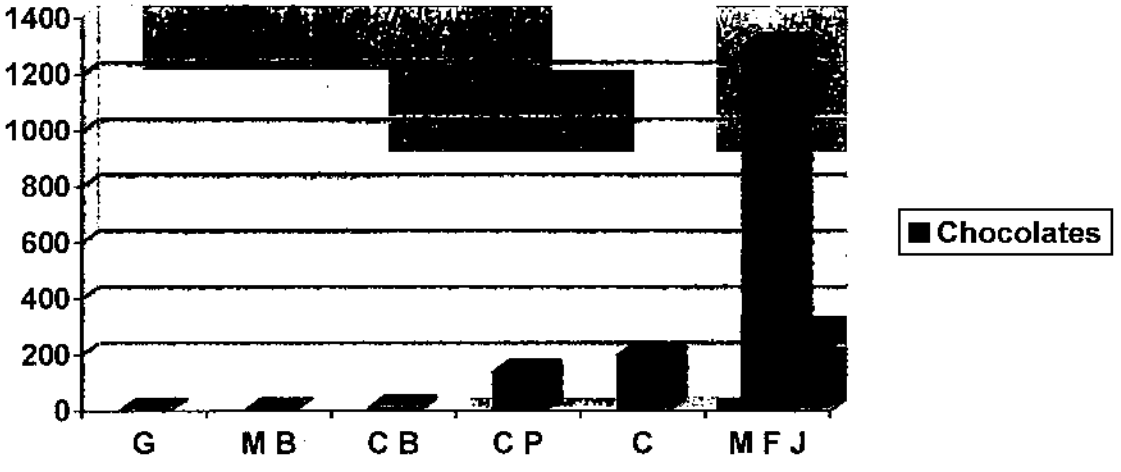


Fig-14: Total Coli form Count of different Chocolates.

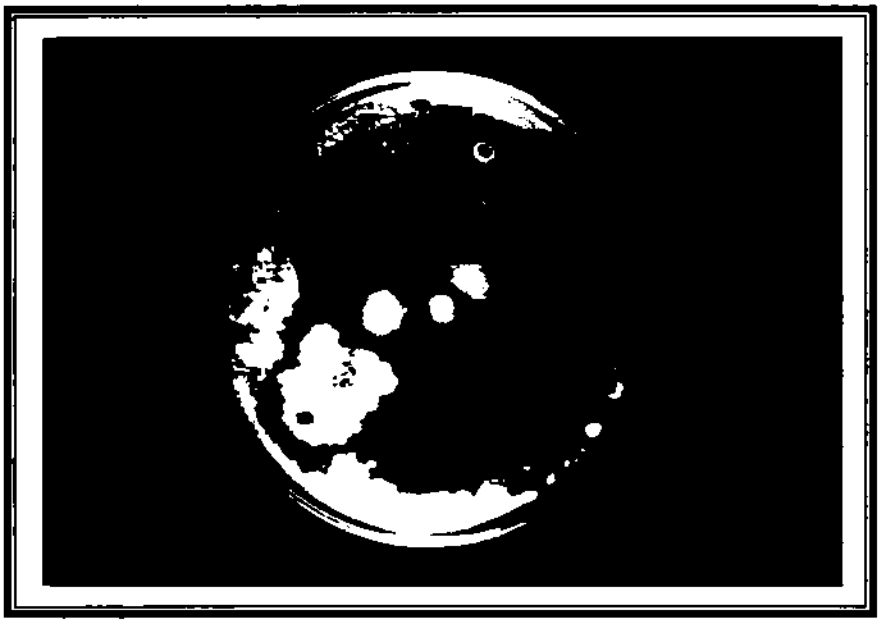


Plate-18: Growth of various coli forms bacterial colonies on Mac Conkey Agar plate from Mini Fruit Jelly (10^{-1}) of different Chocolates.

Plate-19: Growth of various coli forms bacterial colonies on Mac Conkey Agar plate from Chocolate Pops (10^{-1}) of different Chocolates.

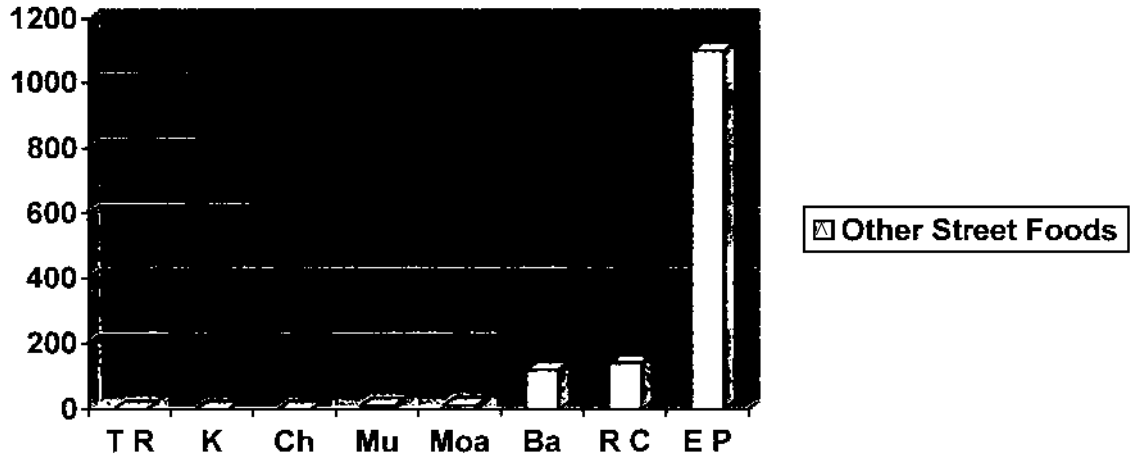
By using Mac-Conkey Agar media, Table-40 and Figure-15 is showing as above the total coli forms of other Street-foods were detected. About 60 % (15 samples) showed positive result for coli form. The mean total coli form was 1.16×10^2 cfu/gm. The highest coli form count 1.1×10^3 cfu/gm in Egg Pudding and the lowest count were 1.0×10 cfu/gm in Muree. About 40 % did not show growth of coli form. On the other hand Tondur Rutee, Khaza and Chanachur are absolutely free from coli form contamination.

Total Coli form Count of other Street-foods is shown on the following Table-40:

Table-40

Total Coli form Count of other Street-foods

Name of the samples	Total number of sample analyzed	Number of sample showing no growth	Number of sample showing growth	Mean total Coli form/gm
Tondur Rutee	02	02	0	0
Khaza	01	01	0	0
Chanachur	01	01	0	0
Muree	02	01	01	1.0×10
Moa	04	01	03	1.1×10
Bakorkhani	02	0	02	1.2×10^2
Rice-cake	09	03	06	1.4×10^2
Egg Pudding	04	01	03	1.1×10^3
Grand Total	**25**	**10 (40 %)**	**15 (60 %)**	**1.16×10^2**



(Name of the samples)

Fig-15: Total Coli form Count of other Street-foods.

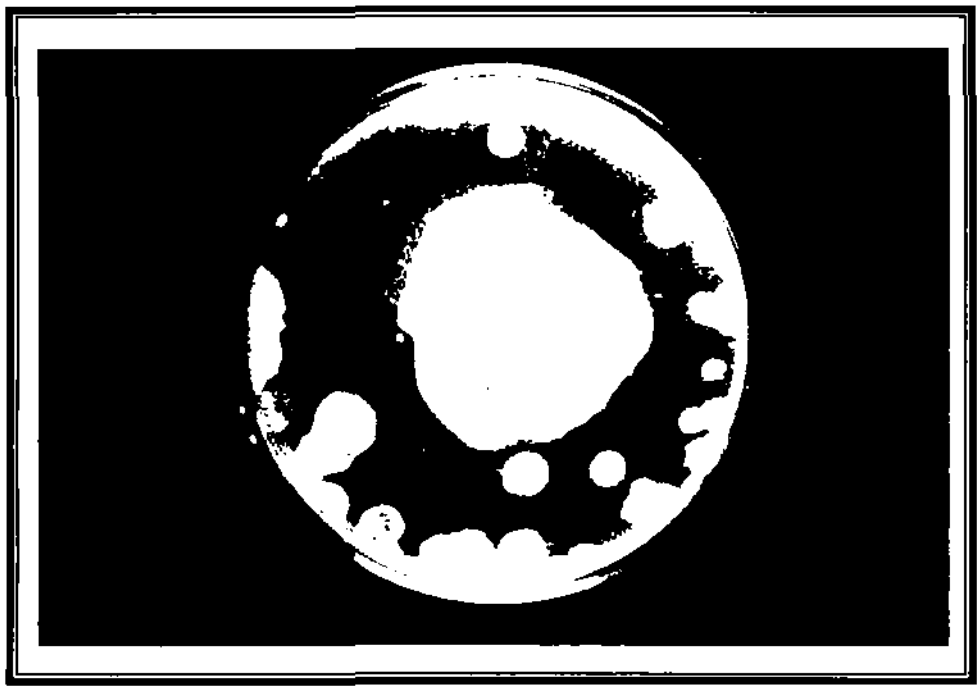


Plate-20: Growth of various coli forms bacterial colonies on Mac Conkey Agar plate from Egg Pudding (10^{-1}) of other street foods.

7.1.4 The Microbial quality with Comparison of Bakery Products, Chocolates and other Street-foods:

According to recommended criteria of ICMSF (2000) for foods, out of 60 Bakery Products, Table-41 and Figure-16 is showing about 26.66 % (16 samples) were *unacceptable. On the other hand, 73.33 % were acceptable food samples.

The Microbial quality of different Bakery Products on the basis of total coli form is shown on the following Table-41:

Table-41

Microbial quality (Acceptable/ Unacceptable) of different Bakery products on the basis of total coli form

Name of the samples	Total number of the sample analyzed	Number of *Acceptable food samples	Number of *Unacceptable food samples	% of *Unacceptable food samples
Biscuit	14	10	04	28.57 %
Bread	06	05	01	16.66 %
Bun	06	04	02	33.33 %
Hot Dog	04	03	01	25 %
Pizza	08	06	02	25 %
Sandwich	08	07	01	12.5 %
Cake	06	05	01	16.66 %
Pastry	04	02	02	50 %
Beef Patties	04	02	02	50 %
Grand Total	**60 (100 %)**	**44 (73.33 %)**	**16**	**26.66 %**

*According to recommended criteria of ICMSF (2000) for foods.

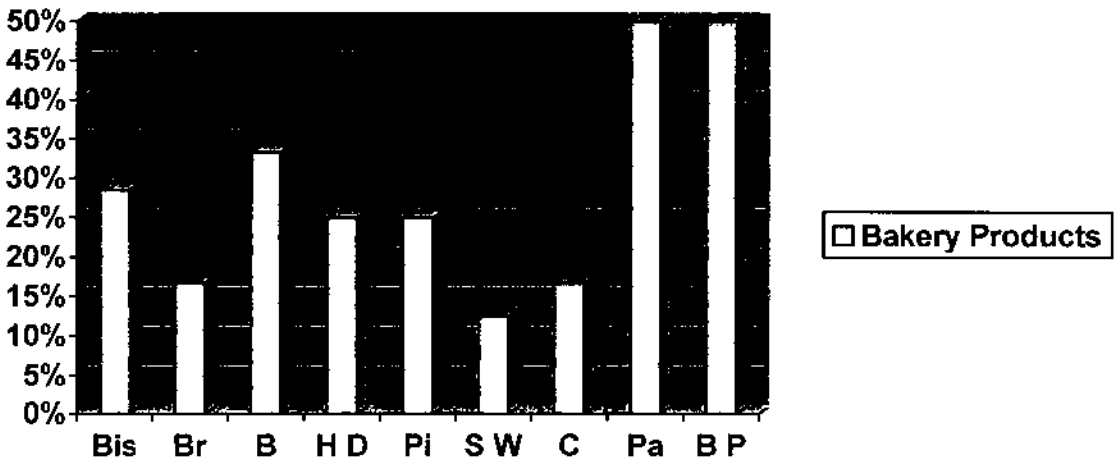


Fig-16: Percentage (%) of *Unacceptable Bakery Products.

According to recommend criteria of ICMSF (2000) for foods, out of 15 Chocolates, Table-42 and Figure-17 is showing about only 13.33 % (02 samples) including Chocolate Biscuits and Gems were unacceptable. Other food such as Candy, Mimi Bar, Chocolate Pops and Mini fruit jelly were acceptable.

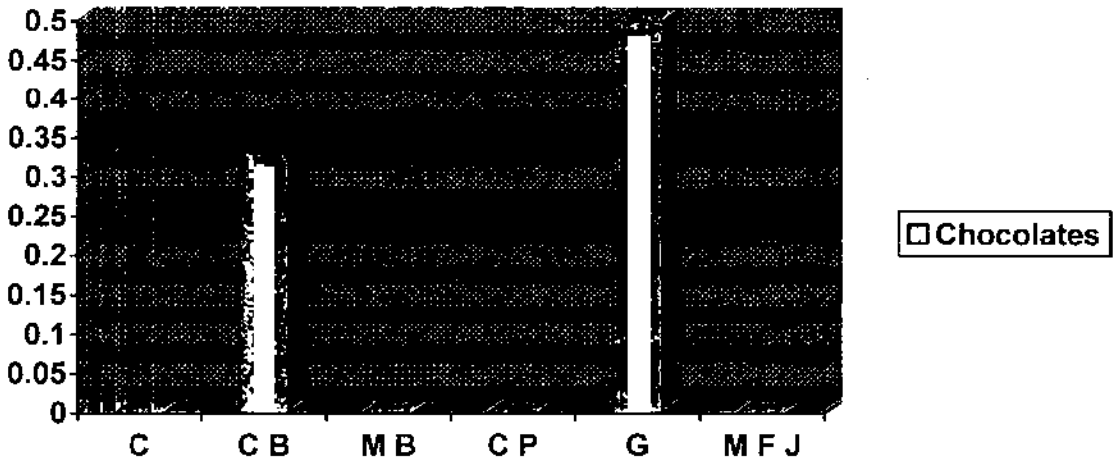
The Microbial quality of different Chocolates on the basis of total coli form is shown on the following Table-42:

Table-42

Microbial quality (Acceptable/ Unacceptable) of different Chocolates on the basis of total coli form

Name of the samples	Total number of the sample analyzed	Number of *Acceptable food samples	Number of *Unacceptable food samples	% of *Unacceptable food samples
Candy	05	05	0	0
Chocolate Biscuits	03	02	01	33.33 %
Mimi Bar	02	02	0	0
Chocolate Pops	02	02	0	0
Gems	02	01	01	50 %
Mini fruit jelly	01	01	0	0
Grand Total	**15 (100 %)**	**13 (86.66 %)**	**02**	**13.33 %**

*According to recommended criteria of ICMSF (2000) for foods.



(Name of the samples)

Fig-17: Percentage (%) of *Unacceptable Chocolates.

According to recommend criteria of ICMSF (2000) for foods, out of 25 other Street-foods, Table-43 and Figure-18 is showing about 44 % (11 samples) were unacceptable. On the other hand, 56 % food samples were acceptable.

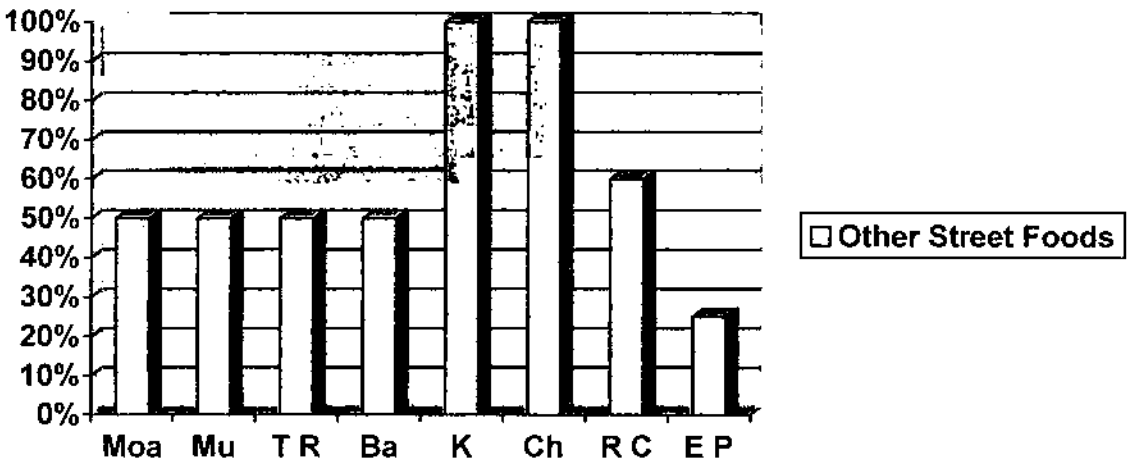
The Microbial quality of other Street-foods on the basis of total coli form is shown on the following Table-43:

Table-43

Microbial quality (Acceptable/ Unacceptable) of other Street-foods on the basis of total coli form

Name of the samples	Total number of the sample analyzed	Number of *Acceptable food samples	Number of *Unacceptable food samples	% of *Unacceptable food samples
Moa	04	02	02	50 %
Muree	02	01	01	50 %
Tondur Rutee	02	01	01	50 %
Bakorkhani	02	01	01	50 %
Khaza	01	0	01	100 %
Chanachur	01	0	01	100 %
Rice-cake	09	05	03	60 %
Egg Pudding	04	03	01	25 %
Grand Total	**25 (100 %)**	**14 (56 %)**	**11**	**44 %**

*According to recommended criteria of ICMSF (2000) for foods.



