# Development of good quality of yogurt in terms of texture, appearance, color, taste and determination of fat percentage in milk and yogurt


**A DISSERTATON SUBMITTED TO THE BRAC UNIVERSITY FOR PARTIAL FULFILLMENT OF THE REQUIREMENT FOR THE DEGREE OF BACHELOR OF SCIENCE (B.Sc) IN MICROBIOLOGY**

**SUBMITTED BY**
**ANILA LABONNYA MODHU**
**STUDENT ID: 12126012**
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**DEPARTMENT OF MATHEMATICS AND NATURAL SCIENCES**
**MICROBIOLOGY PROGRAM**
**BRAC UNIVERSITY**
**MOHAKHALI, DHAKA**

# DECLARATION

This is to declare that the research work embodying the results in this thesis entitled **"Development of good quality of yogurt in terms of texture, flavor, food value and low cost and determining the fat percentage of milk and yogurt."** submitted by Anila Labonnya Modhu, has been carried out by under the joint supervision and guidance of Professor Dr. M Mahboob Hossain, Coordinator, Biotechnology and Microbiology Program, BRAC University in partial fulfillment of B.Sc in Microbiology, at BRAC University, Dhaka. It is further declared that the research work presented hereis original, has not been submitted anywhere else for any degree or diploma.

**Candidate**

**Anila Labonnya Modhu**

**Certified:**

**Prof. Dr. Mahboob Hossain**
Associate Professor,
Microbiology program
Department of Mathematics and Natural Sciences
BRAC University
66, Mohakhali, Dhaka-1212
Bangladesh

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# ABSTRACT

In the present study efforts were made to develop improved quality of yogurt in terms of texture, flavor, colour, taste and low fat content. To achieve this objective, better strains of *Lactobacillus* were isolated from locally available well known branded yogurts of Dhaka city. It was done to optimize the yogurt production with an improved quality yogurt. Total 40 samples were analyzed by recombining many other samples. Of the forty yogurt samples, eight samples found to be of better quality. Later on, from these eight samples two highest scored samples were selected. . Observation of the quality assessment was done by hedonic scaling. Therefore, chemical, physical, microbial and organoleptic analyses for samples were conducted at predetermined days. Yeast and *Lactobacillus spp* was found from better samples. Also the fat content of milk and yogurt showed that yogurt had higher fat content than milk which is healthier to eat. Starter culture found from yogurt isolates may have potential to be used in dairy industries in terms of their high technological and organoleptic characteristics.

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# (LIST OF ABBREVIATIONS)

% percent

αalpha

βbeta

<less than

>more than

≤ less or equal to

≥more or equal to

ºCdegree celsius

ºFdegree fahrenheit

S/sec second

N normality

L/l litre

V volume

µg micro gram

µm micro meter

µl micro litre

cfu colony forming unit

cm centimeter

Conc.Concentration

Min/mins minute

e.g.as example

et.aland others

etcetcetra

Fig.figure

gm/ggram

mgmilligram

kgkilogram

mlmillilitre

KJ kilojoule

Kg/kg kilogram

MW molecular weight

IU International Units

No./no. number

pHnegative logarithm of hydrogen ion concentration

hr/hrshour

yrsyears

i.e.that is

CaCalcium

Mg magnesium

P Phosphorus

Fe Iron

K Potassium

Na Sodium

NA Nutrient agar

NB Nutrient broth

TA Titrable Acidity

ST *Streptoccocus*

T.S.total solids

ppmparts per million

LAB/LB Lactic acid bacteria

SMM skim milk media

TSB Tryptophan soya broth or tryptic soya broth

SPC standard plate count

TCC total coliform count

MRS Man Rogosa Sharpe agar

SNF solids-non-fat

DNA deoxyribonucleic acid

SMP skim milk powder

LDH lactic dehydrogenase

WHOWorld Health Organization

KOH potassium hydroxide

AOAC Association of official Analytical Chemist procedure

BCSIR Bangladesh Council for Scientific and Industrial Research

# CHAPTER ONE: INTRODUCTION

# Introduction

## 1.1 YOGURT

Yoghurt is a fermented milk product obtained from the milk or the milk products by the lactic acid fermentation through the action of *Streptococcus salivarius Lactobacillus thermophilus, Lactobacillusdelbrueckii*. *bulgaricus*. When a sufficient quantity of lactic acid is produced then the milk coagulates and this coagulated milk is called yoghurt.Acidification of milk by fermentation is one of the oldest methods of preserving milk.

Yogurt is a product of the lactic acid fermentation of milk by addition of a starter culture containing *Streptococcus thermophilus and Lactobacillus delbrueckii ssp. bulgaricus*. In some countries less traditional microorganisms, such as *Lactobacillus helveticus and Lactobacillus delbrueckii ssp. lactis*, are sometimes mixed with the starter culture (McKinley, 2005). Although fermented milk products such as yogurts were originally developed simply as a means of preserving the nutrients in milk, it was soon discovered that, by fermenting with different microorganisms, an opportunity existed to develop a wide range of products with different flavors, textures, consistencies and more recently, health attributes. The market now offers a vast array of yogurts to suit all palates and meal occasions. Yogurts come in a variety of textures (e.g. liquid, set and stirred curd), fat contents (e.g. regular fat, low-fat and fat-free) and flavors (e.g. natural, fruit, cereal, chocolate), can be consumed as a snack or part of a meal, as a sweet or savory food. This versatility, together with their acceptance as a healthy and nutritious food, has led to their widespread popularity across all population subgroups (Mckinley, 2005).

Yogurt was introduced to the American diet during the 1940s. By the 1980s, it had become the product for dieters, and the lunch of choice for young women. The use of 2 yogurt as a calcium source has made it one of the most rapidly growing dairy products, but presently it is more than just a calcium source. Yogurt, Kefir, and similar fermented milk products are on the way to becoming major nutraceuticals aimed at treating a variety of disease conditions (Katz, 2001). Yogurt's nutritional profile has a similar composition to the milk from which it is made but will vary somewhat if fruit, cereal or other components are added. Yogurt is an excellent source of protein, calcium, phosphorus, riboflavin (vitamin B2), thiamin (vitamin B1) and Vitamin B12, and a valuable source of folate, niacin, magnesium and zinc. The protein it provides is of high biological value, and the vitamins and minerals found in milk and dairy foods including yogurt are bio-available.

Yogurt particularly the low-fat varieties, provide an array of important nutrients in significant amounts in relation to their energy and fat content, making them a nutrient-dense food. Eating dairy products, such as yogurt, helps to improve the overall quality of the diet and increases the chances of achieving nutritional recommendations, (Mckinley, 2005). Vitamins and minerals may be added and often are for products given to children. Yogurts may be spoonable or drinkable, and may be considered dietary supplements for infant consumption. So they cross the line between dietary supplements, medical foods, and conventional foods (Katz, F. 2001).

Yogurt gels are formed by the fermentation of milk with thermophilic starter bacteria; milk is normally heated at high temperatures (e.g., 85°C for 30 min), which causes the denaturation of whey proteins. Denatured whey proteins interact and cross-link with κ-casein on the surface of casein micelles. There is increased casein-casein attraction as the pH of milk decreases from ~6.6 to ~4.6 during yogurt fermentation, which results in gelation as casein approach their iso-electric

point. Physical properties of 3 yogurt gels, including whey separation play an important role in quality and consumer acceptance. An understanding of gelation process during fermentation is critical in manipulating physical properties of yogurt (Lee and Lucey, 2004).

## 1.2 BIOCHEMISTRY OF YOGURT

Yogurt is a product of the acidic fermentation of milk. The lactose in the milk is converted to lactic acid, which lowers the pH. When pH drops below 5, micelles of caseins, a hydrophobic protein, loses its tertiary structure due to the protonation of its amino acid residues. The denatured protein reassembles by interacting with other hydrophobic molecules, and this intermolecular interaction of caseins creates a structure that allows for the semisolid texture of yogurt (Zourari et al., 1992).

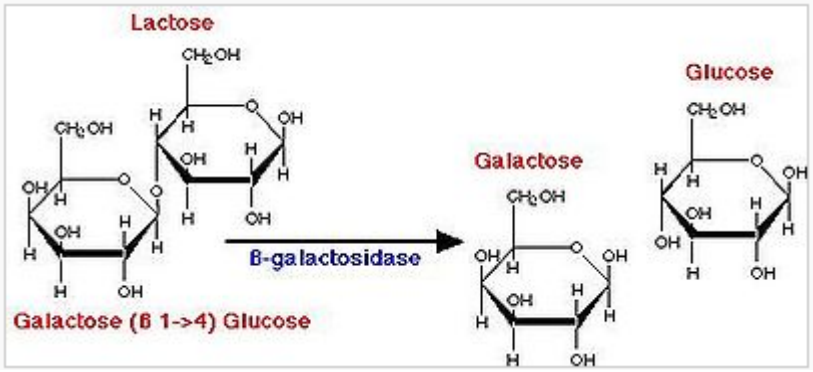


**Fig: 1.1, Lactose catabolism into glucose and galactose**

Yogurt production begins with the breakdown of lactose into glucose and galactose (Fig. 1), a process catalyzed by β-galactosidase. The glucose produced from this catabolic step then enters glycolysis, producing pyruvate. It has been proposed that yogurt bacteria utilize the Embden-Meyerhof-Parnas pathway of glycolysis (Fig. 2). Pyruvate then enters lactate fermentation, also

known as homolactic fermentation, as it produces only lactic acid molecules. In other types of fermentation, such as ethanolic or heterolactic fermentation, the production of ethanol leads to other fermented foods and beverages such as sauerkraut, kimchi, and wine.

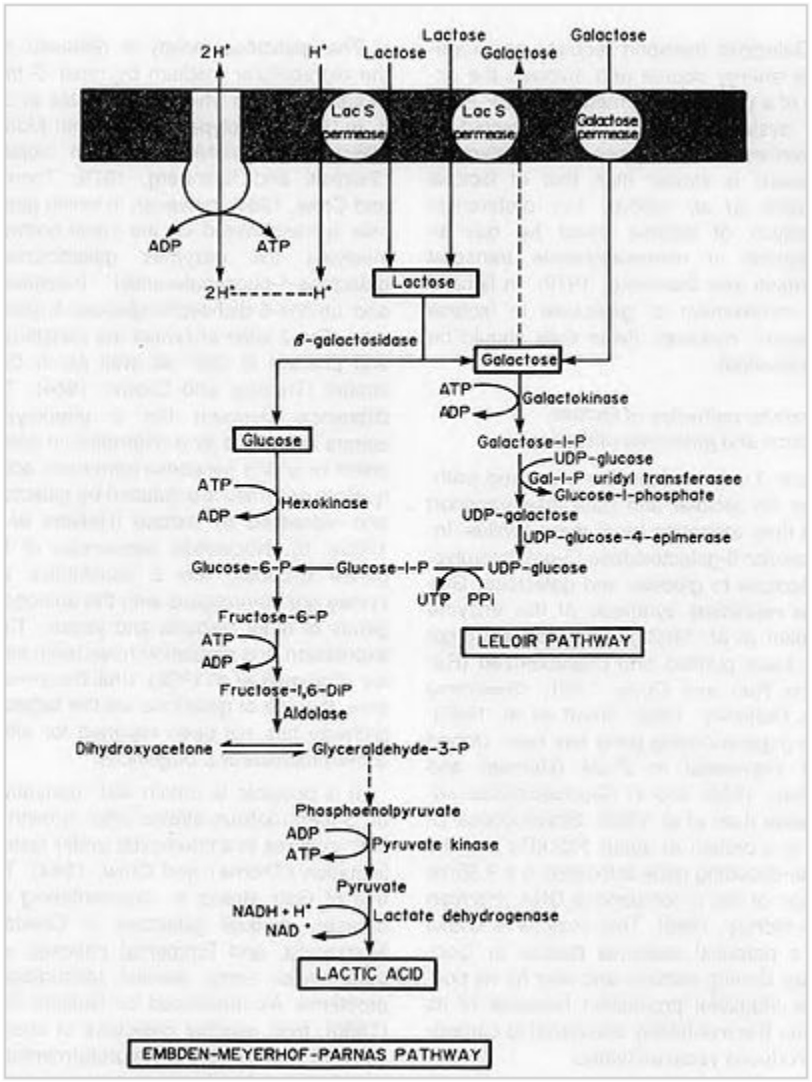


**Fig: 1.2, Embden-Meyerhof-Parnas pathway proposed to be used by*Streptococcusthermophilus* with the homolactic fermentation of pyruvate into lactic acid. This diagram also outlines the conversion of galactose into glucose to be used in the EMP pathway. Courtesy of Zourari et al., 1992.**

The production of lactic acid forms the basic structure and texture of yogurt. However, other molecules contribute to the taste of yogurt. These include acetaldehyde, an important flavor substance in yogurt, and tyrosine, a product of proteolytic activity, but can cause bitterness when the concentration is above 0.5 mg/ml (Guzel-Seydim, Sezgin, &Seydim, 2005).