(Name of the samples)

Fig-18: Percentage (%) of *Unacceptable other Street-foods.

According to recommended criteria of ICMSF (2000) for foods, Table-44 and Figure-19 are showing the comparison of unacceptability of different Bakery products, Chocolates and other Street-foods. 26.66 % of Bakery products, only 13.33 % of Chocolates and 44 % of other Street-foods were unacceptable here.

Comparison of *Unacceptable quality of different Bakery Products, Chocolates and other Street-foods are shown on the following Table-44:

Table-44

Comparison of *Unacceptable quality of different Bakery Products, Chocolates and other Street-foods

Types of the samples	Total number of the samples	Number of contaminated foods	% of contaminated (*Unacceptable) foods
Bakery Products	60	16	26.66 %
Chocolates	15	02	13.33 %
Other Street-foods	25	11	44 %

*According to recommended criteria of ICMSF (2000) for foods.

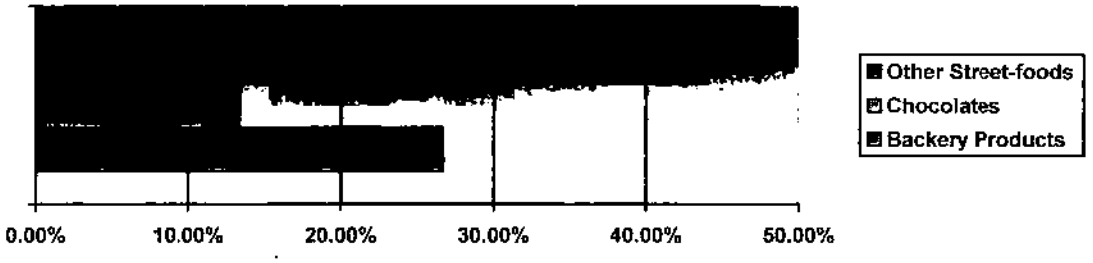


Fig-19: Comparison of *Unacceptable quality of different Bakery products, Chocolates and other Street-foods

7.1.5 Total Fungal Counts (TFC) with Comparison of different types of Bakery Products, Chocolates and other Street-foods:

Identification of Fungi:

The fungi isolated from the PDA and or, Sabouraurd Dextrose Agar Media plate produced from 7 possess:

a) Vegetative phage has definite cell wall.
b) Body true mycelium, profusely branched hyphae possess nucleus,

---------- Fungi.

c) Mycelium septet, asexual spores are not formed within sporangia, rather conidiophores carry those,

---------- Higher Fungi.

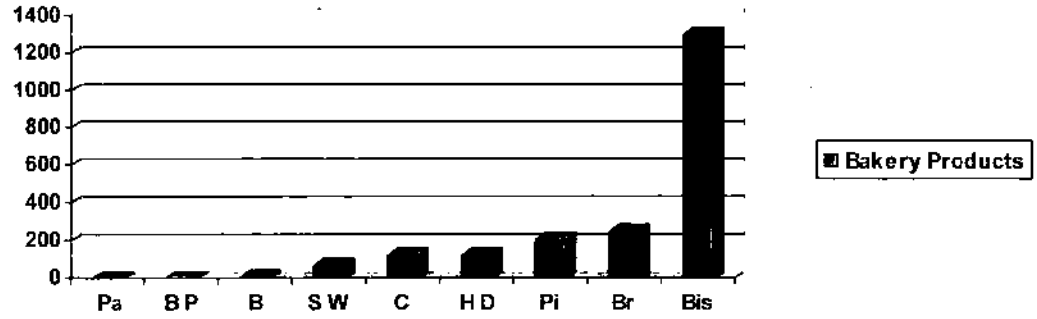
By using 'Sabouraurd Dextrose Agar Media', the total fungal counts of Bakery Products were detected. About 20 % (12 samples) showed positive result for fungi on Table-45 and Figure-20 is showing as above here. The mean total fungal count was 1.14×10^2 cfu/gm. The highest fungal count 1.3×10^3 cfu/gm in Biscuits and the lowest count was only 0.1×10^2 cfu/gm in Bun. About 80 % did not show the growth of fungi. All Pastry and Beef Patties were fully free of fungi.

Total Fungal Counts of different Bakery Products is shown on the following Table-45:

Table-45

Total Fungal Counts of different Bakery Products

Name of the samples	Total number of sample analyzed	Number of sample showing no growth	Number of sample showing growth	Mean total Cfu/gm
Pastry	04	04	0	0
Beef Patties	04	04	0	0
Bun	06	05	01	0.1×10^2
Sandwich	08	06	02	0.7×10^3
Cake	06	05	01	0.12×10^2
Hot Dog	04	03	01	1.2×10^2
Pizza	08	05	03	2.1×10^2
Bread	06	05	01	2.5×10^2
Biscuit	14	11	03	1.3×10^3
Grand Total	**60 (100 %)**	**48 (80 %)**	**12 (20 %)**	**1.14×10^2**



(Name of the samples)

Fig-20: Total Fungal Counts of different Bakery Products.

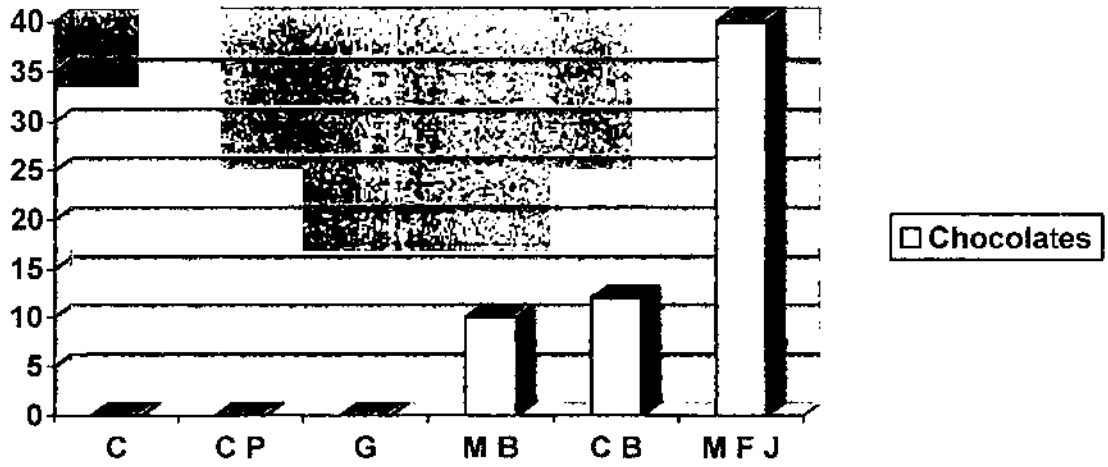
By using 'Sabouraurd Dextrose Agar Media', the total fungal counts of different Chocolates were detected. About only 13.33 % (02 samples) showed positive result for fungi on Table-46 and Figure-21 is showing as above here. The mean total fungal count was 0.56×10^2 cfu/gm. The highest fungal count 1.2 × 10 cfu/gm in Chocolate Biscuits and the lowest count was only 0.1 × 10 cfu/gm in Mimi Bar. About 86.66 % did not show the growth of fungi. All Candy, Chocolate Pops and Gems were fully free of fungi.

Total Fungal Counts of different Chocolates is shown on the following Table-46:

Table-46

Total Fungal Counts of different Chocolates

Name of the samples	Total number of sample analyzed	Number of sample showing no growth	Number of sample showing growth	Mean total Cfu/gm
Candy	05	05	0	0
Chocolate Pops	02	02	0	0
Gems	02	02	0	0
Mimi Bar	02	01	01	0.1 × 10
Chocolate Biscuits	03	02	01	1.2 × 10
Mini fruit jelly	01	01	0	0.4×10^2
Grand Total	**15 (100 %)**	**13 (86.66 %)**	**02 (13.33 %)**	**0.56×10^2**



(Name of the samples)

Fig-21: Total Fungal Counts of different Chocolates.

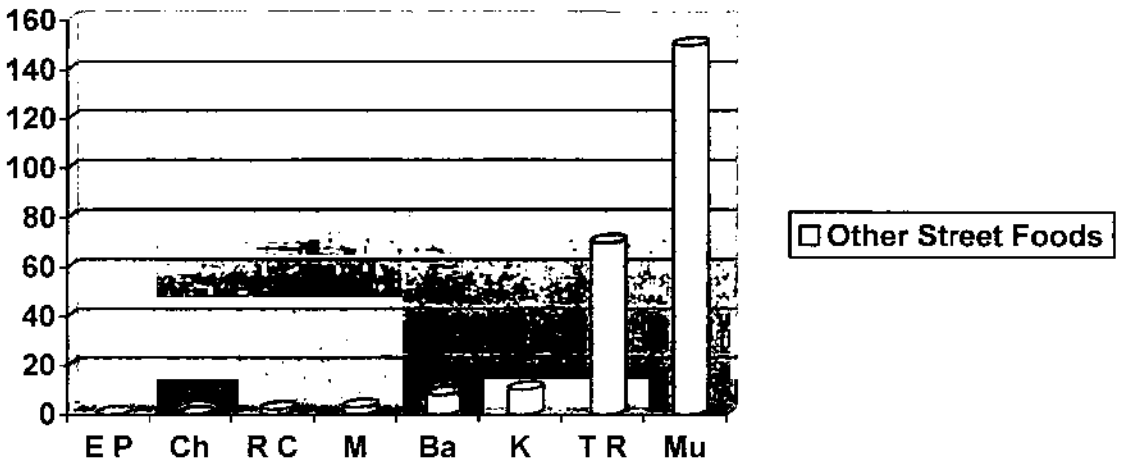
By using 'Sabouraurd Dextrose Agar Media', the total fungal counts of other Street-foods were detected. About 40 % (10 samples) showed positive result for fungi on Table-47 and Figure-22 is showing as above here. The mean total fungal count was 0.6×10^2 cfu/gm. The highest fungal count 1.5×10^2 cfu/gm in Muree and the lowest count was only 0.2×10 cfu/gm in Rice-cake. About 60 % did not show the growth of fungi. All Egg Pudding and Chanachur were fully free of fungi.

Total Fungal Counts of other Street-foods is shown on the following Table-47:

Table-47

Total Fungal Counts of other Street-foods

Name of the samples	Total number of sample analyzed	Number of sample showing no growth	Number of sample showing growth	Mean total Cfu/gm
Egg Pudding	04	04	0	0
Chanachur	01	01	0	0
Rice-cake	09	04	05	0.2×10
Moa	04	03	01	0.3×10
Bakorkhani	02	01	01	0.8×10
Khaza	01	0	01	0.1×10^2
Tondur Rutee	02	01	01	0.7×10^2
Muree	02	01	01	1.5×10^2
Grand Total	**25 (100 %)**	**15 (60 %)**	**10 (40 %)**	**0.6×10^2**



(Name of the samples)

Fig-22: Total Fungal Counts of other Street-foods.

Table-48 and Figure-23 are showing the comparison of fungal contamination of different Bakery products, Chocolates and other Street-foods. 20 % of Bakery products, only 13.33 % of Chocolates and 40 % of other Street-foods were contaminated here.

Comparison of Fungal count of different Bakery Products, Chocolates and other Street-foods are shown on the following Table-48:

Table-48

Comparison of Fungal count of different Bakery Products, Chocolates and other Street-foods

Types of food samples	Total number of sample analyzed	Number of contaminated foods	% of contaminated foods
Bakery Products	60	12	20 %
Chocolates	15	02	13.33 %
Other Street-foods	25	10	40 %

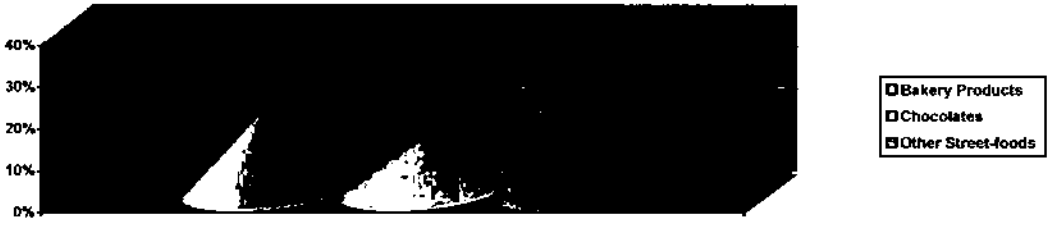


Fig-23: Comparison of Fungal counts of different Bakery Products, Chocolates and other Street-foods.

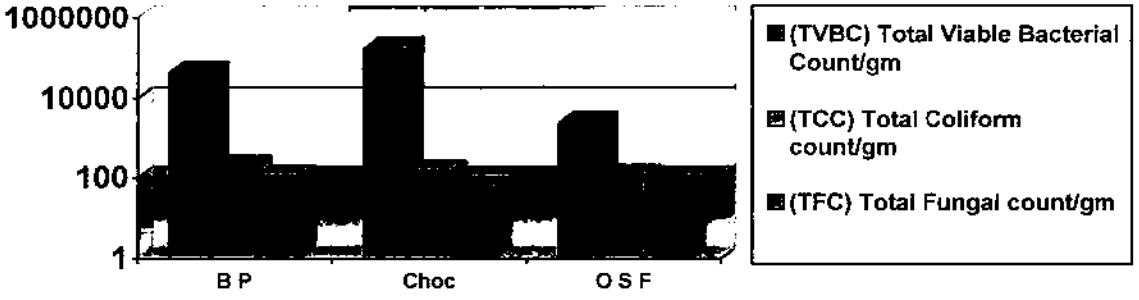
Table-49 and Figure-24 are showing the comparison of total mean Microbial load of different Bakery products, Chocolates and other Street-foods.

Comparison of Microbial load of different Bakery Products, Chocolates and other Street-foods are shown on the following Table-49:

Table-49

Comparison of mean Microbial load of different Bakery Products, Chocolates and other Street-foods

Microbial count of samples	Bakery Products	Chocolates	Other Street-foods
(TVBC) Total Viable Bacterial count/gm	4.3×10^4	1.7×10^5	2.48×10^3
(TCC) Total Coli form count/gm	2.0×10^2	1.48×10^2	1.16×10^2
(TFC) Total Fungal count/gm	1.14×10^2	0.56×10^2	0.6×10^2



(Name of the samples)

Fig-24: Comparison of Microbial loads of different Bakery Products, Chocolates and other Street-foods.

7.2. Isolation of Bacteria:

In the study total 100 samples were examined from varieties of street foods including Bakery Products and Chocolates. All samples were chosen for bacteriological identification and total 24 colonies were isolated and identified up to genus level.

7.3. Characterization:

For the characterization of the isolates the cultural, morphological, biochemical and physiological characters were considered.

7.3.1. Cultural Characterization:

Table-50 shows the cultural characteristics of the isolated strains. From the table we found 15 colonies were circulating and rests of 09 colonies were irregular, 20 colonies had entire margin and 04 had irregular or wavy margin. As of them 13 were white colored, 02 were yellow colored, 07 were cream colored and 02 were orange color.

Cultural characteristics of the strains isolated from different Bakery Products, Chocolates and other Street-foods are shown on the following Table-50:

Table-50

Cultural characteristics of the strains isolated from different Bakery Products, Chocolates and other Street-foods

No. of samples	[Sample] Sample code	Color	Surface	Shape	Edge	Elevation
	[Biscuit]					
01	$BT\text{-}1_1$	White	Rough	Circular	Even	Convex
02	$BS\text{-}2_3$	White	Rough	Irregular	Even	Convex
03	BDC_7	White	Rough	Irregular	Wavy	Raised
04	BB_2	Orange	Smooth	Circular	Even	Convex
	[Bread]					
05	$Br\text{-}4_3$	Cream	Smooth	Irregular	Even	Convex
06	BrO_5	White	Rough	Circular	Even	Convex
	[Bun]					
07	$Bu\text{-}4_2$	White	Smooth	Circular	Even	Convex
	[Hot dog]					
08	$HD\text{-}4_3$	White	Smooth	Circular	Even	Convex
	[Pizza]					
09	$PB\text{-}4_5$	White	Smooth	Circular	Even	Convex
	[Sandwich]					
10	$SC\text{-}4_6$	Cream	Smooth	Circular	Even	Convex
	[Cake]					
11	$C\text{-}4_4$	White	Rough	Irregular	Wavy	Raised
12	CO_6	Orange	Smooth	Circular	Even	Convex
	[Pastry]					
13	$P\text{-}4_4$	Cream	Smooth	Circular	Even	Convex
	[Beef Patties]					
14	$BP\text{-}4_3$	White	Smooth	Circular	Wavy	Raised
	[Chocolates]					
15	CF_2	Cream	Smooth	Irregular	Even	Convex
16	CW_6	White	Smooth	Circular	Even	Convex
	[Moa]					
17	CMO_3	Yellow	Smooth	Circular	Even	Convex
18	CMP_3	Cream	Smooth	Irregular	Even	Raised
19	MMO_1	White	Rough	Irregular	Wavy	Raised
20	MMP_5	Cream	Smooth	Circular	Even	Convex
	[Muree]					
21	MO_1	White	Smooth	Circular	Even	Convex
	[Tondur Rutee]					
22	$TR\text{-}3_4$	Cream	Smooth	Circular	Even	Convex
	[Khaza]					
23	$K\text{-}4_4$	White	Rough	Irregular	Even	Raised
	[Bakorkhani]					
24	$B\text{-}4_2$	Yellow	Smooth	Irregular	Even	Convex

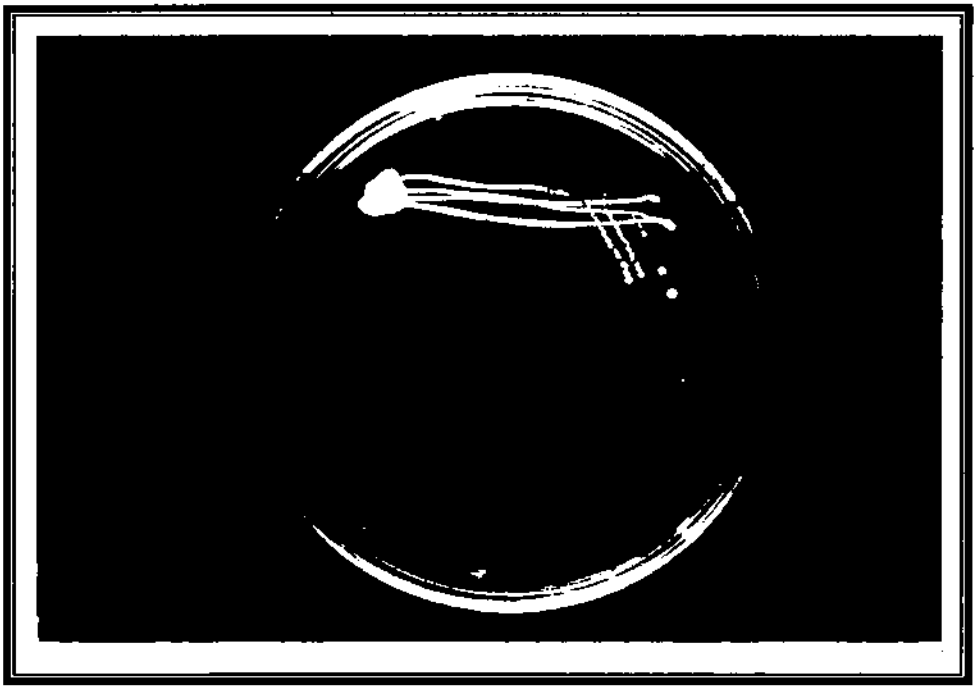


Plate-21: Pure culture of different bacterial strain.

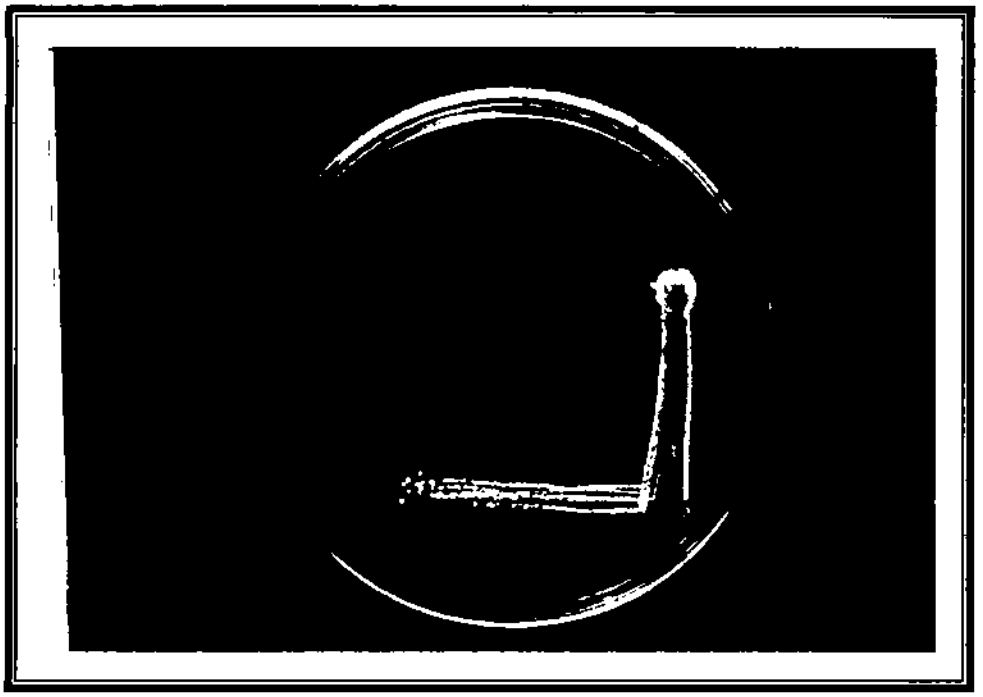


Plate-22: Pure culture of different bacterial strain.

7.3.2. Morphological Characteristics:

Table-51 shows the result of microscopic examination of gram stained slides of all the stains. From which we found that all strains were gram negative; 18 were coccus type and 06 were rod type. According to the gram staining them all was gram positive.

Morphological characteristics of the strains isolated from different Bakery products, Chocolates and other Street-foods are shown on the following Table-51:

Table-51

Morphological characteristics of the strains

No. of samples	[Sample] Sample code	Gram reaction	Shape	Cell arrangement	Presence of spores
	[Biscuit]				
01	$BT\text{-}1_1$	Positive	Rod	Pair	Positive
02	$BS\text{-}2_3$	Positive	Coccus	Cluster	Negative
03	BDC_7	Positive	Rod	Pair	Positive
04	BB_2	Positive	Rod	Pair	Positive
	[Bread]				
05	$Br\text{-}4_3$	Positive	Coccus Coccus	Cluster	Negative
06	BrO_5	Positive		Cluster	Negative
	[Bun]		Coccus		
07	$Bu\text{-}4_2$	Positive		Tetrads	Negative
	[Hot dog]		Coccus		
08	$HD\text{-}4_3$	Positive		Cluster	Negative
	[Pizza]		Coccus		
09	$PB\text{-}4_5$	Positive		Cluster	Negative
	[Sandwich]		Rod		
10	$SC\text{-}4_6$	Positive		Pair	Positive
	[Cake]		Coccus Coccus		
11	$C\text{-}4_4$	Positive		Small tetrads	Negative
12	CO_6	Positive		Cluster	Negative
	[Pastry]		Coccus		
13	$P\text{-}4_4$	Positive		Single, short chain	Negative
	[Beef Patties]		Coccus		
14	$BP\text{-}4_3$	Positive		Cluster	Negative
	[Chocolates]		Coccus Coccus		
15	CF_2	Positive		Tetrads	Negative
16	CW_6	Positive	Coccus Coccus Coccus Coccus	Small tetrads	Negative
	[Moa]				
17	CMO_3	Positive		Cluster	Negative
18	CMP_3	Positive	Rod	Cluster	Negative
19	MMO_1	Positive		Cluster	Negative
20	MMP_5	Positive		Tetrads	Negative
	[Muree]		Coccus		
21	MO_1	Positive		Pair	Positive
	[Tondur Rutee]		Coccus		
22	$TR\text{-}3_4$	Positive		Tetrads	Negative
	[Khaza]		Rod		
23	$K\text{-}4_4$	Positive		Single, short chain	Negative
	[Bakorkhani]				
24	$B\text{-}4_2$	Positive		Pair	Positive

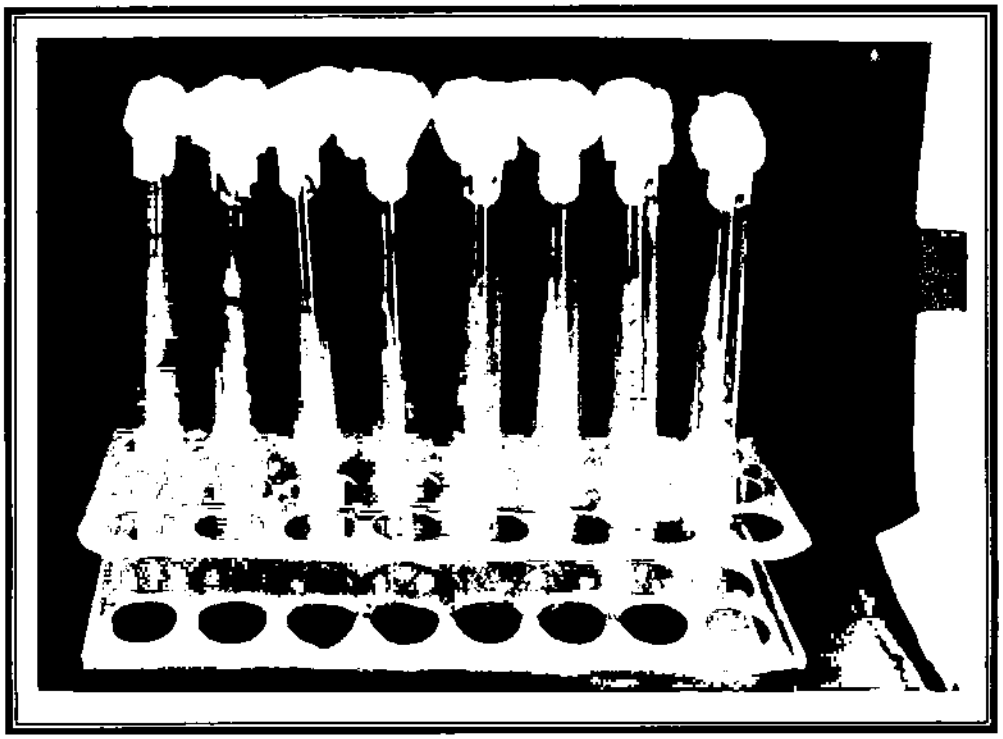


Plate-23: Preservation of bacterial cultures in agar's slants.

7.3.3. Biochemical and Physiological Characteristics of Strains:

Table-52 and Figure-25 are showing the biochemical and physiological characteristics of the isolates. Here only 06 are Bacillus and other 18 was Staphylo coccus. This table shows us all the strains were facultitative anaerobe. From the table we found that out of 24 strains 22 were unable to hydrolysis starch and only 02 were able to hydrolysis starch. According to glucose fermentation test only 02 stains were oxidative and rests of 22 were fermented. In Methyl Red Test only 04 were negative and others were positive. The result of the V P Test showed that only 02 were negative and rests of 22 were positive. In case of Citrate Utilization Test only 06 were positive and 18 were showing positive results. The Glucose Utilization Test showed that acid was produced by all strains except 01 strain and on other hand, no strain produced gas from glucose.

In case of gelatin and casein hydrolysis 18 strains were positive and 06 were negative. From In dole Test we found that 06 were positive and 18 strains were negative. Out of 24 strains 11 strains produced Ammonia from Peptone and 13 did not produce. And Hydrogen Sulphide was produced by no strain.

Out of 24 strains only 01 was catalase negative and all strains were oxidizing negative. According to the result of Urease Test 20 were positive and only 04 were negative.

According to the result of Motility Test 18 strains were non-motile and only 06 strains were motile.

Biochemical and Physiological characteristics of the strains are shown on the following Table-52:

Table-52

Biochemical and physiological characteristics of the strains

No	Sample Code	An Gr	Test for Metabolism of Carbohydrate							Test for Metabolism of Protein					Test for Enzymes				Mis Test	Identity
			St Hy	Glu Fer	MR Test	VP Test	Cit U	Glu Uti		Ge Hy	Ca Hy	In Test	Amm from Pep	H_2S	Catal ase	Oxid ase	Ur Hy	Nit Red	Mot Test	
								Acid	Gas											
1	$BT\text{-}1_1$	+	-	Ferment	-	+	+	-	-	+	+	+	+	-	+	-	+	+	+	Bacillus
2	$BS\text{-}2_3$	+	-	Ferment	+	+	-	+	-	-	-	-	-	-	+	-	+	+	-	Staphylo coccus
3	BDC_7	+	-	Ferment	+	+	+	+	-	+	+	+	+	-	+	-	+	+	+	Bacillus
4	BB_2	+	-	Ferment	+	+	+	+	-	+	+	+	+	-	+	-	+	+	+	Bacillus
5	$Br\text{-}4_3$	+	-	Ferment	+	+	-	+	-	+	+	-	-	-	+	-	+	+	-	Staphylo coccus
6	BrO_5	+	-	Ferment	+	+	-	+	-	+	+	-	+	-	+	-	+	+	-	Staphylo coccus
7	$Bu\text{-}4_2$	+	-	Ferment	+	+	-	+	-	+	+	-	-	-	+	-	+	+	-	Staphylo coccus
8	$HD\text{-}4_3$	+	-	Ferment	-	+	-	+	-	-	-	-	+	-	+	-	+	+	-	Staphylo coccus
9	$PB\text{-}4_5$	+	-	Ferment	+	+	-	+	-	-	-	-	-	-	+	-	+	+	-	Staphylo coccus
10	$SC\text{-}4_6$	+	+	Ferment	+	-	+	+	-	+	+	+	+	-	+	-	+	+	+	Bacillus
11	$C\text{-}4_4$	+	-	Oxidative	+	+	-	+	-	+	+	-	-	-	+	-	-	+	-	Staphylo coccus
12	CO_6	+	-	Ferment	+	+	-	+	-	-	-	-	-	-	+	-	+	-	-	Staphylo coccus
13	$P\text{-}4_4$	+	-	Ferment	+	+	-	+	-	-	-	-	-	-	-	-	+	+	-	Staphylo coccus
14	$BP\text{-}4_3$	+	-	Ferment	+	+	-	+	-	+	+	-	-	-	+	-	+	+	-	Staphylo coccus
15	CF_2	+	-	Ferment	-	+	-	+	-	+	+	-	-	-	+	-	-	-	-	Staphylo coccus
16	CW_6	+	-	Ferment	+	+	-	+	-	+	+	-	+	-	+	-	+	+	-	Staphylo coccus
17	CMO_3	+	-	Ferment	+	+	-	+	-	+	+	-	+	-	+	-	+	+	-	Staphylo coccus
18	CMP_3	+	-	Ferment	+	+	-	+	-	+	+	-	-	-	+	-	+	+	-	Staphylo coccus
19	MMO_1	+	-	Ferment	-	+	-	+	-	+	+	-	-	-	+	-	+	-	-	Staphylo coccus
20	MMP_5	+	-	Oxidative	+	+	-	+	-	+	+	-	-	-	+	-	-	+	-	Staphylo coccus
21	MO_1	+	+	Ferment	+	-	+	+	-	+	+	+	+	-	+	-	+	+	+	Bacillus
22	$TR\text{-}3_4$	+	-	Ferment	+	+	-	+	-	+	+	-	-	-	+	-	-	+	-	Staphylo coccus
23	$K\text{-}4_4$	+	-	Ferment	+	+	-	+	-	-	-	-	+	-	+	-	+	+	-	Staphylo coccus
24	$B\text{-}4_2$	+	-	Ferment	+	+	+	+	-	+	+	+	+	-	+	-	+	+	+	Bacillus

An Gr = Anaerobic Growth
St Hy = Starch Hydrolysis
Glu Fer = Glucose Fermentation
MR Test = Methyl Red Test
VP Test = Voges-Proskauer Test
Cit U = Citrate Utilization
Glu Uti = Glucose Utilization
Ge Hy = Gelatin Hydrolysis
Ca Hy = Casein Hydrolysis
In Test = Indole Test
Amm from Pep = Ammonia from Peptone
Ur Hy = Urea Hydrolysis
Nit Red = Nitrate Red
Mis Test = Miscellaneous Test
Mot Test = Motility Test

Fig-25: Biochemical identity of total samples.