## 1.3 HISTORY OF YOGURT

Milk fermentation is one of the oldest methods practiced by the human beings to preserve milk with an extended shelf life. The exact origination of milk fermentation is not clear; however, it seems that it is dated back to the dawn of the civilization. It has been reported that the early civilizations such as the Samarians, Babylonians, Pharoes and Indians were well advanced in agricultural and animal husbandry practices.

According to the Persian tradition, Abraham owed his fecundity and longevity to the regular ingestion of yogurt, and the emperor Francis I of France was said to be cured of severe diarrheaby consuming yogurt made of goat milk leading to introduce the health benefits of yogurt into the western world in 1542 .The first industrialized production of yogurt was taken place in 1919, in Barcelona, Spain at a company named Danone. Yogurt was firstly introduced to the USA in the early 20th century in the form of tablets especially designed for those with digestive intolerance. However, it became popular in the North America when Dannon, a small-scale yogurt factory started manufacture of yogurt in New York in 1940. Even though, yogurt has been evolved for centuries, it was subjected to a significant and dynamic evolution process in the 20th Century to originate a vast array of products.For instance, fruit yogurts, yogurts with fruit on bottom and blended yogurts were introduced in 1937, 1947 and 1963 respectively.

## 1.4 DIFFERENT NAMES OF YOGURT

It seems that the evolution process of yogurt has taken place in different regions of the world once it had been originated in the Central Asia. This might be the reason of having different types of yogurts and yogurt-like products in different names which are summarized in the Table1.1

**Table 1.1: Yogurt and yogurt-like products originated in different regions of the world**

| Region | Country/island or region of origin | Traditional name of the yogurt or yogurt-like product |
|---|---|---|
| **Europe** | Turkey | Jugurt/eyra/ayran |
| | Balkans | Kisselmleka/naja/yaourt |
| | Balkan mountains | Urgotnic |
| | Greece | Yiaourti |
| | Italy | Cieddu |
| | Sicily | Mezzoradu |
| | Sardinia | Gioddu |
| | Hungary | Tarho/taho |
| | Finland | Viili |
| | Scandinavia | Filmjolk/fillbunke/filbunk/surmelk/taettem |
| | Iceland | Skyr |
| | Yugoslavia | Gruzoviz |
| | Portugal | Iogurte |
| **Eurasia** | Russia | Donskaya/varenetes/kurugna/ryzhenka/guslyanka |
| | Turkestan | Busa |
| | Transcaucasia (South Caucasian state was once extended across the modern-day countries of Armenia, Azerbaijan, and Georgia) | Katyk |
| | Armenia | Mazun/matzoon,matsun, matsoni, madzoon |
| **Middle East and Asia** | Lebanon and some Arab countries | Leban/laban |
| | Egypt and Sudan | Zabady/zabade |
| | Iran and Afghanistan | Mast/dough/doogh |
| | Iraq | Roba/rob |
| | India | Dahi/dadhi/dahee |
| | Mongolia | Tarag |
| | Nepal | Shosim/sho/thara |

Note: Adapted and modified from Tamime and Robinson, 1999 [5]

## 1.5 DIFFERENT TYPES OF YOGURT

Yogurt can be categorized into two different groups namely, standard culture yogurt and bio- or probiotic yogurt. Standard yogurt refers to those made with *L. bulgaricus and S. thermophilus.* These bacteria said to be not actually inhabit gut; however able to stimulate the friendly micro

flora already present in the gut helping to maintain the general intestinal health. On the other hand, bio yogurts are manufactured by culturing beneficial microorganisms that claim to have numerous health benefits once ingested, typically the probiotic strains *of Bifidobacteria and L. acidophilus*. Unlike standard yogurt cultures, these probiotic strains are said to claim more specific health benefits and represent the types of friendly micro flora present in the gut. This type of yogurts are more popular and have a milder, creamier flavor and less acidic. Further, bioyogurts are claimed to aid in digestion and promote good health; however, these probiotic strains should remain live at adequate numbers to claim any health effect. Because of this reason, a term called "Live and Active Cultures" has been introduced recently which refers to the living microorganisms including standard yogurt cultures and probiotic cultures present in the yogurt at the time of manufacture.

Apart from this classification, the yogurt products available in the market are in a wide variety of flavors, textures and forms that suits a vast array of palates and meal occasions. These can be consumed either as a snack, dessert or a part of a meal. Different varieties of yogurt that can be categorized according to the physical and chemical nature, added flavors and post incubational processes are discussed in this section.

a) Based on the chemical composition of the product

Based on the fat content of yogurt, it can be categorized into three major varieties namely, regular yogurt, low-fat yogurt and non-fat yogurt. Regular yogurt is produced from the full fat milk which should contain at least 3.25% of milk fat. On the other hand, low-fat yogurt and non-fat yogurt are produced from low fat milk or partially-skim milk, and skim milk respectively. The fat content along with pH and titratable acidity of these three varieties of yogurt are shown in the Table

**Table 1.2: The composition of regular-, low-fat- and non-fat yogurt**

| Parameter | Regular Yogurt | Low-fat Yogurt | Non-fat Yogurt |
|---|---|---|---|
| Fat (%) | ≥3.25 | 0.5-2.0 | ≤0.5 |
| Solid Non Fat (%) | ≥8.25 | ≥8.25 | ≥8.25 |
| Titratable Acidity (%) | ≥0.9 | ≥0.9 | ≥0.9 |
| pH | ≤4.5 | ≤4.5 | ≤4.5 |

Adapted from FDA and Australia New Zealand Food Standard Code Recommendations [3, 24, 25, 26]

b) Based on the physical nature of the product

The physical nature of yogurt can be solid, semi-solid or fluid. Yogurts that are solid in nature (jelly-like texture) are called as set yogurt that is incubated and cooled in the final packaging. Whereas yogurts which are in semi-solid state and fluid nature called as stirred yogurt and fluid/drinking yogurt, respectively. Stirred yogurts are produced by incubating the mix in a tank followed by breaking by stirring prior to cooling and packaging. Drinking yogurts usually go through a homogenization process in order to reduce the particle size that assured hydrocolloid distribution and stabilization of the protein suspension.

c) Based on the flavor of the product

Addition of flavors would enhance the consumer appeal while produce a variety of products. Flavors can either be added immediately before homogenization or after the homogenization. Yogurts can be categorized into plain-, fruit- and flavored yogurt based on the particular flavor of the yogurt. Plain/Natural Yogurt This is the simplest and the least adulterated form of the yogurt made by lactic acid bacterial fermentation of pasteurized milk in order to produce its characteristic texture and flavor. In other words, it can be defined as

the plain and unsweetened fermented milk product containing no added color or any other additives. Therefore, it is closer to the nutritional value of milk which it is made of, and provides all of the benefits associated with fermentation while supplying fewer amounts of calories. Moreover, plain yogurt gives the pure yogurt taste and contains the richest calcium content among the yogurt products. Flavored Yogurts are available in a vast array of flavors including fruit (apple, apricot, black cherry, black currant, blue berry, lemon, mandarin, raspberry, strawberry, peach), cereal, vegetables, chocolate, vanilla, caramel, ginger, etc. In general, flavors are added to yogurt during production stage and theaddition of flavors not only results a wide array of tastes, but also increases sweetness of the product.

d) Yogurt related products

After the basic incubation process in the yogurt manufacturing, depends on the manufacturing processes employed such as mixing with other mixtures, heat treatment and drying, may results a range of yogurt products namely, pasteurized yogurt, UHT yogurt, dried yogurt, etc. Pasteurized and UHT Yogurt These types of yogurts are produced after the fermentation by subjecting to heat treatment with different time-temperature combinations. Although these types of yogurt products are produced by the manufacturers in order to prolong the shelf life the heat treatment may destroy considerable numbers of live and active cultures present, which would be a disadvantage when considering the health benefits of yogurt consumption. Frozen Yogurt The Pennsylvania Code defines frozen yogurt as a food which is prepared by freezing while stirring a pasteurized mix consisting of the ingredients permitted for ice cream and should contain not less than 3.25% milk fat, not less than 8.25% milk solids non fat and has a titratable acidity of at least 0.3% expressed as lactic acid.

Whereas the low fat version resembles more than 0.5% but less than 2% of milk fat with same amount of milk solid nonfat .

## 1.6 HEALTH BENEFITS OF YOGURT

Yogurt is considered as a nutrient dense food that contains essential nutrients such as protein, vitamins and minerals necessary for growth. Consumption of dairy products such as yogurt helps to improve the overall quality of the diet while increasing the chances of achieving nutritional recommendations such as Recommended Dietary Allowances of each nutrient in daily basis. For instance, milk products including yogurt is a rich source of calcium in bio-available form which is reported to provide 41% of the recommended daily requirement of Calcium for a 5-year old through a serving of 50 g of yogurt . It seems that the health benefits of fermented dairy products including yogurt are well-known for centuries as their health benefits are even mentioned in the Bible and the ancient books of Hinduism . Other than its rich nutritional profile, yogurt is claimed to have many health benefits. Lactose is the main carbohydrate found in milk which is a disaccharide composes of one molecule of glucose and galactose. Lactose is broken down to its simple sugars due to the action of the enzyme, lactase inside the gut. Inadequacy of secretion or interferences to the digestion process of lactase may pass undigested lactose into the large intestine which will then be fermented by colonic micro flora that results gastrointestinal symptoms such as flatulence, diarrhea and abdominal pain. This phenomenon is called as the lactose intolerance.

It has been reported that the lactose intolerance is associated with low calcium intake and bone mineral density most probably unnecessary exclusion of milk and dairy products from the diet. Therefore, it can be concluded that yogurt is effective for the individuals with lactose intolerance

to attain all the benefits of milk products without causing discomforts associated with hypolactasia. It is generally accepted that the optimum balance in the intestinal micro flora is associated with good nutrition and health. Further, *Lactobacilli and Bifidobacteria* are known to be the primary microbial strains associated with this balance. Available research findings suggest that maintaining favorable microbial profile through regular consumption of bio-yogurt results numerous therapeutic benefits. In 1908, the Russian scientist, Metchnikoff suggested that the prolonged life of the Bulgarians was associated with the regular consumption of fermented milk products with lactic acid bacteria.

Yogurt acts as a probiotic carrier food that is considered as an easy food to incorporate probiotics which results high probiotic viability. Bio-yogurt is considered to be an ideal source for the delivery of viable probiotic strains, *L. acidophilus* and *Bifidobacteriumbifidum* which are the most common probiotics used in the dairy industry. However, in order to attain the probiotic effect, it is reported the need of consuming adequate amounts of viable probiotic cells regularly which is known as the therapeutic minimum. Therefore, the consumption should be more than 100 g of bio-yogurt containing more than 106 cfu·mL-1 viable cells. Consumption of probiotics seems to be helpful to maintain good health, restore body vigor and combat intestinal disorders through the therapeutic and beneficial effects associated with them. Probiotics reported to have the therapeutic effects such as prevention of urogenital infections, alleviation of constipation, protection against diarrhea, prevention of infantic diarrhea, prevention of hypercholesterolemia, protection against colon/bladder cancer and prevention of osteoporosis. On the other hand, probiotics claimed to have other beneficial effects such as maintenance of normal intestinal flora, enhancement of the immune system, reduction of the lactose-intolerance and serum cholesterol levels, and enhance anti carc in ogenic activity. Some have recommended fermented milk

products to cure gastrointestinal disorders; for instance, Tissier has recommended the administration of Bifido bacteria to cure infantic diarrhea.