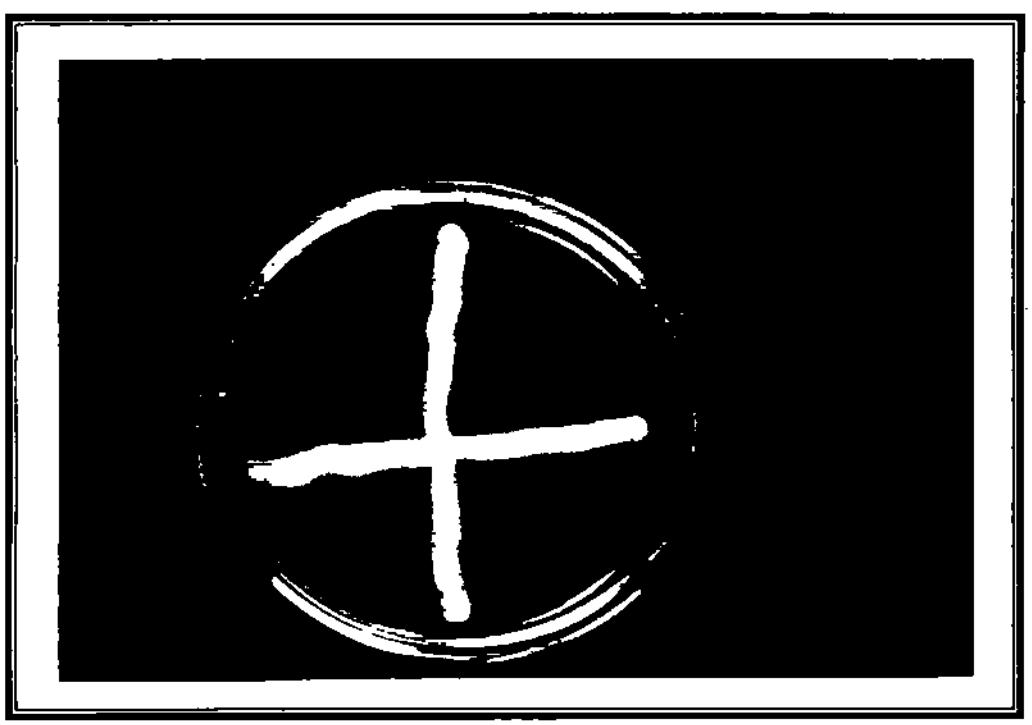


Plate-24: Starch Hydrolysis Test is showing the negative result by Staphylococcus (BB_2).

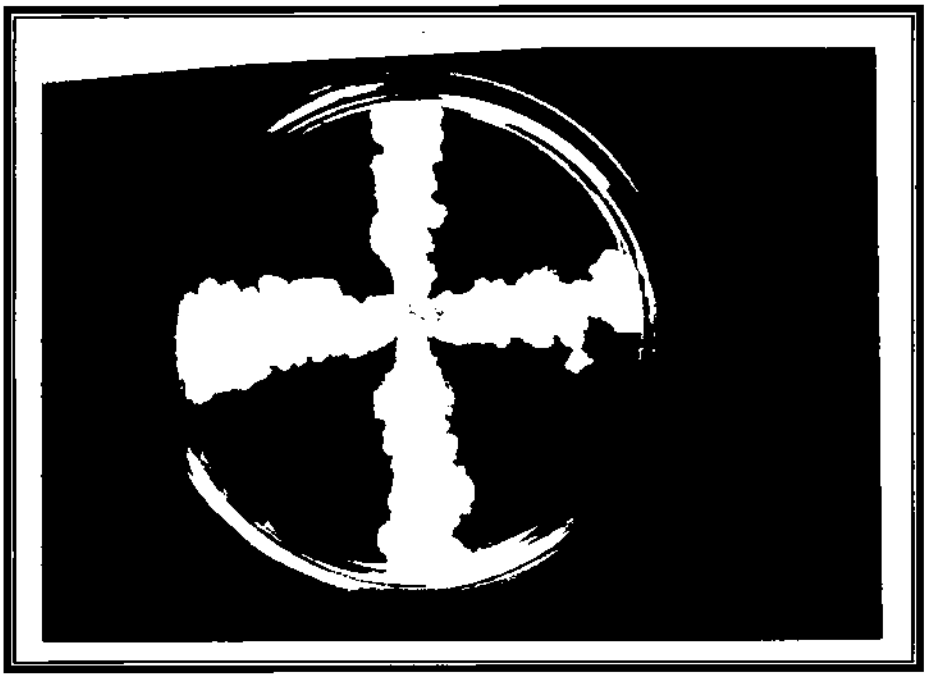


Plate-25: Starch hydrolysis test is showing the positive result by Bacillus ($SC\text{-}4_6$).

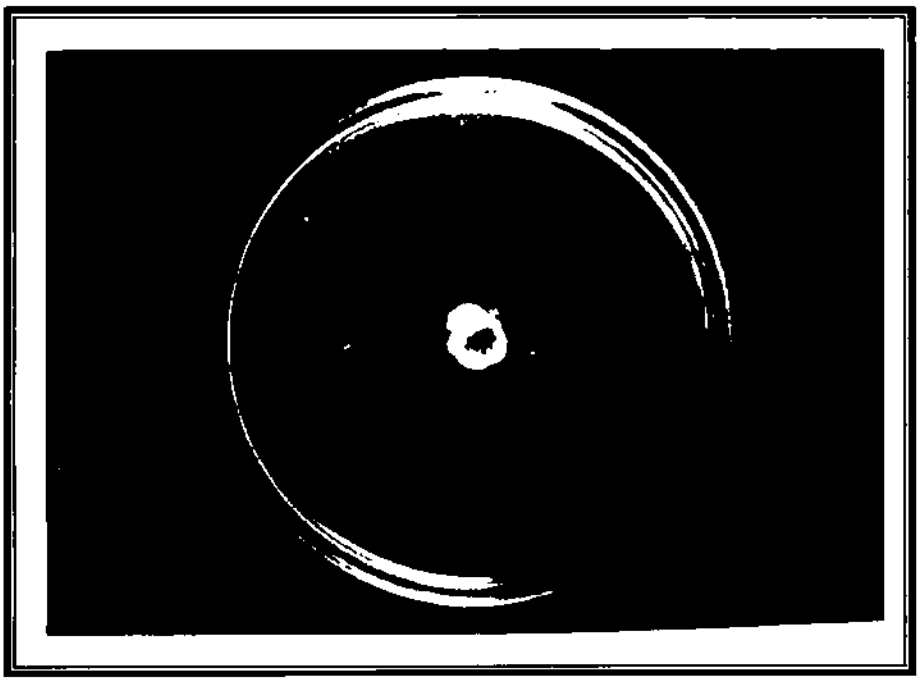


Plate-26: Negative Hydrolysis of Gelatin by Staphylococcus ($BS\text{-}2_3$).

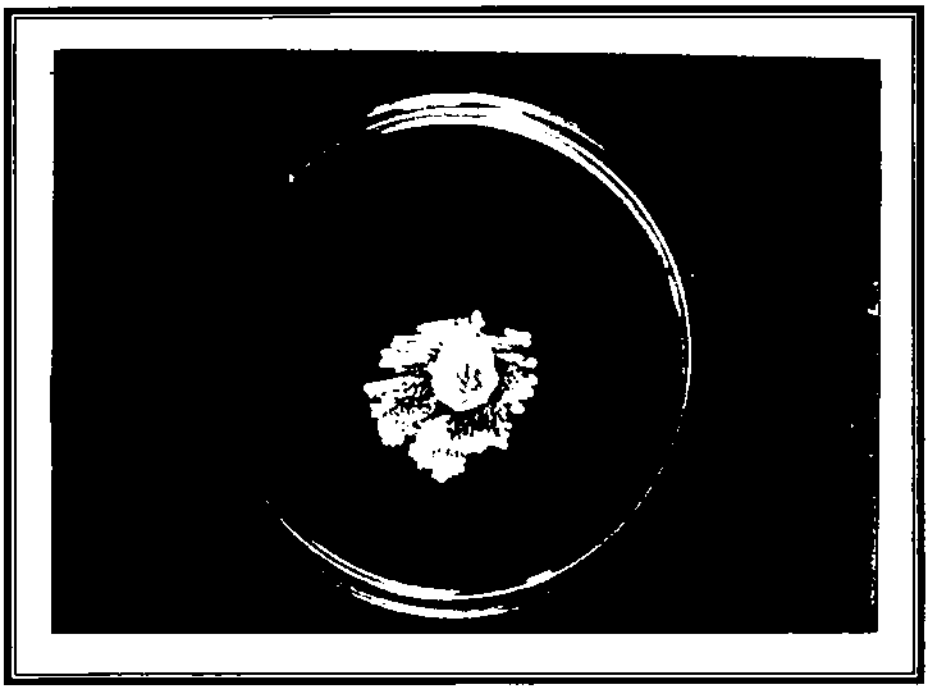


Plate-27: Hydrolysis of Gelatin by Bacillus (BDC_7).

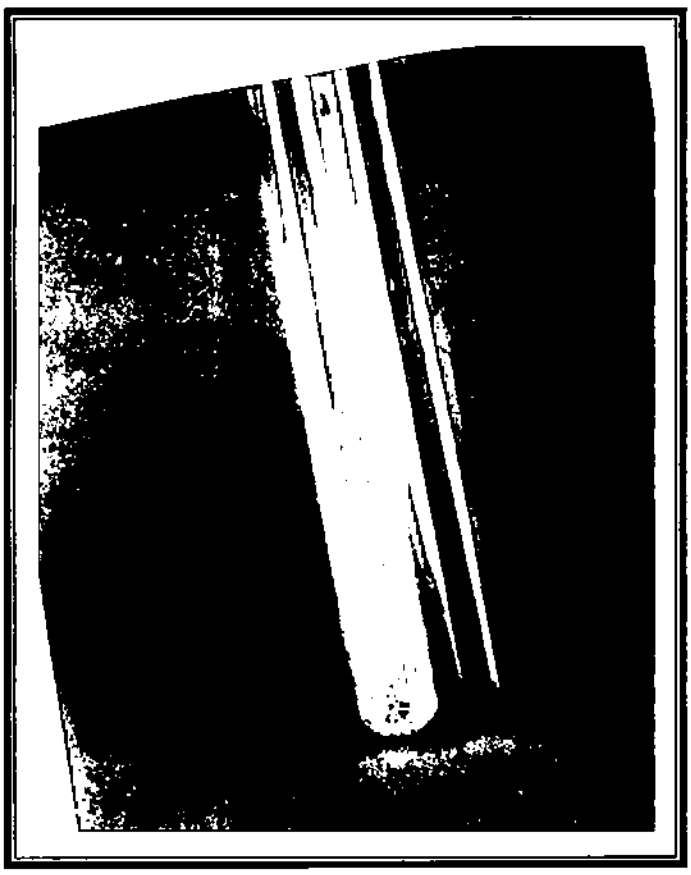


Plate-28: Glucose Utilization Test is showing the positive result by Bacillus (left: $B\text{-}4_2$) and the negative result by Bacillus (right: $BT\text{-}1_1$).

Plate-29: Methyl Red Test is showing the positive result by Bacillus (left: SC-4_6) and the negative result by Staphylococcus (right: CF_2).

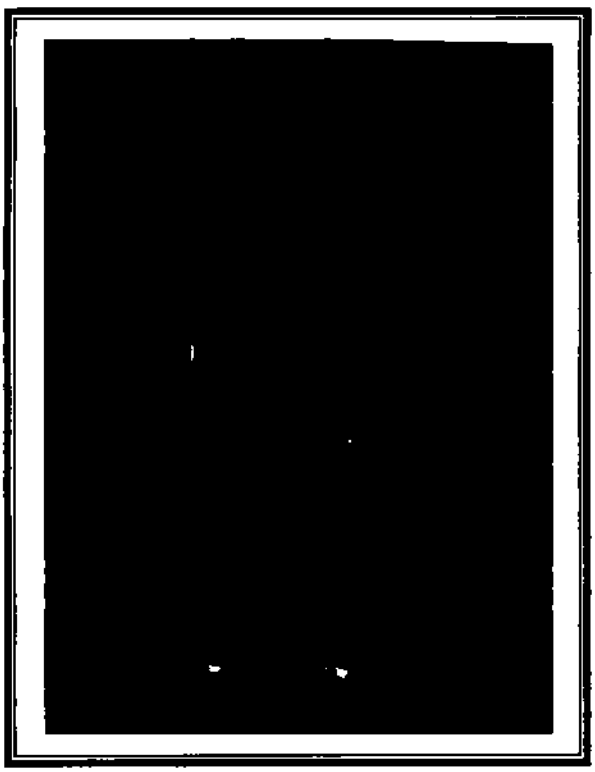


Plate-30: Nitrate Reduction Test is showing the negative result by Staphylococcus (left: MMO_1) and the positive result by Bacillus (left: B-4_2).

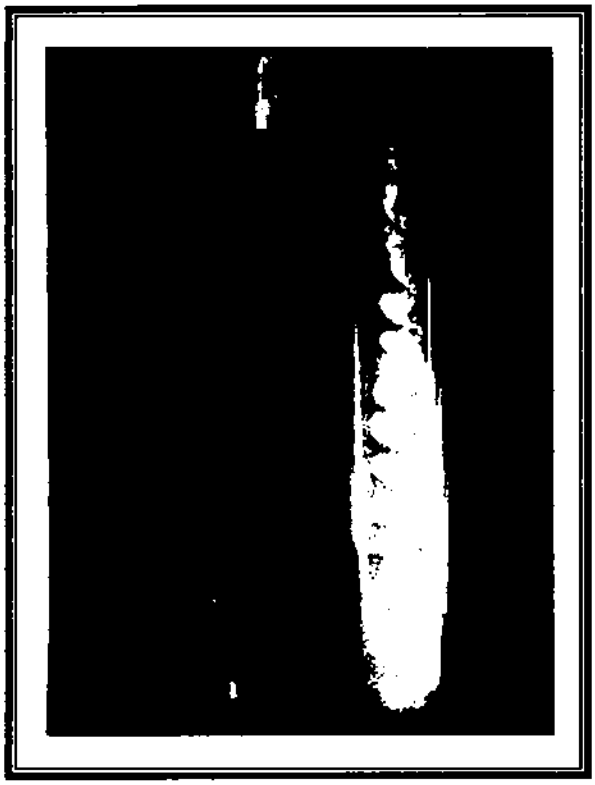


Plate-31: Urea Hydrolysis Test is showing the positive result by Bacillus (left: BT-1_1) and the negative result by Staphylococcus (right: C-4_4).

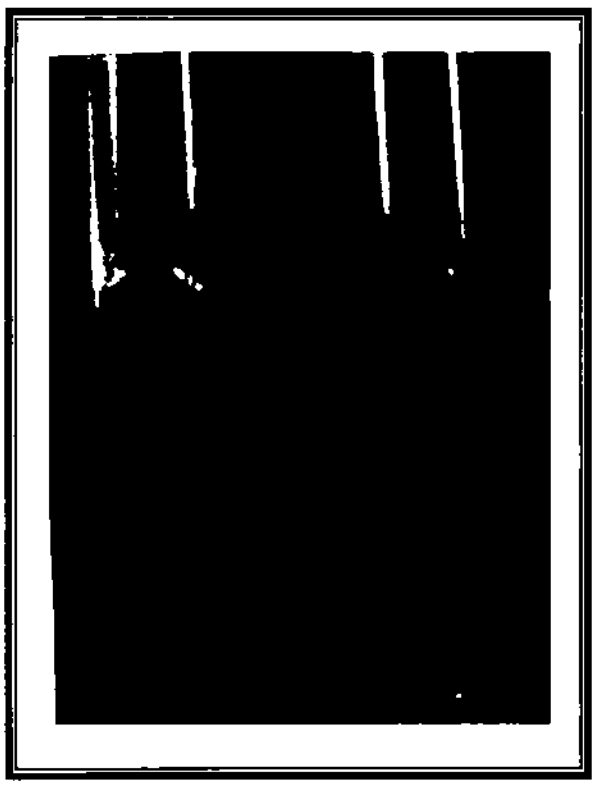


Plate-32: Production of Ammonia from Peptone is showing the positive result by Bacillus (left: BB_2) and the negative result by Staphylococcus (right: TR-3_4).

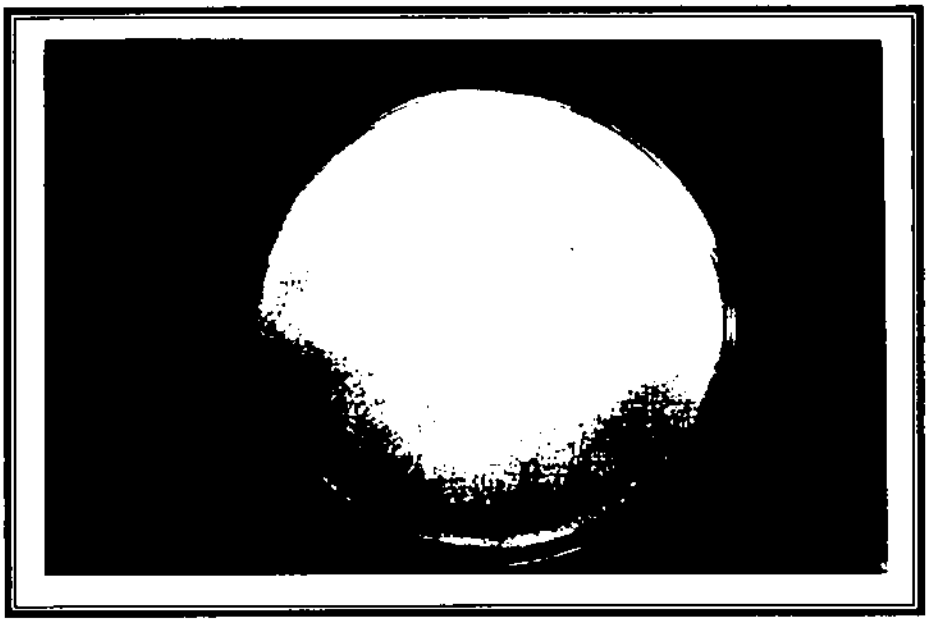


Plate-33: Negative Hydrolysis of Casein by Staphylococcus ($P\text{-}4_4$).

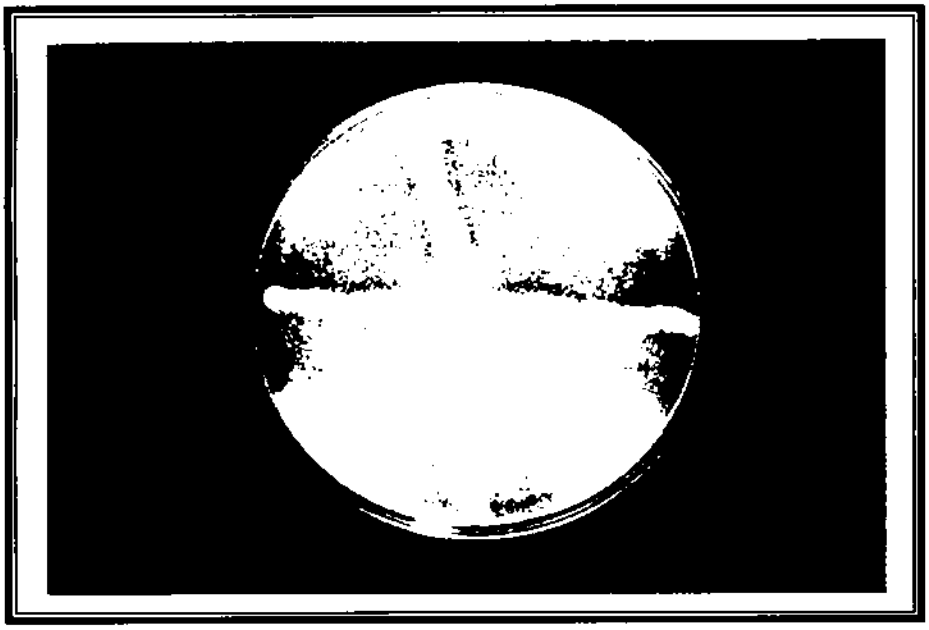


Plate-34: Positive Hydrolysis of Casein by Bacillus (MO_1).

7.4. Physiological Characteristics:

Table-53, 54 and 55 represent here the physiological characteristics of the strains.

7.4.1. Growth of the strains at different concentration of Sodium Chloride (NaCl) of Samples:

From Table-53 we found the effect of Sodium Chloride (NaCl) on the growth of the strains. This table shows that all strains were grown well in presence of Sodium Chloride. But they grew well in 5 % Sodium Chloride than 9 % Sodium Chloride.

Growth of the bacterial strains at different concentration of Sodium Chloride (NaCl) is shown on the following Table-53:

Table-53

Growth of the bacterial strains at different concentration of Sodium Chloride (NaCl)

No. of samples	[Sample] Sample code	Growth of 5 % NaCl		Growth of 9 % NaCl	
		Visual turbidity	Turbidity by Spectra-photometer (% transmittance)	Visual turbidity	Turbidity by Spectra-photometer (% transmittance)
	[Biscuit]				
01	$BT\text{-}1_1$	++	36.80 %	++	54.40 %
02	$BS\text{-}2_3$	++	52.40 %	++	50.60 %
03	BDC_7	++	20.80 %	++	26.00 %
04	BB_2	+++	15.40 %	++	44.20 %
	[Bread]				
05	$Br\text{-}4_3$	++	40.20 %	++	65.00 %
06	BrO_5	++	42.40 %	++	51.20 %
	[Bun]				
07	$Bu\text{-}4_2$	++	52.40 %	++	50.60 %
	[Hot dog]				
08	$HD\text{-}4_3$	+++	40.00 %	+++	63.20 %
	[Pizza]				
09	$PB\text{-}4_5$	+++	56.00 %	+++	67.60 %
	[Sandwich]				
10	$SC\text{-}4_6$	++	23.20 %	++	32.60 %
	[Cake]				
11	$C\text{-}4_4$	+++	10.40 %	+++	25.40 %
12	CO_6	++	30.20 %	++	37.00 %
	[Pastry]				
13	$P\text{-}4_4$	++	43.40 %	++	53.60 %
	[Beef Patties]				
14	$BP\text{-}4_3$	++	52.80 %	++	50.20 %
	[Chocolates]				
15	CF_2	++	30.20 %	++	40.20 %
16	CW_6	++	37.20 %	++	49.20 %
	[Moa]				
17	CMO_3	++	32.60 %	++	46.20 %
18	CMP_3	++	52.00 %	++	62.80 %
19	MMO_1	++	23.80 %	++	34.40 %
20	MMP_5	++	30.60 %	++	40.20 %
	[Muree]				
21	MO_1	+++	38.00 %	++	34.20 %
	[Tondur Rutee]				
22	$TR\text{-}3_4$	++	7.20 %	++	24.60 %
	[Khaza]				
23	$K\text{-}4_4$	+++	16.60 %	+++	28.00 %
	[Bakorkhani]				
24	$B\text{-}4_2$	+++	12.20 %	+++	35.40 %

7.4.2. Growth of the bacterial strains at different temperature of Samples:

Table-54 shows the growth of the strains at different temperature. It clearly represent that all strains were grown well at 4^0-6^0 C, but they were less sensitive to temperature.

Growth of the bacterial strains at different temperature is shown on the following Table-54:

Table-54

Growth of the bacterial strains at different temperature

No. of samples	[Sample] Sample code	Refrigeration temperature (4^0-6^0 C)		High temperature (55^0 C)	
		Visual turbidity	Turbidity by Spectra-photometer (% transmittance)	Visual turbidity	Turbidity by Spectra-photometer (% transmittance)
	[Biscuit]				
01	$BT\text{-}1_1$	++	76.20 %	++	75.40 %
02	$BS\text{-}2_3$	++	48.20 %	++	70.80 %
03	BDC_7	++	68.60 %	++	72.80 %
04	BB_2	++	44.00 %	++	71.20 %
	[Bread]				
05	$Br\text{-}4_3$	++	41.60 %	++	71.60 %
06	BrO_5	++	56.80 %	++	79.40 %
	[Bun]				
07	$Bu\text{-}4_2$	++	56.60 %	++	72.00 %
	[Hot dog]				
08	$HD\text{-}4_3$	++	72.20 %	++	71.00 %
	[Pizza]				
09	$PB\text{-}4_5$	++	43.00 %	++	72.60 %
	[Sandwich]				
10	$SC\text{-}4_6$	++	71.80 %	++	72.20 %
	[Cake]				
11	$C\text{-}4_4$	++	42.00 %	++	72.20 %
12	CO_6	++	58.20 %	++	74.60 %
	[Pastry]		55.20 %		74.20 %
13	$P\text{-}4_4$	++		++	
	[Beef Patties]				
14	$BP\text{-}4_3$	++	48.80 %	++	70.20 %
	[Chocolates]				
15	CF_2	++	56.20 %	++	74.20 %
16	CW_6	++	74.00 %	++	78.20 %
	[Moa]				
17	CMO_3	++	52.40 %	++	72.80 %
18	CMP_3	++	75.80 %	++	78.80 %
19	MMO_1	++	48.20 %	++	71.60 %
20	MMP_5	++	56.60 %	++	74.80 %
	[Muree]				
21	MO_1	++	53.80 %	++	70.00 %
	[Tondur Rutee]				
22	$TR\text{-}3_4$	++	64.40 %	++	78.20 %
	[Khaza]				
23	$K\text{-}4_4$	++	51.60 %	++	74.60 %
	[Bakorkhani]				
24	$B\text{-}4_2$	++	52.20 %	++	76.80 %

7.4.3. Growth of the bacterial strains at different P^H of Samples:

Table-55 shows the growth of the strains at different P^H and that we found they were growing more abundantly at P^H 9.5 than P^H 2.5.

Growth of the bacterial strains at different P^H is shown on the following Table-55:

Table-55

Growth of the bacterial strains at different P^H

No. of samples	[Sample] Sample code	At 9.5 P^H		At 2.5 P^H	
		Visual turbidity	Turbidity by Spectra-photometer (% transmittance)	Visual turbidity	Turbidity by Spectra-photometer (% transmittance)
	[Biscuit]				
01	$BT\text{-}1_1$	+++	19.80 %	++	71.60 %
02	$BS\text{-}2_3$	+++	17.40 %	++	68.00 %
03	BDC_7	+++	18.40 %	++	67.20 %
04	BB_2	++	69.40 %	++	71.20 %
	[Bread]				
05	$Br\text{-}4_3$	+++	19.00 %	++	74.40 %
06	BrO_5	++	55.20 %	++	74.20 %
	[Bun]				
07	$Bu\text{-}4_2$	+++	19.00 %	++	74.40 %
	[Hot dog]				
08	$HD\text{-}4_3$	++	20.20 %	++	72.40 %
	[Pizza]				
09	$PB\text{-}4_5$	+++	17.70 %	++	71.40 %
	[Sandwich]				
10	$SC\text{-}4_6$	++	07.10 %	++	69.00 %
	[Cake]				
11	$C\text{-}4_4$	+++	14.60 %	++	72.00 %
12	CO_6	+++	22.40 %	++	72.80 %
	[Pastry]				
13	$P\text{-}4_4$	+++	05.60 %	++	63.00 %
	[Beef Patties]				
14	$BP\text{-}4_3$	++	24.00 %	++	73.80 %
	[Chocolates]				
15	CF_2	++	51.80 %	++	71.20 %
16	CW_6	+++	19.20 %	++	73.40 %
	[Moa]				
17	CMO_3	++	61.80 %	++	70.20 %
18	CMP_3	+++	07.60 %	++	60.80 %
19	MMO_1	+++	17.40 %	++	74.20 %
20	MMP_5	+++	20.80 %	++	76.40 %
	[Muree]				
21	MO_1	++	57.40 %	++	73.00 %
	[Tondur Rutee]				
22	$TR\text{-}3_4$	+++	55.20 %	++	74.20 %
	[Khaza]				
23	$K\text{-}4_4$	+++	60.80 %	++	78.80 %
	[Bakorkhani]				
24	$B\text{-}4_2$	++	56.00 %	++	76.20 %

7.4.4. Identification of bacterial isolates:

All the strains were characterized by their culture, morphological, physiological and biochemical characters. The characters were compared with those of "Bergey's Manual" (8th edition, 1974) and manual of clinical microbiology (Albert Balows et al, 1991) and were identified tentatively up to genus level. In the total number of 24 isolates 18 were Staphylococcus and 06 were Bacillus.

7.5 Biochemical Analysis with Comparison of different types of Bakery Products, Chocolates and other Street-foods:

Only Moisture and Fat content from Biochemical analysis was done here to compare with the overall contamination of all street food samples. There are two divisions to evaluate total moisture and fat content of all street food samples. One is; less then 5 % of moisture and other is; 5 % and above of moisture.

7.5.1 Moisture content of different types of Bakery Products:

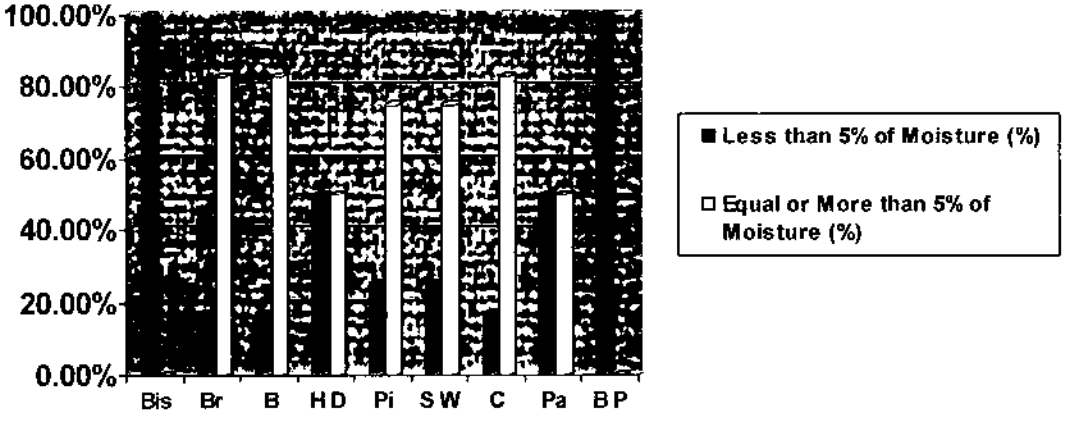
The Moisture content of different types of Bakery Products was evaluated here and presenting on Table-56 and Figure-26. 51.66 % of those samples have 5 % and above of moisture. And 48.33 % have less then 5 % of moisture.

Moisture content of different Bakery Products is shown on the following Table-56:

Table-56

Moisture content of different Bakery Products

Name of the samples	Total number of the samples	Less then 5 % of Moisture (%)	5 % and above of Moisture (%)
Biscuit	14	14 (100 %)	0
Bread	06	01 (17 %)	05 (83 %)
Bun	06	01 (17 %)	05 (83 %)
Hot Dog	04	02 (50 %)	02 (50 %)
Pizza	08	02 (25 %)	06 (75 %)
Sandwich	08	02 (25 %)	06 (75 %)
Cake	06	01 (17 %)	05 (83 %)
Pastry	04	02 (50 %)	02 (50 %)
Beef Patties	04	04 (100 %)	0
Grand Total	**60 (100 %)**	**29 (48.33 %)**	**31 (51.66 %)**



(Name of the samples)

Fig-26: Moisture content of different Bakery Products.

7.5.2 Moisture content of different types of Chocolates:

The Moisture content of different types of Chocolates was evaluated here and presenting on Table-57 and Figure-27. Only 13 % of those samples have 5 % and above of moisture. And 87 % have less then 5 % of moisture.

Moisture content of different Chocolates is shown on the following Table-57:

Table-57

Moisture content of different Chocolates

Name of the samples	Total number of the samples	Less then 5 % of Moisture (%)	5 % and above of Moisture (%)
Candy	05	04 (80 %)	01 (20 %)
Chocolate Biscuits	03	03 (100 %)	0
Mimi Bar	02	02 (100 %)	0
Chocolate Pops	02	02 (100 %)	0
Gems	02	02 (100 %)	0
Mini fruit jelly	01	0	01 (100 %)
Grand Total	**15 (100 %)**	**13 (87 %)**	**02 (13 %)**

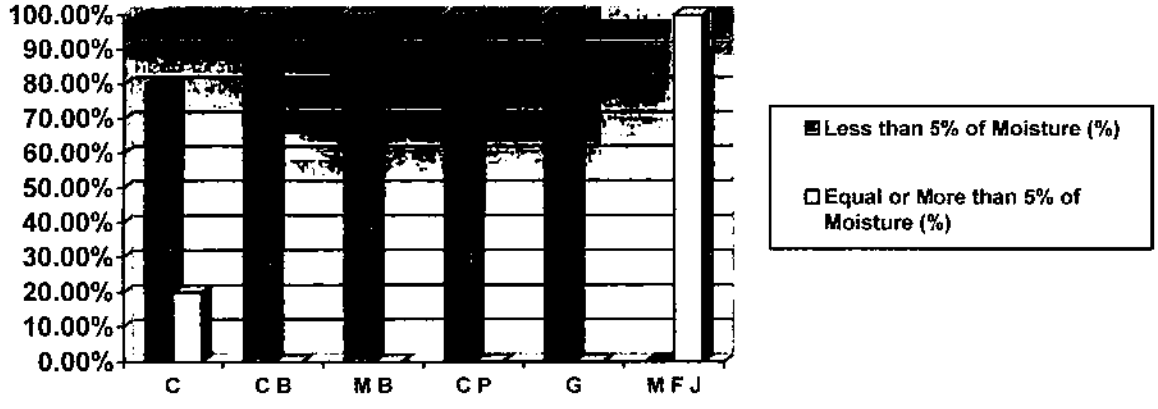


Fig-27: Moisture content of different Chocolates.

7.5.3. Moisture content of different types of other Street-foods:

The Moisture content of other Street-foods was evaluated here and presenting on Table-58 and Figure-28. 48 % of those samples have 5 % and above of moisture. And 52 % have less then 5 % of moisture.