Moreover, yogurt is reported to be beneficial for the treatment of Inflammatory Bowel Disease (IBD) that includes gastrointestinal disorders such as Crohn's disease, ulcerative colitis and pouchitis. The VSL#3 (a mixture of four strains of lactobacilli including *L. casei, L. plantarum, L. acidophilus* and *L. delbrueckii ssp*. bulgaricus, three strains of bifidobacteria including *B. longum, B. breve and B. infantis* and one strain of *S. thermophilus*) found to be effective in maintaining remission in patients with chronic relapsing pouchitis and for the prophylaxis of pouchitis in patients who had ileo-pouch anal anastomosis for ulcerative colitis. On the other hand, Ishikawa et al. (2002) reported that the supplementation of *Bifidobacteria* fermented milk for 1 year was successful in maintaining remission and clamed beneficial preventive effects on the relapse of ulcerative colitis . In adiition, *Saccharomyces boulardii*, non pathogenic yeast, VSL#3 and EcoliNissle 1917 were found to be effective against Crohn's disease. Yogurt consumption is also reported to be effective in cytokine production, T-cell function and natural killer-cell activity, and thereby result an overall immunological enhancement

## 1.7 PROBIOTICS OF YOGURT

The word 'probiotic', derived from the Greek language, means 'for life' (Fuller, 1989) and has had many definitions in the past. Definitions such as 'substances produced by protozoa that stimulate the growth of another' or 'organisms and substances that have a beneficial effect on the host animal by contributing to its intestinal microbial balance' were used. These general definitions were unsatisfactory because 'substances' include chemicals such as antibiotics. The

definition of probiotics has since then been expanded to stress the importance of live cells as an essential component of an effective probiotic. Most recently, Huisin't Veld and Havenaar (1991) broadened the definition of probiotics as being 'a mono- or mixed- culture of live microorganisms which, applied to man or animal (e.g. as dried cells or as a fermented product), beneficially effects the host by improving the properties of the indigenous micro flora. This definition implies that probiotic products, for example bio-yogurt, contain live host by exerting beneficial effects in the gastrointestinal tract.

In order to identify the yogurt products with adequate amounts of beneficial live microorganisms, America's National Yogurt Association recently introduced the Live and Active Cultures Seal. Therefore, according to the National Yogurt Association's guidelines, the refrigerated products should contain at least 100 million live cultures per gram and the frozen products should contain at least 10 million live cultures per gram at the time of manufacture in order to obtain the live and active culture seal.

## 1.8 NUTRITIONAL PROFILE OF YOGURT

Yogurt is a highly nutritious and easily digestible dairy product which is a rich source of more than ten essential nutrients in particular, certain minerals and vitamins. The nutritional composition of yogurt can be varied according to the strains of starter culture used in the fermentation, type of milk used (whole , semi or skimmed milk), species that milk is obtained (bovine, goat, sheep), type of milk solids, solid non-fat, sweeteners and fruits added before fermentation as well as the length of the fermentation process.

**Table1.3: Nutritional composition of different varieties of yogurt (per 100 g)**

| *Component* | *Whole milk yogurt* | *Low fat yogurt* | *Non fat yogurt* | *Greek-style yogurt* | *Drinking yogurt* |
|---|---|---|---|---|---|
| Energy (kcal) | 79 | 56 | 54 | 133 | 62 |
| Protein (g) | 5.7 | 4.8 | 5.4 | 5.7 | 3.1 |
| Carbohydrate (g) | 7.8 | 7.4 | 8.2 | 4.8 | 13.1 |
| Fat (g) | 3.0 | 1.0 | 0.2 | 10.2 | Trace |
| Thiamin (mg) | 0.06 | 0.12 | 0.04 | 0.12 | 0.03 |
| Riboflavin (mg) | 0.27 | 0.22 | 0.29 | 0.13 | 0.16 |
| Niacin (mg) | 0.2 | 0.1 | 0.1 | 0.1 | 0.1 |
| Vitamin B6 (mg) | 0.10 | 0.01 | 0.07 | 0.01 | 0.05 |
| Vitamin B12 (mg) | 0.2 | 0.3 | 0.2 | 0.2 | 0.2 |
| Folate (µg) | 18 | 18 | 8 | 6 | 12 |
| Carotene (µg) | 21 | Trace | Trace | Trace | Trace |
| Vitamin D | 0 | 0.1 | Trace | 0.1 | Trace |
| Potassium (mg) | 280 | 228 | 247 | 184 | 130 |
| Calcium (mg) | 200 | 162 | 160 | 126 | 100 |
| Phosphorus (mg) | 170 | 143 | 151 | 138 | 81 |

Source: The Dairy Council, 2013[20]

However, the general composition of yogurt is more or less similar to that of milk. Therefore, yogurt is a rich source of milk proteins, carbohydrate, minerals such as calcium and phosphorous, and vitamins such as riboflavin (B2), thiamin (B1), cobalamin (B12), folate (B9), niacin (B3) and vitamin A. Milk proteins available in yogurt is in high quality due to its high biological value and provide almost all essential amino acids necessary to maintain good health. In addition, milk proteins available in yogurt contain higher content of proline- and glycine-

contain amino acids than that in whole milk while performing additional body functions such as enhancing calcium absorption and boosting the immune system. Lactose is the main carbohydrate found in yogurt as in other dairy products. Lactose content in raw milk is about 4.6%. However, the original lactose content in milk is lowered by 20-30% during the fermentation process as the lactose coverts into its simple forms of glucose and galactose due to the metabolic activity of lactic acid bacteria. Fat content of yogurt is highly dependable on the fat content of the original yogurt mixture. According to the USDA specifications for yogurt, low-fat yogurt and non-fat yogurt, fat content varies from 0.5-3.25%. However, the fat content of yogurt is highly subjective as some products; for instance Greek style yogurt contains a high fat content as high as 10%. Unlike milk,processes that are employed in yogurt manufacturing such as homogenization and fermentation result in breakdown of some amount of fat into easily digestible and absorbable fatty acids. Vitamins and minerals found in milk and dairy products are in bio-available from where they are available for absorption and use by body. Yogurt as of other dairy products is an exceptional source of several B vitamins in particular, riboflavin and thiamin. It is reported that a 150 serving of whole milk plain yogurt and low-fat plain yogurt will provide 31% and 30% of an adult's daily riboflavin requirement respectively whereas the same amount of serving of each type of yogurts will provide 23% and 45% of an adult's daily thiamin requirement . However, vitamin B12 and B6 are found in significantly lower concentrations than that in milk as *Streptococcus thermophilus* uses these B vitamins for its metabolism. Folic acid/folate content of yogurt can be varied depending on the composition of lactic acid bacteria used as some of the LAB species such as *S. thermophilus*and *Bifidobacteria* synthesize certain vitamins including folate by their own.

## 1.9 YOGURT'S STARTER CULTURE

Specific microorganisms, known as starter cultures, are what determine the body, texture, and flavour of the final yogurt product . These starter cultures initiate every change in the substrate and preserve it by suppressing spoilage and presence of pathogenic flora they can preserve food through the synthesis of lactic acid and antimicrobial substances. This is because the organic acids not only lower the pH but are also toxic for many microorganisms which are what prolongs the shelf life of the substrate It is interesting to note that the preservation and creation of new food products such as beer, wine, cheese, bread and sausages has been widely used long before the discovery of microorganisms.

Because of the starter cultures impact on creating specific products, only fermentation by the two species *Streptococcus thermophilus* and *Lactobacillus delbrueckii subsp. bulgaricus* produce yogurt. Fermentation of milk by any other cultures results in a different dairy product such as butter or cheese. These two species are lactic acid producing bacteria (LAB) and are gram-positive rods and cocci respectively. They do not form spores and are catalase negative, obligatory fermentative, microaerophilic and non-motile. Isolating the specific starter cultures *''L. bulgaricus''* and *''S. thermophilus''* can be done using differential and selective growth agar mediums. Once the starter cultures have been isolated they can be added into a purified milk sample that is void of antibiotics and contaminants which would cause the product to be different than intended

## 1.10 PACKAGING MATERIALS OF YOGURT

A wide range of packaging materials is used for yogurt products (Brody, 2006; Nilsen et al., 2002; Tamime and Robinson, 1999e).

The most popular material by far in current use for spoonable yogurt (either set or stirred) is thermoformed HIPS in the form of small cups or larger tubs, with either an aluminum foil/plastic laminate or a paper/plastic laminate heat-seal lid or closure. These containers may be produced in form–; ll–seal packaging machines or be delivered preformed from packaging materials suppliers. This also helps in heating and softening the HIPS sheet for thermoforming when radiant heating is used (Robertson, 2006b). White is most often used, but other colours are also common. A HIPS sheet for forming into small containers in form–; ll–seal ;llers is usually around 1.0–1.4 mm thick and the containers produced have wall thicknesses of around 0.2 mm. Preformed HIPS containers may have wall thicknesses around 0.25–0.5 mm. Injection-molded polypropylene (PP) containers can be around 0.5–1 mm in wall thickness [Pritchard W.J. (www.itechnik.com.au) 2008, personal communication].

Rectangular paperboard cartons or cups (with or without an aluminum foil layer), glass containers, PP, and blow-molded high density polyethylene (HDPE) containers are all in common use; poly(ethylene terephthalate) (PET), polyvinyl chloride (PVC), polyvinylidene chloride copolymer (PVdC), and polylactate (PLA) have also been used or proposed, and for some specialty products in some markets, ceramic containers have been used (Frederiksen et al., 2003; Tamime and Robinson, 1999e).

For pasteurized, spoonable yogurt products, laminated materials are desirable if a long shelf life is needed, with some having shelf lives of 4–6 months at ambient temperatures. For these products, a low water vapor transmission rate (WVTR) is required to stop the product losing water during shelf life. A good O2 barrier will help to protect the product from oxidation, and a good light barrier will help to delay fading of light-sensitive colors and to avoid light-induced oxidation.

Drinking-yogurt products are becoming increasingly popular. For these products, the most popular containers are HDPE bottles sealed with either aluminum foil laminate heat-seal closures or with low density polyethylene (LDPE) snap or screw caps. Bottles made from other plastics (e.g., PET) may also be used. For bottles it is common for shrink-sleeves to be used for labeling and decoration. For long-life, heat-treated drinking-yogurt products, plastic/alufoil/paperboard laminate cartons with good water vapor, and light barrier properties are also often used.

## 1.11 AIMS AND OBJECTIVES

The aim of this project will mainly focus in isolating the better strain of *Lactobacillusspp* (starters) from the local brands available and found in Bangladesh to maximize the yogurt production with the following categories like; production of yogurt will minimize the time consumption, overall cost and their molecular and biochemical characterization.

- For healthy dietary, yogurt is considered as a good nutritional source. So, the research conducted to produce a good quality of yogurt in terms of texture, appearance, colour and taste
- To produce a good quality of yogurt in terms of low cost as well as from a better bacterial strain.

# CHAPTER TWO: REVIEW OF LITERATURE

# Review of Literature

**Alli*et al*.,2010** studied the microbial assessment and microbiological quality of somecommercially prepared yogurt retailed in Ibadan, Oyo State, Southwestern of Nigeria.Yogurts are ready to drink foods commonly taken for energy production and for health inNigeria but there is paucity of studies done to evaluate their food safety. Therefore this studywas carried out to determine the miroflora of some available yogurts sold in Ibadan. Twentytypes of commercially prepared yogurt products were purchased, from Ibadan in Oyo State,Nigeria and its' environs, transported, processed and analyzed using standard laboratorymethods. A total of 25 different organisms were isolated from 20 yogurt samples with*Lactobacillus bulgaricus, Streptococcus lactis and Saccharomyces cerevisiae*each being themost frequently isolated with frequency of 16.0%. They were also tested to show if their pHproduction was lactose-dependent. There were significant decline in pH in tryptone soy broth($t = -13.88$, $p<0.05$), peptone with lactose ($t = -16.61$, $p<0.05$), and peptone containing milkand lactose ($t = -10.41$, $p< 0.05$). This study has shown that most yogurts in Ibadan containprobiotics isolates including *L. bulgaricus, S. lactis and S. cerevisiae*, which are therefore,beneficial for human consumption.

**Braz*et al.*, 2014**studied the suitability of Transgalactosylated oligosaccharides-mupirocin lithium salt (TOS-MUP) and MRS-clindamycin-ciprofloxacin (MRS-CC) agars, along with several other culture media, for selectively enumerating bifidobacteria and lactic acid bacteria (LAB) species commonly used to make fermented milks. Pure culture suspensions of a total of

13 dairy bacteria strains, belonging to eight species and five genera, were tested for growth capability under various incubation conditions. TOS-MUP agar was successfully used for the selective enumeration of both *Bifidobacteriumanimalis* subsp. *lactis* BB-12 and *B. breve* M-16 V. MRS-CC agar showed relatively good selectivity for *Lactobacillus acidophilus*, however, it also promoted the growth of *Lb. casei* strains. For this reason, MRS-CC agar can only be used as a selective medium for the enumeration of *Lb. acidophilus* if *Lb. casei* is not present in a product at levels similar to or exceeding those of*Lb. acidophilus*. Unlike bifidobacteria and coccus-shaped LAB, all the lactobacilli strains involved in this work were found to grow well in MRS pH 5.4 agar incubated under anaerobiosis at 37 °C for 72 h. Therefore, this method proved to be particularly suitable for the selective enumeration of *Lactobacillus* spp.