Moisture content of other Street-foods is shown on the following Table-58:

Table-58

Moisture content of other Street-foods

Name of the samples	Total number of the samples	Less then 5 % of Moisture (%)	5 % and above of Moisture (%)
Moa	04	02 (50 %)	02 (50 %)
Muree	02	02 (100 %)	0
Tondur Rutee	02	02 (100 %)	0
Bakorkhani	02	02 (100 %)	0
Khaza	01	0	01 (100 %)
Chanachur	01	01 (100 %)	0
Rice-cake	09	04 (44.44 %)	05 (55.55 %)
Egg Pudding	04	0	04 (100 %)
Grand Total	**25 (100 %)**	**13 (52 %)**	**12 (48 %)**

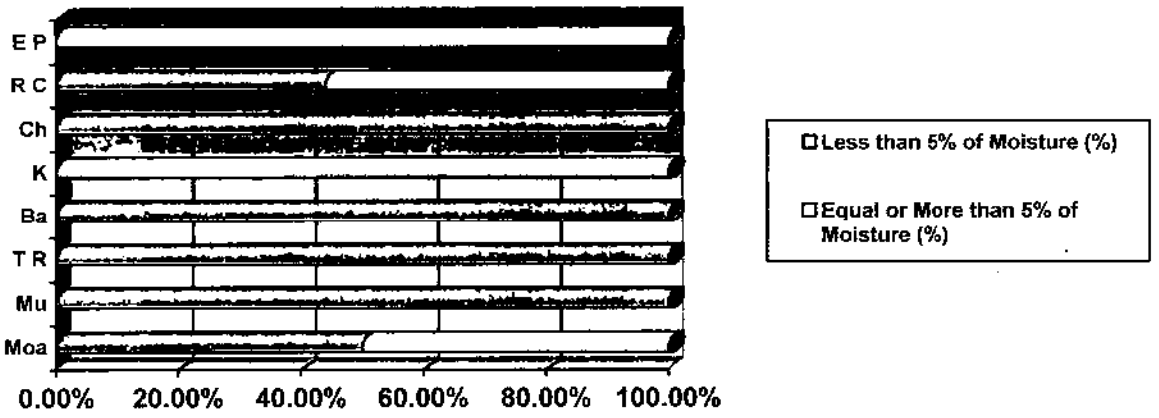


Fig-28: Moisture content of other Street-foods.

7.5.4. Comparison of Moisture content of different types of Bakery products, Chocolates and other Street-foods:

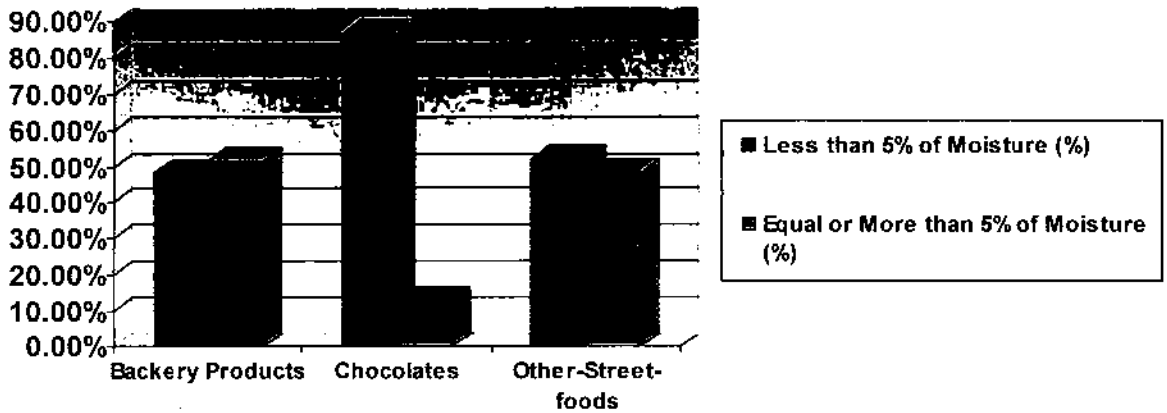
After total calculation of different types of Bakery Products, Chocolates and other Street-foods the comparison of moisture is showing on the Table-59 and Figure-29.

Comparison of Moisture content of different Bakery Products, Chocolates and other Street-foods are shown on the following Table-59:

Table-59

Comparison of Moisture content of different Bakery Products, Chocolates and other Street-foods

Moisture content of the samples	Bakery Products	Chocolates	Other Street-foods
Less then 5 % of Moisture (%)	29 (48.33 %)	13 (87 %)	13 (52 %)
Equal or more than 5% of Moisture (%)	31 (51.66 %)	02 (13 %)	12 (48 %)



(Name of the samples)

Fig-29: Comparison of Moisture content of different Bakery Products, Chocolates and other Street-foods.

7.5.5. Fat content of different Bakery Products:

The Fat content of different types of Bakery Products was calculated here and presenting on Table-60 and Figure-30. 61.66 % of those samples have 5 % and above of fat. And 38.33 % have less then 5 % of fat.

Fat content of different Bakery Products is shown on the following Table-60:

Table-60

Fat content of different Bakery Products

Name of the samples	Total number of the samples	Less then 5 % of Fat (%)	5 % and above of Fat (%)
Biscuit	14	04 (29 %)	10 (71 %)
Bread	06	01 (17 %)	05 (83 %)
Bun	06	01 (17 %)	05 (83 %)
Hot Dog	04	01 (25 %)	03 (75 %)
Pizza	08	02 (25 %)	06 (75 %)
Sandwich	08	04 (50 %)	04 (50 %)
Cake	06	06 (100 %)	0
Pastry	04	0	04 (100 %)
Beef Patties	04	04 (100 %)	0
Grand Total	**60 (100 %)**	**23 (38.33 %)**	**37 (61.66 %)**

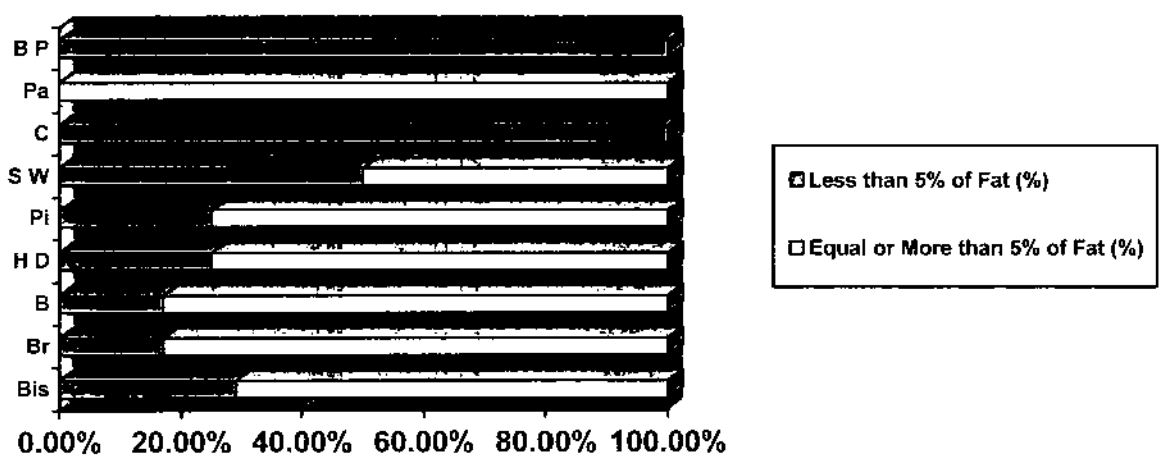


Fig-30: Fat content of different Bakery products.

7.5.6. Fat content of different Chocolates:

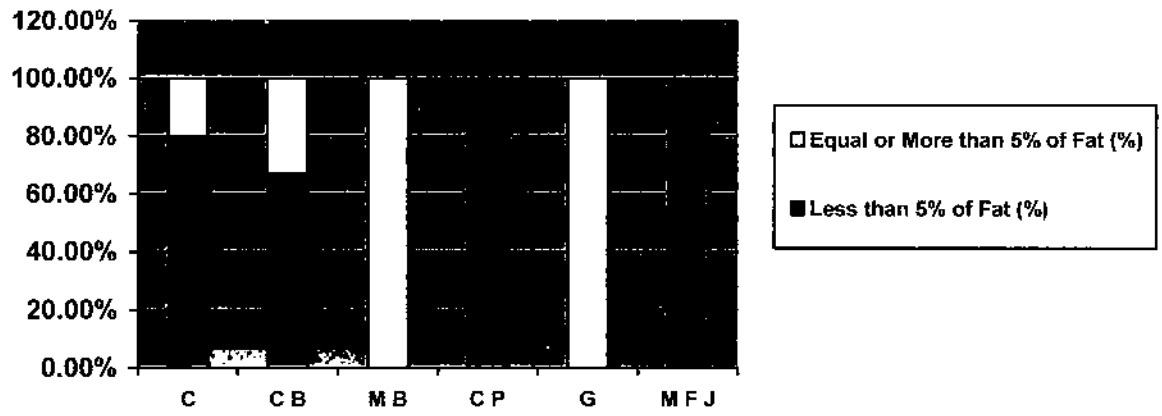
The Fat content of different types of Chocolates was calculated here and presenting on Table-61 and Figure-31. 40 % of those samples have 5 % and above of fat. And 60 % have less then 5 % of fat.

Fat content of different Chocolates is shown on the following Table-61:

Table-61

Fat content of different Chocolates

Name of the samples	Total number of the samples	Less then 5 % of Fat (%)	5 % and above of Fat (%)
Candy	05	04 (80 %)	01 (20 %)
Chocolate Biscuits	03	02 (67 %)	01 (33 %)
Mimi Bar	02	0	02 (100 %)
Chocolate Pops	02	02 (100 %)	0
Gems	02	0	02 (100 %)
Mini fruit jelly	01	01 (100 %)	0
Grand Total	**15 (100 %)**	**09 (60 %)**	**06 (40 %)**



(Name of the samples)

Fig-31: Fat content of different Chocolates.

7.5.7. Fat content of other Street-foods:

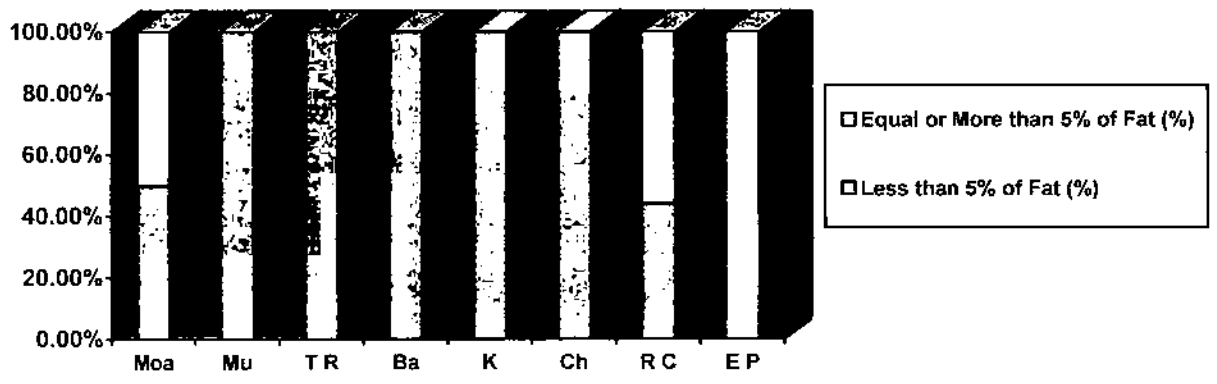
The Fat content of different types of other Street-foods was calculated here and presenting on Table-62 and Figure-32. 44 % of those samples have 5 % and above of fat. And 56 % have less then 5 % of fat.

Fat content of other Street-foods is shown on the following Table-62:

Table-62

Fat content of other Street-foods

Name of the samples	Total number of the samples	Less then 5 % of Fat (%)	5 % and above of Fat (%)
Moa	04	02 (50 %)	02 (50 %)
Muree	02	02 (100 %)	0
Tondur Rutee	02	02 (100 %)	0
Bakorkhani	02	02 (100 %)	0
Khaza	01	01 (100 %)	0
Chanachur	01	01 (100 %)	0
Rice-cake	09	04 (44.44 %)	05 (55.56 %)
Egg Pudding	04	0	04 (100 %)
Grand Total	**25 (100 %)**	**14 (56 %)**	**11 (44 %)**



(Name of the samples)

Fig-32: Fat content of other Street-foods.

After total calculation of different types of Bakery Products, Chocolates and other Street-foods the comparison of fat is showing on the Table-63 and Figure-33.

Comparison of Fat content of different Bakery Products, Chocolates and other Street-foods are shown on the following Table-63:

Table-63

Comparison of Fat content of different Bakery Products, Chocolates and other Street-foods

Fat content of the samples	Bakery Products	Chocolates	Other Street-foods
Less then 5 % of Fat (%)	23 (38.33 %)	09 (60 %)	14 (56 %)
Equal or more than 5% of Fat (%)	37 (61.66 %)	06 (40 %)	11 (44 %)

(Name of the samples)

Fig-33: Comparison of Fat content of different Bakery Products, Chocolates and other Street-foods.

7.6. Correlation between Moisture and Fat contents with Mean Value of Total Microbial load of different Bakery products, Chocolates and other Street-foods:

After finding the TVBC, TCC and TFC of all food samples we calculate the total mean microbial count and compare with the moisture and fat content here. Table-64 and Figure-34 is showing the mean microbial load of Bakery Products is 28.88 %, other street foods are 44 % and different chocolates are only 11.10 %.

Mean Value of Total Microbial load of different Bakery Products, Chocolates and other Street-foods are shown on the following Table-64:

Table-64

Mean Value of Total Microbial loads of different Bakery Products, Chocolates and other Street-foods

Microbial count	Bakery Products	Chocolates	Other Street-foods
(TVBC) Total Viable Bacterial Count (%)	40	6.66	48
(TCC) Total Coli form Count (%)	26.66	13.33	44
(TFC) Total Fungal Count (%)	20	13.33	40
Mean (%)	**28.88**	**11.10**	**44**

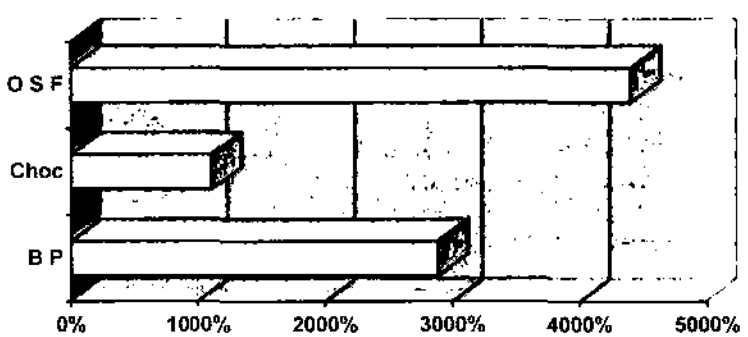


Fig-34: Mean Value of Total Microbial loads of different Bakery products, Chocolates and other Street-foods.

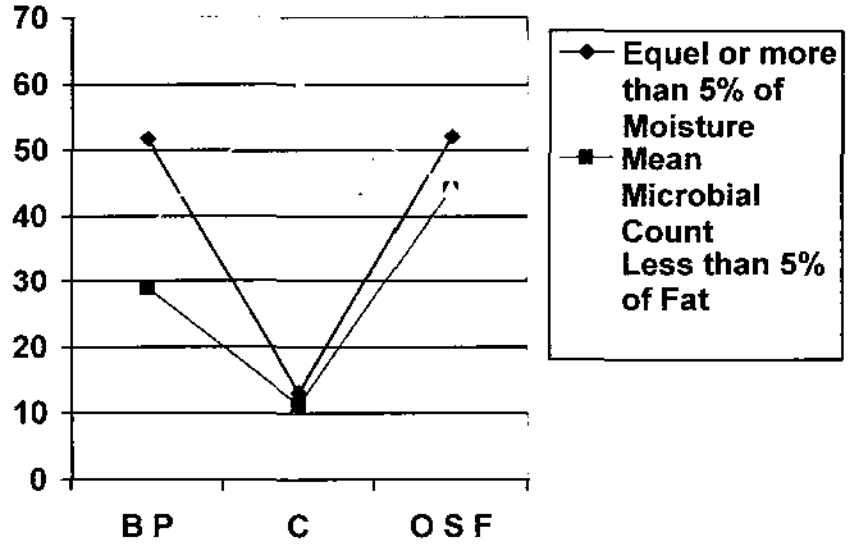
The correlation between Moisture and Fat contents of different Bakery products, Chocolates and other Street-foods with the Mean Value of Total Microbial load are shown on Table-65 and Figure-35. Here we found that when the fat content of food samples is decreasing and the moisture content is increasing, the mean microbial load is simultaneously increasing.

Correlation between Moisture and Fat contents of different Bakery Products, Chocolates and other Street-foods with Mean Value of Total Microbial load are shown on the following Table-65:

Table-65

Correlation between Moisture and Fat contents of different Bakery Products, Chocolates and other Street-foods with Mean Value of Total Microbial load

	Bakery Products (BP)	Chocolates (C)	Other Street-foods (O S F)
Equal or more than 5% of Moisture (%)	51.66	13	52
Mean Microbial Count (%)	28.88	11.10	44
Less then 5 % of Fat (%)	38.33	60	44



(Name of the samples)

Fig-35: Correlation between Moisture and Fat contents of different Samples with Mean Value of Total Microbial load.

Chapter-05

08. Discussion

08. Discussion:

A total of 100 street food samples were collected from Dhaka city zones to analyze here TVBC, TCC, & TFC. The result showed a wide variation of total bacterial count, total coli form count and total fungal count. The total number of Viable Bacteria in different Bakery products detected on standard plate count (SPC) agar as above is shown in Table-28 and Figure-03. Among the samples, 85 % (51 samples) contained mesophilic bacterial cells >10 gm^{-1} range while the rest samples contained bacterial cells <10 gm^{-1}. The highest count was 3.0×10^5 cfu/gm in Pastry and the lowest count was 2.0×10^2 cfu/gm in Biscuits. And the mean count was 4.3×10^4.

The SPC range wise distributions of the bacterial cell in different Bakery Products are also shown on Table-29 and Figure-04. The height 61.66 % (37 samples) showed the acceptable range (01^2 - $<10^4$ gm^{-1}) of total viable bacterial count. This was followed by 23.34 % poor range (01^4 - $<10^5 gm^{-1}$), 5 % safe (01^2 gm^{-1}) and 10 % dangerous (01^5 – above gm^{-1}).

The total number of Viable Bacteria in different Chocolates detected on standard plate count (SPC) agar as above is shown in Table-30 and Figure-05. Among the samples, 93.34 % (14 samples) contained mesophilic bacterial cells >10 gm^{-1} range while the rest samples contained bacterial cells <10 gm^{-1}. The highest count was 1.4×10^5 cfu/gm in Chocolate Pops and the lowest count was 1.3×10^3 cfu/gm in Candy. And the mean count was 1.7×10^5.

The SPC range wise distributions of the bacterial cell in different Chocolates are also shown on Table-31 and Figure-06. The highest 46.66 % (07 samples) showed the acceptable range (01^2 - $<10^4 gm^{-1}$) of total viable bacterial count. This was followed by 33.34 % poor range (01^4 - $<10^5 gm^{-1}$), 6.66 % safe (01^2 gm^{-1}) and 13.34 % dangerous (01^5 – above gm^{-1}).

The total number of Viable Bacteria in other Street-foods detected on standard plate count (SPC) agar as above is shown in Table-32 and Figure-07. Among the samples, 84 % (21 samples) contained mesophilic bacterial cells >10 gm^{-1} range while the rest samples contained bacterial cells <10 gm^{-1}. The highest count was 1.5×10^5 cfu/gm in Moa and the lowest count was 1.2×10^2 cfu/gm in Egg Pudding. And the mean count was 2.48×10^3.

The SPC range wise distributions of the bacterial cell in other Street-foods are also shown on Table-33 and Figure-08. The highest 48 % (12 samples) showed the acceptable range (01^2 - $<10^4 gm^{-1}$) of total viable bacterial count. This was followed by 28 % poor range (01^4 - $<10^5 gm^{-1}$), 08 % safe (01^2 gm^{-1}) and 16 % dangerous (01^5 – above gm^{-1}).

The percentages of unacceptable samples of different Bakery Products are shown on Table-34 and Figure-09, on the basis of cfu/gm. Of the 60 samples 40 % (24 samples) were found seriously contaminated. In comparison to the various samples, the highest sample contamination 75 % was obtained in Hot Dog. A few samples of Biscuit, Bread, Bun, Sandwich, Cake and Pastry were also found to be contaminated. On the other hand Pizza was found to be safe to intake.

The percentages of unacceptable samples of different Chocolates are shown on Table-35 and Figure-10, on the basis of cfu/gm. Of the 15 samples only 6.66 % (01 sample) were found seriously contaminated. In comparison to the various samples, the solely sample contamination was 33.33 % obtained in Chocolate Biscuits. On the other hand, a few samples of Candy, Mimi Bar, Chocolate Pops, Gems and Mini fruit jelly were found to be safe to intake.

The percentages of unacceptable samples of other Street-foods are shown on Table-36 and Figure-11, on the basis of cfu/gm. Of the 25 samples 48 % (12 samples) were found seriously contaminated. In comparison to the various samples, the highest sample contamination 100 % were obtained in Moa, Khaza and Chanachur. On the other hand, other all samples of street foods as Muree, Tondur Rutee, Bakorkhani, Rice-cake and Egg Pudding were also found to be contaminated.

Table-37 and Figure-12 are showing the comparison of different Bakery Products, Chocolates and other Street-foods. 40 % of Bakery Products, 48 % of Other Street-foods and only 6.66 % of Chocolates were contaminated here.

By using Mac-Conkey Agar media, Table-38 and Figure-13 is showing as above the total coli forms of Bakery Products were detected. About 40 % (24 samples) showed positive result for coli form. The mean total coli form was 2.0×10^2 cfu/gm. The highest coli form count 1.0×10^3 cfu/gm in Cake and the lowest count were only 1.3×10 cfu/gm in Beef Patties. About 60 % did not show growth of coli form. On the other hand Bun, Bread and Biscuits are completely safe from coli form contamination.

By using Mac-Conkey Agar media, Table-39 and Figure-14 is showing as above the total coli forms of Chocolates were detected. About 46.66 % (07 samples) showed positive result for coli form. The mean total coli form was 1.48×10^2 cfu/gm. The highest coli form count 1.3×10^3 cfu/gm in Mini fruit jelly and the lowest count were only 1.2×10 cfu/gm in Chocolate Biscuits. About 53.34 % did not show growth of coli form. On the other hand Gems and Mimi Bar are completely free from coli form contamination.

By using Mac-Conkey Agar media, Table-40 and Figure-15 is showing as above the total coli forms of other Street-foods were detected. About 60 % (15 samples) showed positive result for coli form. The mean total coli form was 1.16×10^2 cfu/gm. The highest coli form count 1.1×10^3 cfu/gm in Egg Pudding and the lowest count were 1.0×10 cfu/gm in Muree. About 40 % did not show growth of coli form. On the other hand Tondur Rutee, Khaza and Chanachur are absolutely free from coli form contamination.

According to recommended criteria of ICMSF (2000) for foods, out of 60 Bakery Products, Table-41 and Figure-16 is showing about 26.66 % (16 samples) were *unacceptable. On the other hand, 73.33 % were acceptable food samples.

According to recommend criteria of ICMSF (2000) for foods, out of 15 Chocolates, Table-42 and Figure-17 is showing about only 13.33 % (02 samples) including Chocolate Biscuits and Gems were unacceptable. Other food such as Candy, Mimi Bar, Chocolate Pops and Mini fruit jelly were acceptable.

According to recommend criteria of ICMSF (2000) for foods, out of 25 other Street-foods, Table-43 and Figure-18 is showing about 44 % (11 samples) were unacceptable. On the other hand, 56 % food samples were acceptable.

According to recommended criteria of ICMSF (2000) for foods, Table-44 and Figure-19 are showing the comparison of unacceptability of different Bakery Products, Chocolates and other Street-foods. 26.66 % of Bakery Products, only 13.33 % of Chocolates and 44 % of other Street-foods were unacceptable here.

By using 'Sabouraurd Dextrose Agar Media', the total fungal counts of Bakery Products were detected. About 20 % (12 samples) showed positive result for fungi on Table-45 and Figure - 20 is showing as above here. The mean total fungal count was 1.14×10^2 cfu/gm. The highest fungal count 1.3×10^3 cfu/gm in Biscuits and the lowest count was only 0.1×10^2 cfu/gm in Bun. About 80 % did not show the growth of fungi. All Pastry and Beef Patties were fully free of fungi.

By using 'Sabouraurd Dextrose Agar Media', the total fungal counts of different Chocolates were detected. About only 13.33 % (02 samples) showed positive result for fungi on Table-46 and Figure-21 is showing as above here. The mean total fungal count was 0.56×10^2 cfu/gm. The highest fungal count 1.2×10 cfu/gm in Chocolate Biscuits and the lowest count was only 0.1×10 cfu/gm in Mimi Bar. About 86.66 % did not show the growth of fungi. All Candy, Chocolate Pops and Gems were fully free of fungi.

By using 'Sabouraurd Dextrose Agar Media', the total fungal counts of other Street-foods were detected. About 40 % (10 samples) showed positive result for fungi on Table-47 and Figure-22 is showing as above here. The mean total fungal count was 0.6×10^2 cfu/gm. The highest fungal count 1.5×10^2 cfu/gm in Muree and the lowest count was only 0.2×10 cfu/gm in Rice-cake. About 60 % did not show the growth of fungi. All Egg Pudding and Chanachur were fully free of fungi.

Table-48 and Figure-23 are showing the comparison of fungal contamination of different Bakery Products, Chocolates and other Street-foods. 20 % of Bakery Products, only 13.33 % of Chocolates and 40 % of other Street-foods were contaminated here.

Table-49 and Figure-24 are showing the comparison of total mean Microbial load of different Bakery Products, Chocolates and other Street-foods.

In the study total 100 samples were examined from varieties of street foods including Bakery Products and Chocolates. All samples were chosen for bacteriological identification and total 24 colonies were isolated and identified up to genus level. For the characterization of the isolates the cultural, morphological, biochemical and physiological characters were considered. Table-52 and Figure-25 are showing the biochemical and physiological characteristics of the isolates. Here only 06 (25 %) are Bacillus and other 18 (75 %) was Staphylo coccus.

The presence of some strains of these isolated organisms in large amount in food may be harmful and cause of bacterial food poisoning (WHO. Tech Rep, 1976). Great majority the world were caused by enterotoxim Staphylo coccus aureus (Bryan, 1977). The physiological tests of the strain shows that they can grow well in 5 % of NaCl than 9 % of NaCl, at 55^0 C than 4-6^0 C temperature and at basic P^H - 9.5 than acidic P^H - 2.5. Table-53, 54 and 55 represent here the physiological characteristics of the strains.

Only Moisture and Fat content from Biochemical analysis was done here to compare with the overall contamination of all street food samples. There are two divisions to evaluate total moisture and fat content of all street food samples. One is; less then 5 % of moisture and other is; 5 % and above of moisture. The Moisture content of different types of Bakery Products was evaluated here and presenting on Table-56 and Figure-26. 51.66 % of those samples have 5 % and above of moisture. And 48.33 % have less then 5 % of moisture.

The Moisture content of different types of Chocolates was evaluated here and presenting on Table-57 and Figure-27. Only 13 % of those samples have 5 % and above of moisture. And 87 % have less then 5 % of moisture.

The Moisture content of other Street-foods was evaluated here and presenting on Table-58 and Figure-28. 48 % of those samples have 5 % and above of moisture. And 52 % have less then 5 % of moisture.
The Fat content of different types of Bakery Products was calculated here and presenting on Table-60 and Figure-30. 61.66 % of those samples have 5 % and above of fat. And 38.33 % have less then 5 % of fat.

The Fat content of different types of Chocolates was calculated here and presenting on Table-61 and Figure-31. 40 % of those samples have 5 % and above of fat. And 60 % have less then 5 % of fat.

The Fat content of different types of other Street-foods was calculated here and presenting on Table-62 and Figure-32. 44 % of those samples have 5 % and above of fat. And 56 % have less then 5 % of fat.

Finally the findings of the study is; with the help of TVBC, TCC and TFC count of all food samples we calculate the total mean microbial count and compare with the moisture and fat content here. Table-64 and Figure-34 is showing the mean microbial load of Bakery Products is 28.88 %, other street foods are 44 % and different chocolates are only 11.10 %.

The correlation between Moisture and Fat contents of different Bakery Products, Chocolates and other Street-foods with the Mean Value of Total Microbial load are shown on Table-65 and Figure-35. Here we found that when the fat content of food samples is decreasing and the moisture content is increasing, the mean microbial load is simultaneously increasing.

This study will help the policy makers as well as relevant authorities to make needful action. As people move from different parts of a country, the urban centers also become a melting pot including their own food habits. Preparation and sale of Street-foods is an activity and almost universal in developing countries, and is also present in the industrial world even though it may not play the same traditional role. This activity has reached new dimensions as a result of rapid urbanization.

It is now widely accepted that where a food represents an actual or potential health hazard microbiological examinations should be made and microbiological specification, where appropriate, should be established for the food (WHO Tech. Report Survey, 1976: 598).

Preparation and sale may be carried out in the same place. Hygienic requirements are however, similar in both cases. Food should be prepared in a clean, well-lighted place protected from strong sun, dust, rain and wind. It should be away from sources of contaminants such as solid and liquid wastes, and from animals, including pets as well as pests. Equipment and surfaces in the place of preparation should be such that they can be cleaned easily and preferably made or covered with impervious materials. Preparation should not be carried out on or near the ground.

In some confectionary and industries the food handlers use additives to improve taste, appearance, texture of their foods and for preservation. Food additives, which are observed during the study are coloring agents, saccharin and soda.

The Codex Code of General Principles of Food Hygiene should be used as guide, with particular attention. Raw materials and ingredients should be obtained from known and reliable sources and not from clandestine dealers. In any case, the dealer should be guarantee food-grade standard for the ingredients. It should be assured that materials remain whole some during transport, storage and handling through processing, cooking and sale. Transport should be affected without undue exposure to heat, contaminants, pollutants, pests and other causes of spoilage. In many shops keeping of food is not so good. They should be kept free from contamination from the environment like dust and foreign matter.

In small bakeries one of the most critical problem is they have no proper or direct access to a water supply for using in the food, washing, cleaning and other operations. Or some where water tops may run only for a few hours during the day and sometimes not for days. So, water supply needs particular attention in street food operations. As for a possible, the production and sales units should have their own supplies of potable water whether it is from a central system or on individual source, such as a hand pump. If potable water is not available, a suitable source of safe water should be used. Special care should be taken to assure that such water is maintained in a sanitary state.

Most of the food handlers do not wash hands before preparing foods and they are also not so educated. Personal hygiene of them is very poor. So, all street food handlers should be educated, trained, encouraged or supervised to shop their business promptly if at any time they suffer from diarrhea or vomiting or have boils, sores or unclean on exposed parts of the skin. Resumption of business after recovery may be subject to authorization by the appropriate food control authority. Food handlers should wear clean and proper clothing according to prevailing local standards. Where feasible, food handlers should be encouraged to wear clean overall aprons preferably white or light in color.

Food handlers should wash their hands with soap and water after handling raw foods, before handling baking foods, after using the toilet, after handling unsanitary objects such as garbage containers and after contact with toxic substances such as pesticides and disinfectants. In the preparation and sale of food, food handlers should refrain from unhygienic and unsightly practices, such as chewing betel nut or gum, smoking tobacco, touching mouth, tongue, nose, eyes etc and splitting sneezing and coughing on or near food. All waste should be handled and disposed of in such a manner as to avoid contamination of

food and water and the environment. In particular, access to food waste by pests as well as by animals should be avoided. The following types of waste should if possible were disposed of separately. Liquid waste except oil and fat should be emptied into the nearest sewer or drain. Some from of a trap should be used to ensure that only liquid waste is discharged into the sewer or drain. Remains of food may be separated and kept for feeding animals. Utensils on which food has been served to customers should not be licked by animals to clean them. Other solid waste should be kept in covered containers to be removed at least once daily to the public garbage collection system. The containers should be cleaned daily.

It was observed that some of them adulterate the foods by lower grade. They mixed fried oil or burned Mobil oil. They collect decomposed sweets and mixes with sweet products. They also buy low grade raw materials. Care should be taken that they do not utilize contaminated or hazardous raw materials and ingredients. Special care is required to assure that they use only permitted additives and only in quantities, which are approved by appropriate authorities.

We select and collect here only locally produced Bakery Products, Chocolates and other Street-foods. So far, there is no regular market and street monitoring system for quantitative assessment of these items and no scientific report published yet. Considering the situation, I have undertaken this study aimed to assess the general specifications, the qualitative and quantitative aspects of ingredients of Bakery products, Chocolates and other Street-foods available in the market and street of Dhaka city zones. All the samples were collected on payment from all Zones of the city using probability proportionate to size multistage stratified random sampling method. For this type of analysis, I visit all those confectioneries, bakeries and food processing factories of these selected samples were processed to collect the original ingredients, adulterant food items and BSTI approval, date of manufacture and expiry information. I found the food handlers were mixing some special adulterant items there. I had no choice about any laboratory test, but only organoleptic test (like tongue test) for the lack of our laboratory and reagent facilities. We collect from them some loose items and only container source, which they are mixing to those food products. We record some of them and made a list, which are as follows:

1. Urea Fertilizer
2. Textile Dye
3. Chemical Sweeteners
4. Bad Milk Products
5. Harmful Fat items
    a. Fat item (Tolu) using for Soap making
    b. Very low quality Dalda or Palm Oil
    c. Burnt Soya bean Oil
    d. Burnt Mobil Oil

1. Urea Fertilizer:

Urea fertilizer is hugely used in all kinds of bakery products, mainly biscuits, breads, cakes and muree, tondur rutee, bakorkhani etc in everywhere in Dhaka city zones. The food handlers and factory workers claimed that those pile of urea fertilizers contain ammonia, hydroxide or hydroxyl.