**Lee *et al.*,2010**studied the formation and physical properties of yogurt. Yogurt gels are atype of soft solid, and these networks are relatively dynamic systems that are prone tostructural rearrangements. The physical properties of yogurt gels can be qualitativelyexplained using a model for casein interactions that emphasizes a balance between attractive(e.g., hydrophobic attractions, casein cross-links contributed by calcium phosphate nano-clusters and covalent disulfide cross-links between caseins and denatured whey proteins) andrepulsive (e.g., electrostatic or charge repulsions, mostly negative at the start of fermentation)forces. Various methods are discussed to investigate the physical and structural attributes ofyogurts. Various processing variables are discussed which influence the textural properties ofyogurts, such as total solids content, heat treatment, and incubation temperatures. A betterunderstanding of factors contributing to the physical and structural attributes may allowmanufacturers to improve the quality of yogurt.

**Ana *et al.*, 2006** studied the simultaneous effects of total solids content, milk base, heattreatment temperature and sample temperature on the rheological properties of plain stirredyogurt. Response surface methodology was used to establish a relationship between totalsolids content, milk base, heat treatment temperature, and sample temperature, andconsistency index, flow behavior index, and apparent viscosity of plain stirred yogurts.Statistical treatments resulted in developments of mathematical models. All samplespresented shear thinning fluid behavior. The increase of the content of total solids (9.3-22.7%) and milk base heat treatment temperature (81.6-98.4 °C) resulted in a significantincrease in consistency index and a decrease in flow behavior index. Increase in the sampletemperature (1.6-18.4 °C) caused a decrease in consistency index and increase in flowbehavior index. Apparent viscosity was directly related to the content of total solids.Rheological properties of yogurt were highly dependent on the content of total solids in milk.

**Mohammed *et al.*, 2007** studied the stirred yogurt samples produced by Blue Nile DairyCompany. The stirred yogurt was purchased from the market (sixty samples). They weretransported to the Faculty of Animal Production, laboratory to assess the chemical andmicrobiological content and shelf life of stirred yoghurt. Chemical and microbiologicalexaminations were carried out on 1, 2, 4, 6, 8 and 10 days of manufacturing. Ten samplesfrom six batches were examined for fat, protein, lactose, ash, total solids, solids-non-fat andmeasurement of pH, acidity, enumeration of lactic acid bacteria and total bacterial counts.The chemical analysis for stirred yogurt results showed that the means were: fat 2.17-4.51,protein 2.66-3.97, lactose 8.45-9.58, ash 0.73-0.92, total solids 15.75-16.57, solids-non-fat11.73-13.58, acidity 0.93-1.12, pH 3.81-4.19, and viscosity 61.98-6.95. The highest logcounts for Strep to

coccusthermophilus and Lactobacillus bulgaricus were 7.15-7.51 and 7.21-7.50, respectively. The log total bacterial count (cfu) is 7.27-7.68. The results indicated thatthe storage period had significant (P> 0.001) effect on the chemical composition except onthe total solids and viscosity. Also there was significant (P>0.01) effect of the storage periodon the microbiological tests.

**Reyhan*et al.*, 2008** researches about viable Lactobacillus bulgaricus and Streptococcusthermophilus numbers in the market yogurts.The industrial production of yogurt isincreasingly developed in the world. Yogurt is a fermented milk product obtained fromfermentation of Lactobacillus bulgaricus and Streptococcus thermophilus strains. In Turkey,yoghurt is produced by the two ways; one of them is a traditional method without usingstarter culture in small dairy plants and the second production method by using industrialstarter culture in modern plants. In this study yogurt samples were collected from the localmarkets which were produced traditional process and produced in modern plants by additionof starter cultures, their viable L. bulgaricus and S.thermophilus bacteria numbers, coliform,Escherichia coli, yeast and mould counts, pH values were determined and compared eachother. Yogurts have pH values between 3.95-4.23, viable S. thermophilus and L.bulgaricusnumbers were determined between 10 7 -10 8 cfu/g for yogurts producing with starter culture,10 5 -10 6 cfu/g and 10 6 -106 7 cfu/g for yogurts producing with traditional methods,respectively. Coliforms, E. coli, yeast and mould counts have at low numbers for all yogurtsamples. As the result, yogurts which are produced by starter cultures have high numbers ofyogurt bacteria means that yogurts produced by using starter cultures have higher therapeuticand/or antimicrobial properties beside of their organoleptic characteristics.Importance

ofyogurt production by using starter cultures should be known and advantages of using startercultures in fermentation products should be stated.

**Donovan*et al.,* 2014** discussed on the health effects of yogurt. Yogurt has been part of thehuman diet for thousands of years, and during that time a number of health benefits havebeen associated with its consumption. The goal of the First Global Summit on the HealthEffects of Yogurt was to review and evaluate the strength of current scientific knowledgewith regard to the health benefits of yogurt and to identify areas where further research isneeded. The evidence base for the benefits of yogurt in promoting bone health, maintaininghealth throughout the life cycle, improving diet quality, and reducing the incidence ofchronic diseases, such as obesity, metabolic syndrome, and cardiovascular disease, waspresented. When assessing a complex food matrix, rather than specific nutrients, scientistsand consumers are faced with new challenges as to how a food item's quality or necessitywould be judged as part of an individual's whole diet. To tackle this challenge, speakersdescribed methods for assessing the nutrient density of foods and its application to yogurt,use of yogurt for lactose intolerance, and the cost-effectiveness of yogurt and dairy productsin reducing health care expenses

**Zahoor*et al.,* 2002** studied the viability of Lactobacillus bulgaricus as yogurt cultureunderdifferent preservation methods. In present study, Lactobacillus bulgaricus (yogurtstarter culture) was isolated from indigenous sources and preserved by three differentmethods namely on agar slopes, under oil and in liquid form conditions using MRS medium.Best method of preservation was suggested on the basis of viability, morphology and

Gram'sstaining ability of culture during storage of two months. Viability checks were made at 0, 15,30, 45 and 60 days of storage. Under oil preservation method was found to be the bestmethod for maintenance and preservation of starter culture.

**Istikhar*et al.,* 2009**discussed thequality comparison of probiotic and natural yogurt. Thestudy was conducted to evaluate and compare the quality of probiotic and natural yogurt.Several samples of probiotic and natural yogurt were bought from supermarkets in Middlesborough (UK) and analyzed for physico-chemical, microbiological and organo lepticproperties. Physico-chemical analysis showed that probiotic yogurts have more pH, fat andsolid not fat (SNF) contents compared to natural yogurt. While natural yogurts have higher Total Titrable Acidities (TTA) and total solids contents, compared to probiotic yogurts. Organoleptically, probiotic yogurt was found more acceptable compared to natural yogurt.

However, the fat contents of natural yogurt are lower and that might affect the overallacceptability of the yogurt. Similarly, an increase in the TA of the natural yogurt might affectthe quality of the product. Microbiological analysis found no significant variation in totalviable count between probiotic and natural yogurt.

# CHAPTER THREE: MATERIALS AND METHOD

# Materials and Methods

## 3.1 COLLECTION OF MARKET SAMPLES

### 3.1.1 Milk

For doing this project three type of milk was chosen. Among them two were raw milk which needed to be heated up and then use another one is pasteurized UHT milk which was ready to use.

a) Milkvita: Most popular and available packaged raw milk of Bangladesh. It is produced by the Bangladesh milk producer's co-operative union which is supervised under government.

b) Aarong milk: "Aarong" milk is a product of BRAC dairy under most popular NGO of Bangladesh. It also came as afull cream raw milk.

c) PRAN UHT milk :**"PRAN"** is currently one of the most admired food & beverages brands among the millions of people of Bangladesh and other 106 countries of the world where **PRAN** Products are regularly being exported.. Shelf safe milk is what the industry calls UHT or Ultra High Temperature, meaning that the milk has been pasteurized at a higher temperature, but for a shorter time, to preserve taste and nutrition. UHT Milk is the most common product, for ready to use/ drink function. It comes in a "30 fino aseptic packaging".

### 3.1.2 Starter Culture

Here, many commercially available branded yogurts have been used. It has been limited to the branded yogurt because we can determine the starter quality and source as well as overall expected also most popular taste of these region. So for this, we have collected samples from Rosh, Aarong, Bikrampur, Premeium Sweets, Igloo, Vagyakul, Nimtoli, Solna, Moronchand, Star, Mughal sweets, Muslim.

### 3.1.3 Combination and labeling of different starter culture

Above samples were re-combined and labeled for utmost result to be taken place. At nitial stage 32 samples were recombined using Aarong and Milkvita's raw milk. In the second stage another round of eight recombined samples were and used it with Pran UHT milk. Name of all 40 recombined samples were enlisted in table 4.1 and table 4.2 respectively.

## 3.2 **PLACE OF EXPERIMENT**

The experiment took place on Microbiology and Biotechnology Laboratory of BRAC University, building number-ub02. First screening took place on $10^{th}$ floors lab under lab supervisor Ms. Asma Afzal and furthermore experiment took place on $18^{th}$ floor, under lab supervisor Ms. Shammi Akhter.

## 3.3 FERMENTATION OF YOGURT

Manufacturing of yogurt is an ancient technique, which dates back to thousands of years, and the knowledge has transferred generation to generation. However, during the last few decades, it became more rational due to improvement of various fields such as microbiology, biochemistry

and food engineering. Today it is a complex activity combined with art and science. The generalized process of yogurt making is comprised of modifying the original composition of milk, pasteurizing the yogurt mix, fermentation at thermophilic temperatures (40-45 ºC), cooling and addition of fruits and flavors. The production steps in manufacture of stirred- and set yogurt are illustrated in the Figure

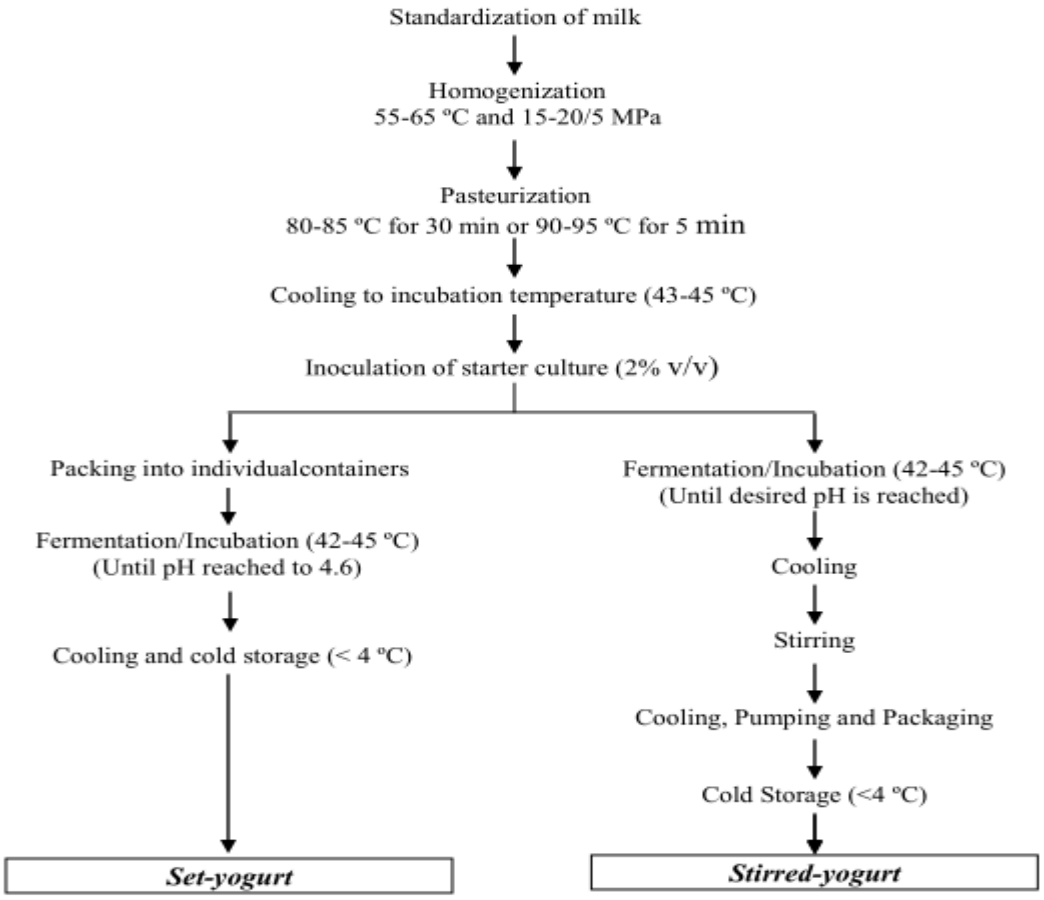


**Figure 3.1: Manufacturing process of set- and stirred-yogurt (Adapted from Lee and Lucey, 2010)**

### 3.3.1 Ingredients for yogurt manufacture

Yogurt is made with a variety of ingredients including milk, sweeteners, stabilizers, fruits, flavors, and bacterial cultures. Milk is the main ingredient used in yogurt

manufacturing. The type of milk to be used depends on the variety or type of theyogurt that will be prepared. For instance, whole milk is used for full fat/regular yogurt, partially skimmed milk is used for low fat yogurt and skimmed milk is used for nonfat yogurt. Cream/butter fat is used to adjust the fat content whereas skim milk powder, whey protein concentrate are used to elevate the total solid content of the yogurt mix. Stabilizers are usually added to the mix in order to increase the body and texture leading to an increase in firmness, prevents whey separation/syneresis, and aids in uniform distribution of ingredients. In addition, sweeteners are added to increase the flavor and consumer appeal.