Urea fertilizers are used as an important adulterant item to make the food products whitish, big, puff up, overall looking so attractive, crisper and save from the attack of fungus for a long time.

2. Textile Dye:

Different types of Textile dyes were hugely used in various food processing factories in Dhaka city. Including chocolate producing factories and some confectioneries, a singe confectionery is using 11-18 different kinds of textile colors here, such as, Black SM, Sun Yellow RCH, Orange SE, Scarlet 4BS, Sky Blue FB, Rhodamine B, Green PLS, Fast Brown BRLL, Yellow 3gx, Orange GR POP, Bordeaux BW, Fast Red 5B, Turquoise Blue GL, Brown CN, Metanil Yellow, Orange ll, Auramine Blue and VRS etc. Most yellow items are colored with Metanil Yellow and red items with Rhodamine B; recently revealed sources from the Consumers Associations of Bangladesh (CAB) [Daily News Paper]. Some dishonest food producers used those harmful coloring agents only to save their money.

3. Chemical Sweeteners:

The food processing factories are using very harmful chemical sweeteners like Sodium Cyclamate to sweeten their products, which is banned world wide. Some dishonest food producers used it only to save their money.

4. Bad Milk Products:

Very low graded and exceeded or without expiry dates milk powder and unrefined palm oil mixed condensed milk are using the substitute of milk in some of the factories. The food producers can get those harmful adulterant items very cheaper rate. That's why they are mixing as adulterant to prepare some milk-cream biscuits and chocolates.

5. Harmful Fat items:

Some dishonest food producers used these bad fat items only to save their money.

a. **Fat item (Tolu) using for Soap making:**
   Now-a-days this is used instead of vegetable oils in some bakery products here. But recently this item import is banned by Bangladesh Standard and Testing Institute (BSTI) authority.

b. **Very low quality Dalda or Palm Oil:**
   Very low quality or unrefined Palm oil and Dalda are using also hugely in many bakery and confectioneries.

c. **Burnt Soya bean Oil:**
   Some dishonest food makers use burnt Soya bean oil again and again to save money.

d. **Burnt Mobil Oil:**
   Some dishonest bakery product producers use it to adulterate their products instead of vegetable oils.

While doing this type of analysis, we found these harmful and toxic chemicals are hugely used in mainly different Bakery Products and other Street-foods; some less in Chocolates. The height amount of toxic chemical items are using in different Bakery Products. Recently our 'Mobile Court' says that 96 % bakery and confectioneries are used those adulterants.

According to Professor Dr. Mobin Khan, Chairman Liver section of Bangabandhu Medical University of Dhaka, "If one consumes those adulterated food items regularly then it may cause grave illness like cancer, kidney failure, liver and even brain damage at any time."

According to some other experts, "Consumption of such harmful chemicals could lead to a host of health problems, including skin reactions like dermatitis, allergies, asthma, breathing problems, stomach upsets, jaundice, typhoid, dysentery, blood dysentery, peptic ulcers and bone marrow depression leading to leukemia."

According to Associate Professor Miah Masud, head of the gastro-ontology department of Dhaka Medical College Hospital, "The effects could be short and long-term. The short-term effects could be diarrhea, abdominal pain and vomiting. But the long-term effects could be very serious as pancreas cancer, intestine cancer and asthma."

Most of the food handlers do not use uniform and hand gloves and always sweating at the time of food preparing. Some of them mixing freeze stocked old, spoiled and bad-smelled beef to prepare Beef-patties. They must have **"Health Certificate"** given by local 'Civil Surgeon'. But in fact majority of them don't have this type of any document. Also most of the factories don't have own laboratory or any food expert, nutritionist or chemist.

The food handlers washed their hand before preparing food and majority of them use only plain water for washing but most of them don't washed their hand before serving food. And in this way food could be contaminated. Some of them use soap for doing of the vended place properly. The majority of them use tap water to the food at the time of preparing, which is also very unhygienic and can contaminate food. It was observed that most of the confectionary and bakeries places are not protected from dust and open foods are available. Water is reused for cleaning. Some places of city handlers' uses river water to clean utensils duty to lack of water. The vending places are also near sewerage and filthy environment is present some where. Above these favorable conditions can produce microorganism like E Coli, salmonella, staph aurcues fecal coli form, V-cholera etc and food may be contaminated by these organisms and may caused diarrhea, dysentery and other diseases (WHO study).

There are some ill effects of street food on health. Majority of street food consumer were effected in diarrhea and stomach ache (PAHO, 1985). It may be mentioned that very few consumers believed that there is an intoxication problem may caused by street food which is supported by WHO study (1992) in different developing countries. In spite of ill effects there are some advantages of some street food some house less people (floating) are quite development on street food vending can help to solve unemployment problem cheaper street food is also accessible. The quality of food can be developed by increasing public awareness and increasing capital. The activity of police is not satisfactory. Their activity is not always fare problem water is not available in street food at all. Terrorism is another problem in street food selling and vending.

The study presents the investigation on the efficacy of different methods of treatments in decreasing the bacterial load of different Bakery products, Chocolates and other Street-foods.

Some products don't have any manufacture or expiry dates. Majority of these types of factories are baking their foods not so far from the capital city but in silence area in very unhygienic and unhealthy environment. The health department of the city corporation has to take some action to close those types of food production factories.

BSTI authorities permit using coloring agents only in jam, jelly, juice and sauce, but they do not know whether those food colors are available in the market or not. They also do not know what kind of color food producers are using as manpower for scrutinizing the situation is limited. BSTI do not permit any color for confectionery and sweetmeat items. But they have very limited manpower to monitor these things in the whole country, which is impossible to monitor it regularly. When the food producers send to BSTI office any sample of their products, they meet the overall standard. But after that they change their position and start producing below standard food for undue profit. It is not possible for them to supervise all those things due to their limited workers.

The microbiological analysis of different types of street-foods suggested that in most of the higher class or Standard-I & Standard-II food shops proper sanitary and hygienic condition were maintained throughout the preparation to storage. In some cases street-foods were kept within glass decorated showcase under lighting. This condition is very dangerous because it may create an optimum growth temperature for food borne microorganisms and thus foods can be spoiled or unsafe for consumption. It is not exposed to proper heating in microwave oven before eating.

The interior environment of some confectioneries was unhygienic that may contribute the contamination of foods. In some cases, it was found that food utensils and other equipments were washed out by dirty water and every time they use the same water. Many servers are not aware of food contamination. But most of them have microwave oven heating foods before serve to consumers.

The food samples collected from lower class or Standard-III & Standard-IV confectioneries were found to be dangerous. Most of them were microbiologically unsatisfactory or unacceptable to consume on the basis of bacterial count and fungal growth. The storage condition of all most all bakeries were unhygienic. They keep the foods at room temperature for a long period of time. They have no heating device to heat the foods before serve to consumers. Water is re-used one after another for washing purpose. Foods are prepared and stored in most hygienic condition. They store foods on open container. Foods are easily contaminated from outside environment. This storage condition rather acts as an incubator for the growth of microorganisms. Servers are illiterate and they have no knowledge about safety and hygiene. All these may cause higher contamination in these Bakery Products, Chocolates and other street-foods.

Street foods have become popular for various reasons. They are available at the places where required - around factories, offices, schools and universities, transit points, market places etc. With more people joining the labor force and working away from home, street foods are the most accessible source of food intake. Many people lack proper housing facilities and often simultaneously cooking facilities. With increasing costs and difficulty in procuring fuel, the only alternative is to fall back on street foods. Street foods also provide different verity.

While there are many positive aspects of street foods, where are also several negative aspects, so improvement of the street food situation will succeed only when both the positive and negative aspects are fully taken into consideration. The negative aspects include food-borne disease through street food, encroachment on road sides and pavements, creation of problems of hygiene and sanitation of environment as well as the personal hygiene of street food sellers and consumers, potential disturbance in the lives of other citizens and a possible contribution to the deterioration of the law and order situation with in the city.

Lower classic street food sellers or vendors are less able to defend themselves against corruption or exploitation. If a code or any mandatory requirement to allow or encourage corrupt or exploitative practices. Although the actual strategies for improving street food can only be developed after, appropriate studies and other information on local conditions and practices are available. Availability of the means and resources to implement provisions of any mandatory requirement, e.g. potable water and energy supplies, cleaning and disinfecting facilities and waste disposal, should be kept in mind in developing appropriate strategies. Street food vendors should be officially recognized as a part of the food supply system and where possible, included in urban development buildings incorporate street food sells centers in their design. Among other benefits, this recognition may enable some seller to obtain loans for improving their simple business. Training of food handlers in personal hygiene and safe handling and preparation of food, as practicable under local Street vending conditions is an essential part of any strategy to improve the safety and quality of street foods.

A complementary education programmed for the consumer and the community is also strongly encouraged and will help to assure compliance by sellers under pressure of customer demands. It also demands proper attention to improve the quality, safety and nutritional aspects. It needs the involvement of the relevant government authorities to take the control measures through proper legislation and regulation to avoid public health hazards. Control authorities have to be particularly vigilant on these occasions and take measures to enforce hygienic practice.

Chapter-06

09. Conclusion

09. Conclusion:

Microbial examination of food serves a dual purpose: To protect the health of consumer and to ensure the quality of the food. To make the best use of limited resources in money and manpower it is essential tests be applied at those points in the chain of food protection and processing that offer maximum benefit in terms of protection of health or quality.

Every food has a Self life. In all street foods Biscuits are dry than Cakes. And those are safer from spoilage than egg products like Cakes, Pastries, Egg Puddings and cereal based as Breads, Buns, Hot Dogs, Pizza, Sandwiches, Tondur Rutee or Rice-cakes. Those were mostly unacceptable. High moisture content foods as Mini Fruit Jelly were more contaminated than low moisture content foods as Muree. On the other hand, low fatty foods as Bakorkhani are more contaminated than fat-rich foods as Mimi Bar. Finally this study teaches us to develop our awareness and we have to improve our hygienic condition to decrease the microbial load of street foods.

According to Dr. Hussainuddin Shekhor, Associate Professor, Department of Bio-chemistry and Micro-biology, University of Dhaka, "1.8 million people are suffering from verities of illness and loss of any limb even for whole life due to intake unacceptable or adulterated foods every year in our country. Medicine expenses for them all are 10 Thousand Crore Taka only each year." And according to other Experts, "91 % of our total foods are adulterated and the tendency of this type of food adulteration is higher then any other country in Bangladesh. But we don't have any adulteration measuring equipments for our food experts. In some of the developed countries have special simple pricing kids or equipments to measure the food adulteration for every general passing consumer." Much organization including Ministry of Environment wants to import some special equipment for this purpose, but till today they can't apply it. The Magistrates ordered their punishments like fine; jail only depends on their guess.

Now-a-days for the publicity of the newspaper and electronic media coverage of the dangerous bad effect of the consumption of adulterated foods the consciousness of the consumers is increasing day by day. Following in formations are concluded from the study:

- Street food vendors earned considerable income when compared with national minimum wage.
- Good sanitation practice is not a feature in the preparation and presentation of these foods.
- People of low income group having minimum education are mostly the consumers of Std-IV type street foods.
- Street food consumers are prone to diarrhea disease.
- Street food maintains good nutritional status in spite of high morbidity rate.
- The contamination rate is high in high moisture and low fat content foods.

Considering the important of street foods, it cannot be neglected and has to be improved in order to guarantee its safety and wholesomeness to the consumers while maintaining its nutritional value. From health perspectives the study shows the need to improve the food handling practice that will promote good sanitation and hygiene. It needs the involvement of the relevant government authorities to take control measure through proper legislation and regulations.

The present study confined to Dhaka city zones, so that results do not represent the whole county. There is no available restaurant directory or list of confectionery or vendors in the country. The study requires enough time and fund, but these were not available for the present investigation.

Unfortunately it is very difficult to monitor the consumers affected by taking street foods in Bangladesh. It is there fore; better to monitor the hygienic conditions of the restaurants to prevent contamination chance. Safe foods will save lives. All of us want our food to be free or harmful microorganisms. So, we can do our part by learning to handle, cook and preserve food properly and by making smart choices when we buy food at a store or eat at a street food sells centre.

Chapter-07

10. Limitations of the Study

10. Limitations of the Study:

I faced a number of difficulties and limitations while doing this research work. Those are as follows:

a. A major limitation of my research work was shortage of laboratory reagents and necessary equipment facilities. If I got more lab facilities I could have done this research in a much better way with adulteration measurement and can complete it more days before.

b. I studied on the quality aspects of some Bakery products, Chocolates and other Street-foods here. Because of ingredients mixing, food preparation and take some photos, I need to enter and talk with those food preparing labors. I spend more time with them and asked a lot of questions. They were not always very co-operative. For the 'Mobile Court Judgment Group's by the setting of our Government, those people were always very worried and tensed to answer anything and even to allow to enter anyone there. Because they can fine a big amount of money and can arrest those people or sealed the factory at once sometimes. So, in some confectionery, food processing factories and bakeries, the working peoples were quite accustomed to this kind of watching and therefore, sometimes they do not want to answer any question or even do not want to talk. Or some of the respondents were not able to give proper answer always.

Chapter-08

11. Recommendations

11. Recommendations:

We should recognize Street-foods as an important part of our urban life. So some necessary steps could be taken so that the food handlers are obliged to prepare and serve their food taking hygienic care and not to pollute the environmental where they do their merchandise. Realizing the quality aspects of different Street-foods to determine the fitness for human consumption, as well as, their potential for health hazards, it is recommended to take early steps to recognize and assist this informal sector in order to be able to initiate necessary actions to upgrade its performance.

It may therefore be advisable under certain circumstances to commence with a limited project and subsequently expand the work based on the initial experience. Keeping this approach in view, it is suggested that the Governments identify and select the capital city, which they might develop as a model for a Street-food programmed. In the city, a beginning could be made with certain selected areas which should have or be provided with, the necessary infrastructure and facilities.

The necessary steps are as follows:

1. Training and health educations should be given to the food handlers, Street-food sellers as hawkers and vendors in proper food handling practices and at least, about the sources of food contamination.
2. Appropriate heat treatment should be adopted during preparation of foods to inactive pathogenic microorganisma.
3. License should be given to the all Street-food sellers and distributors.
4. A certain places should be selected only for vending.
5. Measures should be taken to remove harmful substance as food additives, adulterants, arsenic from water lead etc. Other measures may be taken as direction of ICMSF.
6. Preparing and disseminating education material and media messages on food safety issues, including food hygiene, sanitation, good or bad effects of adding food color, flavor and other additives and adulterants and nutrition issues for Street-foods sellers and customers.
7. Loan or financial assistance should be given to the shop-keepers and vendors.
8. Consumers should also careful about the food, what they buy and eat, its hygienic status, cleanliness of the shop-keepers and cover of water they will conscious about the environment as the vicinities of the business. There should be a health and nutritional education for them to assist them in food choice as well as recognizing visual sign of unsanitary conditions, which will lead to health problem.

9. Legislation and regulations should be prepared and implemented, which could provide for appropriate Street-food handling guidelines including codes of practice. Monitoring and inspection of food qualities, facilities for both Street-food sellers and customers should be maintained by the authority routinely.

10. Development of codes of practice for Street-foods based on risk analysis, taking into consideration both the potential hazards and the possible control measures so as to provide consumer protection.

11. Establishing a Street-food unit or cell at the local body level or by the Government and setting up of an inter-departmental committee or task-force like mobile court with a group of some members like magistrate, chemist, nutritionist etc on Street-foods for routine inspection.

12. If any food handler, food processing labors or owner of the bakery, confectionery or chocolate factories mixed any harmful chemicals to their foods or any shop-keepers to sell any food product exceeded or without expiry date obviously immediately fine to them a huge amount of money, issued warrant and file cases under the Special Power Act -1974 with the help of police and lawyers against every responsible persons. Not only punish them to jail but also rule should be made even for death-punishment, because mixing of toxic chemical items are equal to murder someone.

13. Immediately seized and destroy all adulterated food products after identify and shut down the factories.

It is duty to the Government to look and arrange the administration so that food handlers maintain their personal hygiene, environmental hygiene and quality of food. It is the duty of the administration to ensure that the people in general who are dependent partially or fully on Street-food can always get good foods. If these recommendations are adopted and implemented effectively, the benefits of Street-food will be enhanced.

Chapter-09

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12. Acknowledgements:

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Chapter-11

14. Biographies

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