### 3.3.2 Milk Standardization

Milk solid content of yogurt seems to be varied from 14-15% in commercial yogurt products and the minimum milk solids non fat content varies from 8.2-8.6% according to the standards andregulations of many countries. According the Codex Alimentarius Commissionyogurt should have a minimum protein content of 2.7% and a maximum fat content of 15% . In order to achieve this, the FAO/WHO standards specifies that milk should be standardized with the minimum SNF and milk fat content of 8.2% and 3% respectively for yogurt manufacture. The average composition of bovine milk comprised of 4.5% lactose, 3.3% protein, 3.5% of fat and 0.7% mineral matter. Therefore, it is obvious that the composition of yogurt is varied according to the variety, and yogurt mixture should therefore standardize accordingly in such a way that produce an end product with not less than 2.7% of protein and less than 15% of milk fat with a titratable acidity not less than 0.3% expressed as percentage of lactic acid. Stabilizers such as

pectin and gelatin are added to the yogurt mix in order to attain the characteristic properties of yogurt namely, texture, mouth feel, appearance, viscosity and to inhibit the whey separation. However, both over-stabilization and under-stabilization may cause quality defects as the over-stabilization results a“jello-like” springy body of yogurt, whereas the under-stabilization causes “runny body” or whey separation.

### 3.3.3 Homogenization

Homogenization treatment reduces the diameter of fat globules to less than 1µm and ensures uniform distribution throughout the food matrix, thus considered as an important processing step especially for yogurt with high fat content. Consequently, it results no distinct creamy layer on surface of the yogurt and improves consistency of the yogurt. Homogenization is accomplished by using a homogenizer or viscolizer where the milk is forced through small openings at a high pressure in which the fat globules are broken up due to the shearing forces. Typically, milk is homogenized using pressures of 10-20 and 5MPa in first and second stages, respectively for over 10-17 min. More recently, ultra-high pressure homogenization has been introduced to the commercial yogurt manufacture leading to an increase in yogurt firmness and water holding capacity comparatively to that of the conventional homogenization process.

### 3.3.4 Heat Treatment

It is generally considered that the heat treatment of milk is an essential step in yogurt manufacturing process that greatly influences the microstructure and physical properties of yogurt. Heat treatment has a number of beneficial effects as it will destroy the

microorganisms present in milk or yogurt mixture which can potentially interfere with the controlled fermentation process, will denature the whey proteins that will give the final product a better body and texture, and will release the compounds in milk that stimulate growth of the starter culture microorganisms. In addition, it will help some ingredients to achieve the required state to form gels and protein lattice, that affects the final texture and viscosity of the product while aids in removing dissolved oxygen in the milk and thereby assists the starter culture growth as they are sensitive to oxygen. Heat treatment is a continuous- or batch-process involves heating of milk to relatively high temperature and hold in there for pre-determined time period. The time-temperature combinations for the batch heat treatments that are commonly employed in the commercial yogurt making include 85 º for 30 min and 90-95 ºC for 5 min. Alternative time-temperature combinations available for the milk pasteurization are summarized in the Table2.

**Table 3.1: Time-temperature combinations for milk pasteurization**

| Type of Pasteurization | Process | Temperature (ºC) | Holding time |
|---|---|---|---|
| Low Temperature Long Time (LTLT) | Batch | 62.8 | 30 min |
| High Temperature Short Time (HTST) | Continuous | 71.7 | 15 s |
| Higher Heat Shorter Time (HHST) | Continuous | 88.3 | 1 s |
| Ultra-pasteurization | Continuous | 137.8 | 2 s |
| Ultra High Temperature (UHT) | Aseptic | 135-150 | 4-15 s |

Source: Food and Drug Administration, 2011 [19]

Despite the time-temperature combination used, it is a must to fulfill the minimum requirement to destroy the most heat resistant pathogen currently recognized in milk, Coxiella burnetii that cause Q-fever in humans. Heat treatment of milk is important to destroy unnecessary pathogenic organisms and enzymes present in milk.

### 3.3.5 Inoculation and Fermentation

After the heat treatment, the yogurt mixture is cooled to 43-46 ºCprior to the addition of yogurt starter culture bacteria at aconcentration of about 2 % (v/v). This temperature range isoptimal for the thermophilic microorganisms used in the yogurt starter culture. The typical standard yogurt culture consistsof *S. thermophilus*and *L. delbrueckii*subsp. *bulgaricus* in 1:1ratio. Inoculation of starter cultures usually takes place in asealed hygienic stainless steel vessel. However, the place offermentation is different to each other in set-and stirred yogurtmanufacture. It is usually occurred in individual containers andin large hygienic stainless steel vats in set- and stirred yogurtmanufacturing processes, respectively. Incubation temperature ismaintained and monitored at optimal level throughout thefermentation process for few hours (2.5-3 h) until the pH andacidity reached their desired levels prior to discontinue thefermentation process by rapid cooling. During the fermentationprocess, due to the metabolic activity of the lactic acid bacteriaused, lactose converts into lactic acid which coagulates milkproteins along with the production of certain volatile compoundsthat gives its characteristic flavor and aroma.

### 3.3.6 Cooling

When yogurt has reached the desired pH (4.5-4.6), it will thenoften blast chilled to refrigerated temperatures (<10 ºC) in orderto stop the fermentation process and thereby stops further aciddevelopment. In the manufacture of set-yogurt, yogurts aredirectly transferred to a cold store or blast chilled in coolingtunnels. On the other hand, in the manufacture of stirred-yogurt,cooling is first performed by agitating the coagulum in the jacketed fermentation vat in order to produce smoothenedproduct before filling to

containers According to the USDASpecifications, after the final steps in manufacturing and/orpackaging, the yogurt should be cooled and maintained attemperatures less than 7.2 ºC

## 3.4 QUALITY ASSESMENT BY SENSORY AND ORGANOLEPTIC EVALUATION OR PHYSICAL TEST (HEDONIC SCALE)

The most widely used scale for measuring food acceptability is the 9-point hedonic scale. David Peryam and colleagues developed the scale at the Quartermaster Food and Container Institute of the U.S. Armed Forces, for the purpose of measuring the food preferences of soldiers. The scale was quickly adopted by the food industry, and now is used not just for measuring the acceptability of foods and beverages, but also of personal care products, household products, and cosmetics.

**Table3.2: Hedonic Scale**

| 9-Point Hedonic Scale | |
|---|---|
| 9 | Like Extremely |
| 8 | Like Very Much |
| 7 | Like Moderately |
| 6 | Like Slightly |
| 5 | Neither Like nor Dislike |
| 4 | Dislike Slightly |
| 3 | Dislike Moderately |
| 2 | Dislike Very Much |
| 1 | Dislike Extremely |

It was invented for the purpose of measuring the food preferences of soldiers of US Army. Due to its popularity it is now not only used for food industry but as well as for personal care products, household products, and cosmetics. The verbal anchors of the scale were selected so that the psychological distance between successive scale points is approximately equal. This equal-interval property helps justify the practice of analyzing the responses by assigning

successive integer values (1, 2, 3, … up to 9) to the scale points and testing differences in average acceptability using parametric statistics. The reliability, validity and discriminative ability of the scale was proven in food acceptance tests with soldiers in the field and in the laboratory, as well as in large-scale food preference surveys.

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## 3.5 MICROBIOLOGICAL TEST

### 3.5.1 Determination of the Bacterial Load

#### 3.5.1 a) Media Used

For carrying out yogurts differentiation and culturing it, we have used Nutrient Agar (NA) and de Man, Rogosa and Sharpe agar (MRS). They are described below.

**MRS(de Man, Rogosa and Sharpe agar) agar:** The MRS formulation was developed by de Man, Rogosa and Sharpe to replace a variable product (tomato juice) and, at the same time, provide a medium which would support good growth of lactobacilli in general, even those strains which showed poor growth in existing media. MRS medium is superior to the tomato juice medium of Briggs and the meat extract tomato juice medium of de Man. It gives more profuse

growth of all strains of *lactobacilli*, especially the difficult and slow growing strains of*Lactobacillus brevis* and *Lactobacillus fermenti.*

MRS Agar and Broth were designed to encourage the growth of the `lactic acid bacteria' which includes species of the following genera: *Lactobacillus, Streptococcus, Pediococcus* and *Leuconostoc*. All these species can produce lactic acid in considerable amounts. They are Gram-positive, catalase and oxidase negative and are fastidious in their nutritional requirements. Growth is enhanced considerably by microaerobic conditions. Generally the `lactic acid bacteria' show delayed growth and smaller colony size than other micro-organisms. They may be overgrown in non-selective media, especially if incubation is required for 2-4 days.

MRS medium is selective for lactobacilli but some growth of leuconostocs and pediococci may occur. Selectivity can be altered by pH adjustment. Lactobacilli will tolerate lower pH levels than streptococci (pH 5.0-6.5) with pediococci and leuconostocs growing best within this range. Inhibitors of the main groups of competitor microflora include thallous acetate, sodium acetate, sorbic acid, acetic acid, sodium nitrite, cycloheximide and polymyxin. These substances can be used at varying concentrations and combinations but, inevitably, a compromise has to be reached between selectivity and productivity of the organism(s) sought. MRS Agar with sorbic acid has been described. This is MRS medium with its pH reduced to 5.7 and 0.14% sorbic acid added (equating to 0.2% potassium sorbate).

An evaluation of media for selective enumeration of *Lactobacillus acidophilus* and *Bifidobacterium*species showed that minor adjustments to the basic formula of MRS Agar can readily be made to optimise its performance for determining the content of *Lactobacillus acidophilus* and *Bifidobacterium*spp. in the presence of other lactic acid bacteria

which are present in yoghurt.*Lactobacilli* are microaerophilic and generally require layer plates for aerobic cultivation on solid media. Submerged or surface colonies may be compact or feathery, and are small, opaque and white.

Technique:

1. Products to be examined for *lactobacilli* content are macerated or diluted in a diluent such as quarter-strength Ringer solution, and further dilutions are made in MRS Broth.
2. Diluted sample in 1ml volumes are added to sterile dishes, and molten MRS Agar (45°C) is poured into the dish and mixed thoroughly.

3. When the medium has set, another layer of uninoculated MRS Agar is poured over the surface to produce a layer-plate.

4. Plates are incubated as described below. It is important that adequate moisture vapour is present in the atmosphere above the agar because drying of the plates during incubation will concentrate the selective factors on the surface and make the medium inhibitory. The presence of carbon dioxide stimulates growth and plates should be incubated in an atmosphere of 5% $CO_2$.

Incubation carried out under anaerobic or microaerophilic conditions:To identify the presumptive Lactobacillus colonies, select isolated colonies on the agar medium. Stain a smear from each and pick off into MRS Broth (CM0359), individually. An advantage of this broth is that any other micro-organisms, originally lying dormant in the selective agar, are not given the opportunity to multiply, as may occur in a non-selective broth. Incubate the broths at temperatures and times similar to those used for the MRS Agar; they can then be examined microscopically and further sub-cultured to MRS Agar for subsequent confirmation and identification of species.

Storage conditions and Shelf life**:** Store the dehydrated medium at 10-30°C and use before the expiry date on the label. Store the prepared plates at 2-8°C.

Appearance **:**

Dehydrated medium: Dark straw coloured powder

Prepared medium: Amber coloured gel

**Nutrient Agar**is used for the cultivation of bacteria and for the enumeration of organisms in water, sewage, faeces and other materials.

Composition (Ingredients Gms / Litre)

Peptic digest of animal tissue 5.000

Beef extract 3.000

Agar 15.000

Final pH ( at 25°C) 6.8±0.2

Directions : Suspend 23.00 grams in 1000 ml distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. If desired, the medium can be enriched with 5 - 10% v/v sterile defibrinated bloods Mix well and pour into sterile Petri plates.

Principle And Interpretation : Nutrient Agar is a basic culture medium used for maintenance or to check purity of subcultures prior to biochemical or serological tests from water and Dairy. This medium may be used as slants or plates for routine work with non-fastidious organisms. Nutrient Agar, pH 6.8 has relatively simple formulation which provides the necessary nutrients for the growth of many microorganisms which are not very fastidious. Many bacteria have the optimum pH growth range of 6.6 to 7.0. Wetmore and Gochenour maintained cultures of

Malleomyces and Pseudomonas on Nutrient Agar to which glycerol was added. Greenberg and Cooper employed this medium in cultivation of Staphylococci for the preparation of vaccines and antigens. Nutrient Agar has relatively simple formulation which provides the necessary nutrients for the growth of many microorganisms which are not very fastidious. Beef extract contains vitamins, organic nitrogen compounds, salts and little carbohydates. Peptic digest of animal tissue provide amino acids and long chain peptides for the organisms.

Quality Control

Appearance : Cream to yellow homogeneous free flowing powder

Gelling: Firm, comparable with 1.5% Agar gel

Colour and Clarity of Prepared: medium Yellow coloured clear to slightly opalescent gel forms in Petri plates

Reaction: Reaction of 2.3% w/v aqueous solution at 25°C.

pH : 6.8±0.2 pH 6.60-7.00

### 3.5.1 b) Techniques Employed

Only two types of techniques have been used. Spread plate and streaking. On the initial phase spread plate technique had been used. But the yogurt is so thick in texture so later on we had to use streaking technique. On second and third phase of screening the plates were streaked wid the newly recombined yogurt samples to collect the desired strain of *Lactobacillus.*

All the petri-plates were labeled carefully, taken duplicates for each plate and even a negative control was taken too. The colonies formed were counted after 24-48 hours incubation at 37 °C in an inverted position.

### 3.5.1 c) Enumeration of Bacterial Load

After having 24-48 hrs incubation, the plates had well-spaced colonies which were selected for counting. The counting of colony from the selected plates was done normally by visual observation. The number of colonies or viable aerobic bacterial count per ml was calculated by multiplying the average number of colonies per plate by the reciprocal of the dilution. The calculated results were expressed as colony forming units (cfu) per µl of the Here:cfu/ml = cfu/plate x dilution factor, where, cfu is the colony forming unit

### 3.5.1 d) Purification of the Isolates

The desired strain was again streaked on NA media for furthermore characterization. Strains collected from both medias were characterised depending upon color, form, elevation, margin, surface etc. from the selected and isolated colonies Gram stain slides were prepared and were observed under microscope. Presumptive colonies were inoculated again into MRS broth and incubated at 37 ºC for 24-48 hours. The resulted colonies were reexamined on to MRS agar through repeated four ways of streaking plate method. When a plate gave only one type of colony it was considered to be pure.

### 3.5.2 Morphological and Cultural Studies of Selected Isolates

Morphological characters of selected isolates can be observed by cultural and microscopicmethods. By the cultural method colony characteristics on agar plates, agar slants, growth in liquid broth media can be observed. But microscopic methods generally used for the study of size, shape, color, arrangement etc. With a view to identify the selected strains the following morphological characters were studied:

**Agar Colony :** The selected isolates were streaked on MRS agar medium for their morphological characters such as size, shape (rhizoidal, irregular, circular, undulate, spindle, etc), edge (curled, lobate, entire, crenate, dentate, etc) elevation ( flat, concave, convex, umbonateetc), opacity, surface (smooth, rough etc) and color of the colony (mostly white, off white, yellowish).

### 3.5.3 Microscopic Characteristics

For the study of the shape, size and colony morphology of the selected culture were identified based on microscopic and Gram staining method. The arrangement of the cells whether present singly in chains or in clusters were also observed.

 Fixed Stained Smears: The techniques were used to obtain information on shape, anatomy and taxonomic features of the cells that cannot easily be seen in unstained materials. For this purpose much importance was given to the studies. For good staining different steps were taken. -Cleaning of the Slides: New slides were rubbed with a piece of clean tissue paper and washed with 95% ethanol solution. When a slide is required for use, it was removed from alcohol using forceps and then heated over a spirit lamp to burn off the alcohol. The slide was then allowed to cool keeping the heated surface on the upside.

-Preparation of Smear: A portion of bacterial culture was taken out by a sterilized loop and was suspended 0.9% physiological saline. A drop of the suspension was taken on a slide and a very thin film was made which was allowed to air dry. This method was followed in almost all types of staining except flagella staining where a slightly different method was used.

-Fixation of the Smear: The smear was fixed by slightly heating the slide over a spirit lamp. The temperature should be sufficient to fix the cells to the slide. Otherwise the cells may become destroyed.

-Gram staining : The fixed smear was flooded with ammonium oxalate crystal violet solution for 1 minute (Frobisher et al., 1957). This was gently rinsed off and an iodine solution was applied for 30 seconds. Followed by gentle washing with water, ethyle alcohol (95%) was then applied for 20 seconds to decolorize the stain. Finally safranine was used as a counter stain for 3 minutes. Then the slide was gently rinsed off with water and blotted dry. The result was recorded as gram positive and gram negative.

### 3.5.4 Maintenance & Preservation of the Isolates

The organisms were identified based on colony morphology, Gram staining. After selecting the isolates which mostly were lactobacillus species and yeasts collected from the local yogurt samples, they were then maintained in selected media as these species generally show well growth and population in liquid broth media like tryptophan soya broth (TSB) and skimmed milk (SKM) during the course of study and then preserved as stock culture in the refrigerator at 4 ºC. Occasional sub culturing (3/4 weeks) was done to keep the cultures in active condition.

## 3.6 RoeseGotlieb Method for determining fat percentage of yogurt

**Principle**

The sample is treated with ammonia and ethyl alcohol; the former to dissolve the protein and the latter to help precipitate the proteins. Fat is extracted with diethyl ether and petroleum ether.

Mixed ethers are evaporated and the residue weighed. This method is considered suitable for reference purposes. Strict adherence to details is essential in order to obtain reliable results.

**Apparatus**

1. i) Mojonnier fat extraction flask or any other suitable extraction tube (as per ISI specification).
2. ii) Bark Cork or ground glass stopper are usually unaffected by the fat solvents.

iii) 100 ml flat bottom flask with G/G joint or stainless steel or aluminum dishes of 5.5 cm height and 9 cm diameter or glass bowl.

**Reagents**

1. a) Concentrated Ammonium Solution- Specific gravity 0.8974 at 16°C
2. b) Ethyl alcohol (95%-96% v/v)
3. c) Diethyl ether, peroxide-free-specific gravity 0.702

   It may be maintained free from peroxides by adding wet zinc foil (approximately 80 cm / L, cut in strips long enough to reach at least half-way of the container) that has been completely immersed in dilute acidified copper sulphate for 1 minute and subsequently washed with water.

1. d) Petroleum ether- boiling range 40-60°C
2. e) Mixed Solvent

   Prepared by mixing equal volumes of the ether and light petroleum.

**Procedure**

Weigh accurately about 10 g of sample (liquid milk), transfer to extraction tube. Add 1.0 ml of ammonia sp. gr. 0.8974; mix well in the lower bulb thoroughly. Add 10 ml ethyl alcohol and mix by allowing the liquid to flow backwards and forwards between the two bulbs. Allow the tube to cool in cold, running water or by immersing in chilled water. Add 25 ml of diethyl ether (peroxide free), close with bark cork or glass stopper which is wetted with water before insertion, and shake vigorously for 1 minute. Open the tube and then add 25 ml light petroleum ether, close the tube, and shake again vigorously for a minute. Let the tube stand on the flat bottom of the lower bulb until the upper ethereal layer is clear and separated completely from the aqueous layer, usually not less than 30 minutes, or centrifuge until clear. If there is a tendency to form emulsion, a little alcohol may be added to help separation of the layers.

Decant off as much as possible of the supernatant layer into a suitable flask by gradually bringing the cylindrical bulb of the tube into a horizontal position. When as much as possible has been poured off, wash the outside of the neck of the tube and the cork or stopper with mixed solvent, collecting the rinsing in the flask. With the Mojonnier tube in a vertical position, wash the inside of the neck with 4 to 5 ml of mixed solvent, and decant. Repeat twice extraction of the liquids remaining in the extraction tube using 15 ml of ether and 15 ml of petroleum solvent every time. Distil carefully the solvents from the flask and dry the flask containing the residual milk fat in an air oven at 102 ± 2°C for two hours, cool in a desiccator and weigh. Heat the flask again in the oven for 30 min. Cool in desiccator and weigh. Repeat the process of heating and cooling and weighing until the difference between two successive weights does not exceed 1 mg. Wash out the fat from the flask with petroleum ether carefully leaving any insoluble residue in

the flask. Dry the flask in the oven, cool and weigh as before. The difference in weights represents the weight of fat extracted from the milk.

Make a blank determination using the specified quantities of reagent throughout, and water in place of milk, deduct the value found. If reagent blank is more than 0.5 mg purify or replace reagents. Difference between duplicate determinations obtained simultaneously by the same analyst should not be more than 0.03 gm fat/100 gm product.

**Calculation**

**Fat percent w/w =** Weight of fat x 100/Weight of yogurt

# CHAPTER FOUR: RESULTS

# Results

To determine good quality of yogurt, present study was done by taking samples from various branded yogurts in Dhaka. It was done in two phases. The first phase was to recombine first set of yogurt samples and then the second phase came with better recombined yogurt samples conducting from the first phase. All the screening was done based on the parameters of texture, appearance, colour and taste. Each sample was tasted by five people. These data were collected from our co-researchers, students and teachers of the lab. The evaluation was done by the hedonic scaling where the products should be ranked from 0-9.

## 4.1 Organoleptic Quality Assessment

Yogurts qualities were determined by the process of hedonic scaling. It is most widely used scale for measuring food acceptability in the 9-point scaling parameter. Therefore in this study the quality assessment was conducted by this most popular method.

### a) First set of recombination:

At initial stage, thirty two samples were tasted by the people. Table 4.1 shows name chart of recombined samples, where “m” denotes the milk company whose milk was used.

**Table 4.1: Name of recombined 32 samples of the first screening**

| Sample no. | Sample sources | Sample no. | Sample sources |
|---|---|---|---|
| 1 | Rosh + Aarong (Milkvita m) | 17 | Nimtoli + Solna(Milkvita m) |
| 2 | Aarong + Bikrampurn (Milkvita) | 18 | Solna + Moronchan(Milkvita m)d |
| 3 | Bikrampur + Rosh (Milkvita m) | 19 | Moronchand + Nimtoli (Milkvita m) |
| 4 | Aarong + Rosh +Bikrampur (Milkvita m) | 20 | Nimtoli+ Solna + Moronchand(Milkvita m) |
| 5 | Rosh + Aarong (Aarong m) | 21 | Nimtoli + Solna(Aarong m) |
| 6 | Aarong + Bikrampur (Aarong m) | 22 | Solna + Moronchand (Aarong m) |
| 7 | Bikrampur + Rosh(Aarong m) | 23 | Moronchand + Nimtoli(Aarong m) |
| 8 | Aarong + Rosh +Bikrampur (Aarong m) | 24 | Nimtoli+ Solna + Moronchand(Aarong m) |
| 9 | Premium sweets+ Igloo(Milkvita m) | 25 | Star + Mughal(Milkvita m) |
| 10 | Igloo+ Vagyakul(Milkvita m) | 26 | Mughal+ Muslim(Milkvita m) |
| 11 | Vagyakul + Igloo(Milkvita m) | 27 | Muslim + Star(Milkvita m) |
| 12 | Premium sweets +Vagyakul + Igloo(Milkvita m) | 28 | Star+ Mughal+ Muslim(Milkvita m) |
| 13 | Premium sweets+ Igloo(Aarong m) | 29 | Star + Mughal(Aarong m) |
| 14 | Igloo+ Vagyakul(Aarong m) | 30 | Mughal+ Muslim(Aarong m) |
| 15 | Vagyakul + Igloo(Aarong m) | 31 | Muslim + Star(Aarong m) |
| 16 | Premium sweets +Vagyakul + Igloo(Aarong m) | 32 | Star+ Mughal+ Muslim(Aarong m) |

Initial recombined samples were tasted by five people for ranking them in hedonic scale. As the quality and Organoleptic point both increases in a proportional way from 0-9. Each sample was ranked on the basis of texture, appearance, colour and taste.

Table 4.2 shows the remarks of first 32 recombined samples given by the observers. Each sample was tasted by five people to get a mean value. And calculating the means together helped to design the second set of samples for further experiments.

**Table 4.2: Hedonic data of first set of yogurts**

| Sample no. | Texture | | | | | Appearance | | | | | Colour | | | | | Taste | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 6 | 7 | 8 | 6 | 8 | 8 | 9 | 7 | 6 | 9 | 5 | 6 | 8 | 6 | 8 | 6 | 8 | 5 | 7 | 7 |
| 2 | 8 | 9 | 8 | 6 | 9 | 6 | 7 | 8 | 5 | 8 | 9 | 6 | 7 | 9 | 5 | 7 | 8 | 7 | 6 | 8 |
| 3 | 7 | 6 | 8 | 9 | 6 | 8 | 6 | 8 | 9 | 7 | 7 | 9 | 8 | 9 | 9 | 9 | 7 | 9 | 7 | 8 |
| 4 | 8 | 9 | 6 | 9 | 4 | 8 | 6 | 8 | 8 | 6 | 4 | 7 | 8 | 8 | 8 | 8 | 8 | 6 | 5 | 9 |
| 5 | 8 | 9 | 6 | 8 | 9 | 6 | 8 | 6 | 8 | 9 | 6 | 8 | 0 | 7 | 7 | 7 | 6 | 7 | 9 | 8 |
| 6 | 6 | 7 | 8 | 6 | 8 | 9 | 7 | 9 | 7 | 9 | 7 | 8 | 7 | 8 | 7 | 7 | 7 | 5 | 7 | 9 |
| 7 | 7 | 8 | 6 | 6 | 8 | 9 | 8 | 7 | 9 | 7 | 8 | 7 | 7 | 6 | 8 | 9 | 7 | 8 | 8 | 8 |
| 8 | 7 | 8 | 7 | 8 | 7 | 6 | 7 | 6 | 7 | 7 | 7 | 6 | 7 | 7 | 8 | 6 | 7 | 7 | 8 | 6 |
| 9 | 8 | 9 | 6 | 8 | 9 | 6 | 8 | 6 | 8 | 9 | 6 | 8 | 0 | 7 | 7 | 7 | 6 | 8 | 9 | 8 |
| 10 | 6 | 7 | 8 | 6 | 8 | 9 | 7 | 9 | 7 | 9 | 7 | 8 | 7 | 8 | 7 | 7 | 7 | 5 | 7 | 9 |
| 11 | 7 | 8 | 7 | 8 | 7 | 6 | 7 | 6 | 7 | 7 | 7 | 6 | 7 | 7 | 8 | 6 | 7 | 7 | 8 | 6 |
| 12 | 6 | 7 | 8 | 6 | 8 | 9 | 7 | 9 | 7 | 9 | 7 | 8 | 7 | 8 | 7 | 7 | 7 | 5 | 7 | 9 |
| 13 | 7 | 8 | 6 | 6 | 8 | 9 | 8 | 7 | 9 | 7 | 8 | 7 | 7 | 6 | 8 | 9 | 7 | 8 | 8 | 8 |
| 14 | 8 | 7 | 9 | 8 | 9 | 9 | 7 | 8 | 9 | 7 | 8 | 9 | 7 | 9 | 8 | 8 | 9 | 7 | 7 | 8 |
| 15 | 7 | 8 | 7 | 8 | 7 | 6 | 7 | 6 | 7 | 7 | 7 | 6 | 7 | 7 | 8 | 6 | 7 | 7 | 8 | 6 |
| 16 | 7 | 6 | 8 | 9 | 6 | 8 | 6 | 8 | 9 | 7 | 7 | 9 | 8 | 9 | 9 | 9 | 7 | 9 | 7 | 8 |
| 17 | 8 | 9 | 6 | 9 | 4 | 8 | 6 | 8 | 8 | 6 | 4 | 7 | 8 | 8 | 8 | 8 | 8 | 6 | 5 | 9 |
| 18 | 7 | 8 | 7 | 8 | 7 | 6 | 7 | 6 | 7 | 7 | 7 | 6 | 7 | 7 | 8 | 6 | 7 | 7 | 8 | 6 |
| 19 | 8 | 9 | 6 | 9 | 4 | 8 | 6 | 8 | 8 | 6 | 4 | 7 | 8 | 8 | 8 | 8 | 8 | 6 | 5 | 9 |
| 20 | 7 | 6 | 8 | 9 | 6 | 8 | 6 | 8 | 9 | 7 | 7 | 9 | 8 | 9 | 9 | 9 | 7 | 9 | 7 | 8 |
| 21 | 8 | 9 | 6 | 9 | 4 | 8 | 6 | 8 | 8 | 6 | 4 | 7 | 8 | 8 | 8 | 8 | 8 | 6 | 5 | 9 |
| 22 | 6 | 7 | 8 | 6 | 8 | 9 | 7 | 9 | 7 | 9 | 7 | 8 | 7 | 8 | 7 | 7 | 7 | 5 | 7 | 9 |
| 23 | 7 | 8 | 6 | 6 | 8 | 9 | 8 | 7 | 9 | 7 | 8 | 7 | 7 | 6 | 8 | 9 | 7 | 8 | 8 | 8 |
| 24 | 7 | 8 | 7 | 8 | 7 | 6 | 7 | 6 | 7 | 7 | 7 | 6 | 7 | 7 | 8 | 6 | 7 | 7 | 8 | 6 |
| 25 | 8 | 9 | 6 | 9 | 4 | 8 | 6 | 8 | 8 | 6 | 4 | 7 | 8 | 8 | 8 | 8 | 8 | 6 | 5 | 9 |
| 26 | 7 | 6 | 8 | 9 | 6 | 8 | 6 | 8 | 9 | 7 | 7 | 9 | 8 | 9 | 9 | 9 | 7 | 9 | 7 | 8 |
| 27 | 8 | 9 | 6 | 9 | 4 | 8 | 6 | 8 | 8 | 6 | 4 | 7 | 8 | 8 | 8 | 8 | 8 | 6 | 5 | 9 |
| 28 | 7 | 8 | 7 | 8 | 7 | 6 | 7 | 6 | 7 | 7 | 7 | 6 | 7 | 7 | 8 | 6 | 7 | 7 | 8 | 6 |
| 29 | 8 | 9 | 6 | 8 | 9 | 6 | 8 | 6 | 8 | 9 | 6 | 8 | 0 | 7 | 7 | 7 | 6 | 8 | 9 | 8 |
| 30 | 6 | 7 | 8 | 6 | 8 | 9 | 7 | 9 | 7 | 9 | 7 | 8 | 7 | 8 | 7 | 7 | 7 | 5 | 7 | 9 |
| 31 | 7 | 8 | 6 | 6 | 8 | 9 | 8 | 7 | 9 | 7 | 8 | 7 | 7 | 6 | 8 | 9 | 7 | 8 | 8 | 8 |
| 32 | 8 | 9 | 6 | 9 | 4 | 8 | 6 | 8 | 8 | 6 | 4 | 7 | 8 | 8 | 8 | 8 | 8 | 6 | 5 | 9 |

Fig: 4.1-fig 4.32 shows the appearance and texture of yogurts sample from first set of recombination. Yogurt making procedure was fully conducted in the microbiology lab. No added sugar, flavor and colour was present. As a result, all white milk turned creamy white. The figure also shows the bottom line to see the texture and consistency of each sample.




**Fig4.1: Sample-1 Fig4.2: Sample-2 Fig4.3: Sample-3 Fig4.4: Sample-4**




**Fig4.5: Sample-5 Fig4.6: Sample-6 Fig4.7: Sample-7 Fig4.8: Sample-8**




**Fig4.9: Sample-9 Fig4.10: Sample-10 Fig4.11: Sample-11 Fig4.12: Sample-12**

**Fig4.13: Sample-13 Fig4.14: Sample-14 Fig4.15: Sample-15 Fig4.16: Sample-16**

**Fig4.17: Sample-17 Fig4.18: Sample-18 Fig4.19: Sample-19 Fig4.20: Sample-20**

**Fig4.21: Sample-21 Fig4.22: Sample-22 Fig4.23: Sample-23 Fig4.24: Sample-24**

**Fig4.25: Sample-25 Fig4.26: Sample-26 Fig4.27: Sample-27 Fig4.28: Sample-28**




**Fig4.29: Sample-29** **Fig4.30: Sample-30** **Fig4.31: Sample-31** **Fig4.32: Sample-32**

## b) Second set of recombination:

On the second stage, only eight samples were screened. From the result of initial stage, another set of better recombination was observed. The mean value by hedonic scale was higher than the initial stage.

Table 4.3 shows name chart of recombined samples, where "m" denotes the milk company which had been used.

**Table4.3: Name of recombined 8 samples of screening**

| Sample no. | Sample sources | Cultured date |
|---|---|---|
| A | Bikrampur + Rosh (Pran Milk) | 06.03.2016 |
| B | Nimtoli+Bikrampur (Pran Milk) | 06.03.2016 , 03.04.2016 |
| C | Rosh + Nimtoli(Pran Milk) | 06.03.2016 |
| D | Solna+ Bikrampur(Pran Milk) | 13.03.2016 |
| E | Solna+Nimtoli (Pran Milk) | 13.03.2016 , 10.04.2016 |
| F | Bikrampur+Rosh+Nimtoli(Pran Milk) | 20.03.2016 |
| G | Rosh + Nimtoli+ Solna(Pran Milk) | 20.03.2016 |
| H | Rosh + Solna(Pran Milk) | 20.03.2016 |

On the second recombination stage, only eight samples were tested. This time all the parameters were tested individually to judge the specific characteristics of each sample. From this test, it was easier to find out the most desired yogurt sample.

Table 4.4 shows the hedonic scaling of second set of yogurt samples developed after recombination among eight samples on the basis of texture, appearance, colour and taste.

**Table 4.4: Hedonic data of second set of yogurts**

| Sample no. | Texture | | | | | $\bar{x}$ | Appearance | | | | | $\bar{x}$ | Colour | | | | | $\bar{x}$ | Taste | | | | | $\bar{x}$ | $\bar{x}$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A | 7 | 8 | 7 | 7 | 9 | 7 | 6 | 7 | 6 | 7 | 7 | 6.5 | 6 | 7 | 6 | 8 | 7 | 6.8 | 9 | 8 | 7 | 6 | 7 | 7.4 | 6.9 |
| B* | 8 | 7 | 9 | 8 | 9 | 8 | 9 | 7 | 8 | 9 | 7 | 8 | 8 | 9 | 7 | 9 | 8 | 8.1 | 7 | 8 | 9 | 9 | 8 | 8.1 | 8 |
| C | 8 | 7 | 8 | 7 | 7 | 7.4 | 8 | 7 | 7 | 8 | 7 | 6 | 7 | 6 | 8 | 8 | 7 | 7.2 | 7 | 8 | 6 | 7 | 8 | 7.2 | 6.95 |
| D | 6 | 8 | 7 | 6 | 8 | 7 | 6 | 7 | 6 | 7 | 7 | 7 | 8 | 7 | 7 | 6 | 8 | 7.2 | 8 | 7 | 7 | 8 | 7 | 7.2 | 7.1 |
| E* | 8 | 9 | 8 | 8 | 9 | 8.5 | 7 | 8 | 9 | 9 | 9 | 8.4 | 8 | 9 | 9 | 8 | 9 | 8.6 | 8 | 9 | 8 | 9 | 7 | 8.1 | 8.4* |
| F | 7 | 8 | 7 | 8 | 7 | 7.4 | 8 | 7 | 8 | 7 | 7 | 7.4 | 8 | 7 | 7 | 7 | 8 | 7.4 | 6 | 7 | 8 | 7 | 8 | 7.2 | 7.3 |
| G | 7 | 6 | 7 | 8 | 7 | 7 | 7 | 8 | 7 | 6 | 6 | 6.9 | 8 | 7 | 6 | 8 | 6 | 7 | 6 | 9 | 8 | 6 | 7 | 7.2 | 7.12 |
| H | 7 | 8 | 7 | 9 | 8 | 7.8 | 6 | 7 | 8 | 7 | 9 | 7.4 | 8 | 7 | 9 | 7 | 8 | 7.9 | 7 | 8 | 9 | 7 | 9 | 7.9 | 7.75 |

*Represents best yogurt samples

Fig 4.33 to Fig 4.40 shows that overnight growth of eight yogurt samples from second set of recombination. No sugar, flavor and colour was present. As a result the entire colour went from milk white to creamy white. The figure also shows the bottom line to see the texture and consistency of each sample.

From the figure, sample B and E have the good consistency in texture and appearance. As at the initial stage only two samples had been collected, so sample B and E were of better quality.




**Fig4.33: Sample A Fig4.34: Sample B Fig4.35: Sample C Fig4.36: Sample D**

**Fig4.37: SampleE Fig4.38: SampleF Fig4.39: SampleG Fig4.40: SampleH**

## Organoleptic Quality Assessment by Hedonic Scaling

After the second set of recombined yogurt samples, all the data were presented in a bar diagram form to show the comparison of all samples. The figures where individually all the quality assessments are shown below.

### 4.1.1 A) Texture Acceptability

Mean score of texture in the yogurt sample of A, B, C, D, E, F, G, H are 7, 8, 7.4, 7, 8.5, 7.4, 7, 7.8, respectively. In texture acceptability test, from fig4.41, Hedonic scale showed that the yogurt sample E was excellent and B was considered very good texture quality.

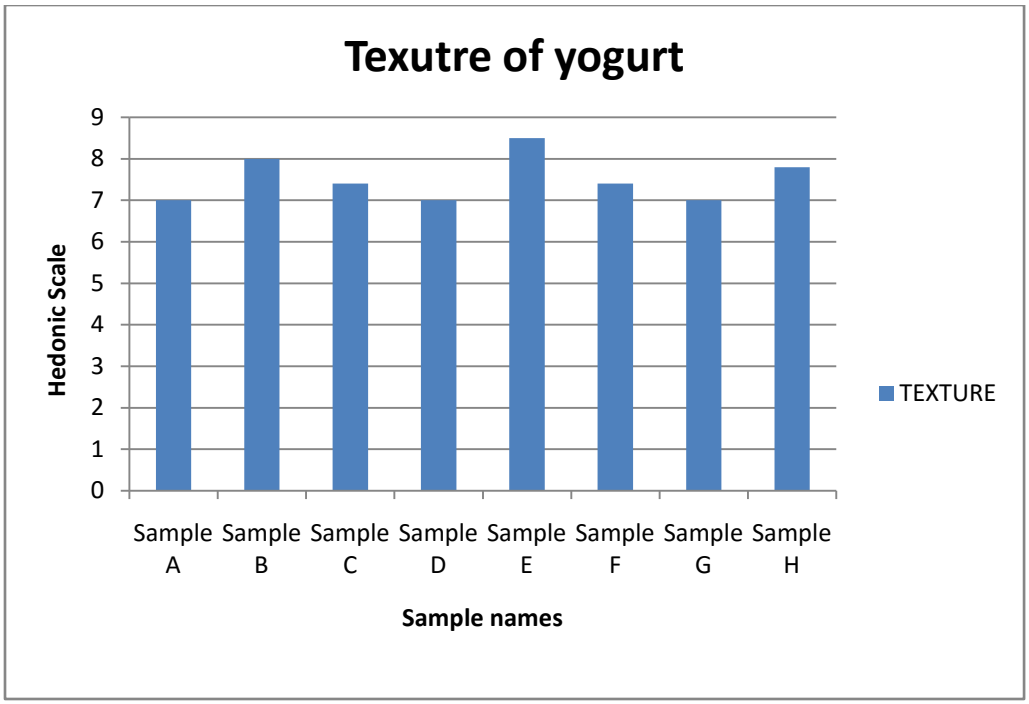



**Fig 4.41: Mean of texture of second sets yogurts combination chart**

### 4.1.1 B) Appearance Acceptability

The flavors mean score were 6.5, 8, 6, 7, 8.4, 6.4, 7.4, 6.9, and 7.4 respectively. In the flavor acceptability test, from Fig. 4.42, Hedonic scale showed that the yogurt sample E was excellent (mean score is 8.4), sample B considered as very good and sample F had poor taste quality.

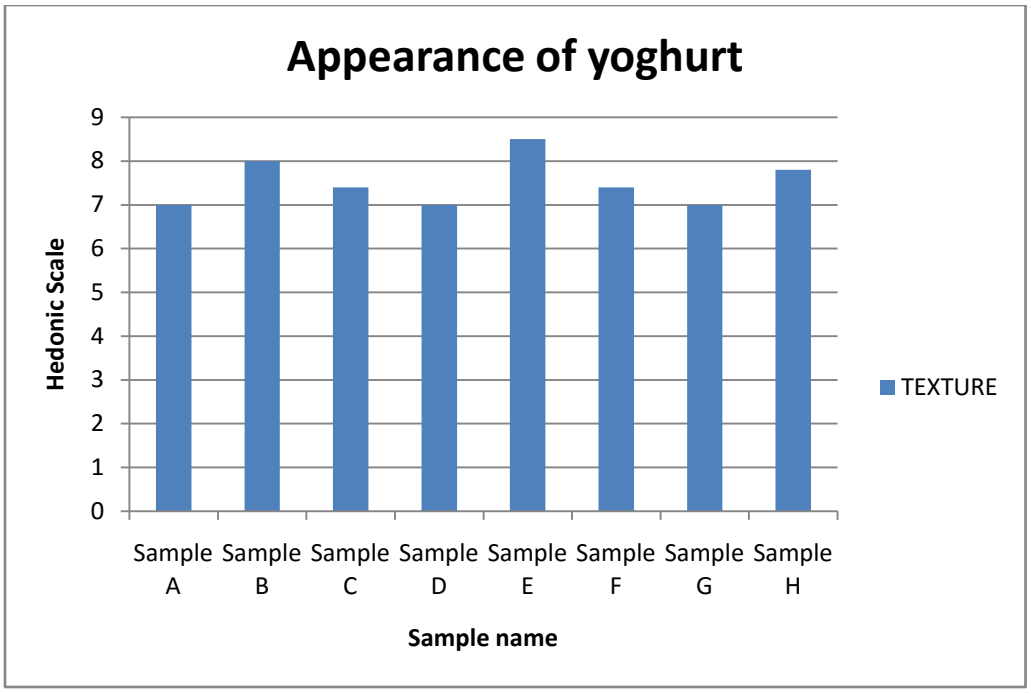



**Fig 4.42: Mean of appearance of second sets yogurts combination chart**

### 4.1.1 C) Colour Acceptability

It appeared that both sample B and E obtained the highest score for its colour (mean is 8). The mean for others were 7, 8, 7, 8, 9, 8, 6, 8for A, B, C, D, E, F, G, H and so on. In the color acceptability test, from Fig 4.43, Hedonic scale showed that the yogurt sample B and E both were excellent. Others A, C, D, F, G and H are considered as acceptable quality.

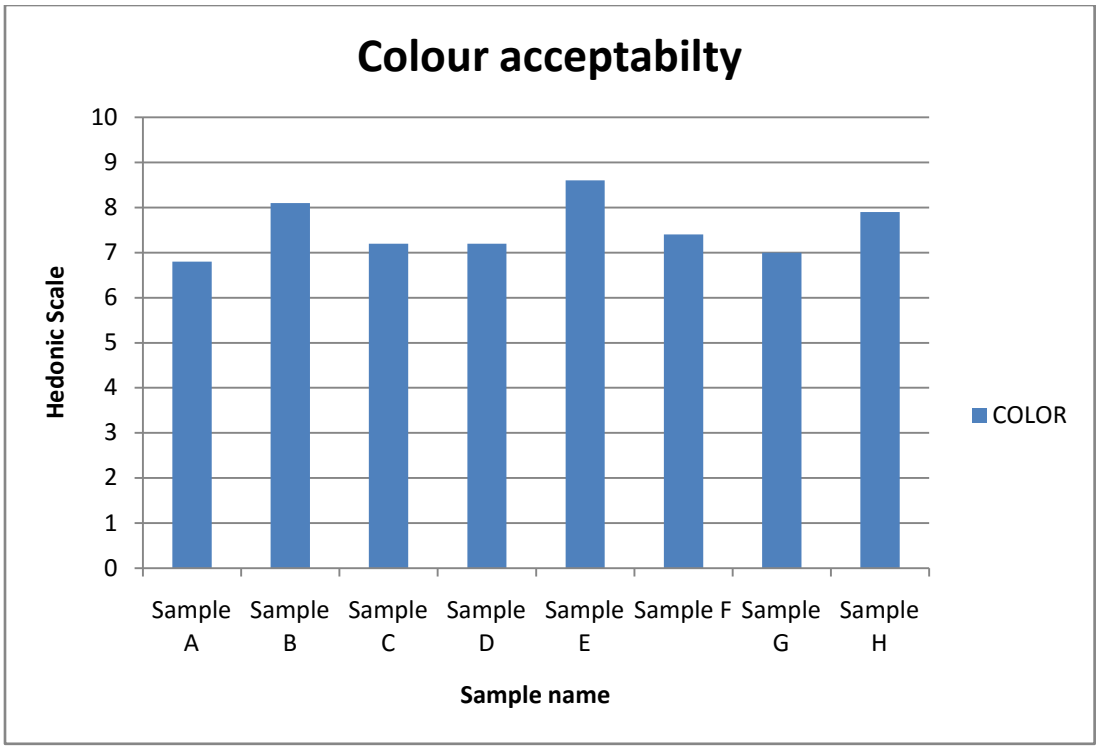



**Fig 4.43: Mean of colour of second sets yogurts combination chart**

### 4.1.1.1 D) Taste Acceptabil

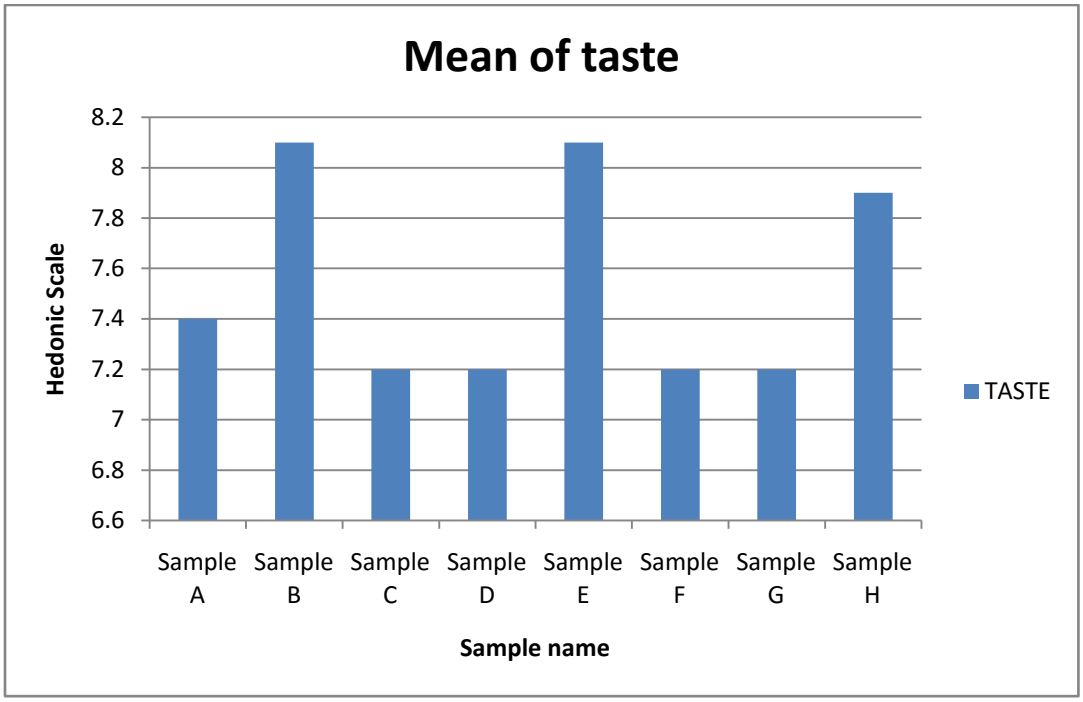



**Fig 4.44: Mean of taste of second sets yogurts combination chart**

The taste score of A, B, C, D, E, F, G, H were 7.4, 8.1, 7.2, 7.2, 8.1, 7.2, 7.2 and 7.9 respectively. In the taste acceptability test, from Fig4.44, Hedonic scale showed that yogurt sample B and E both were excellent (mean score 8.1) and sample H considered very good in taste quality.

From the second set of recombination yogurt study, Hedonic scale ranked all the samples on the scale of 0-9. Table 4, shows the mean of each sample

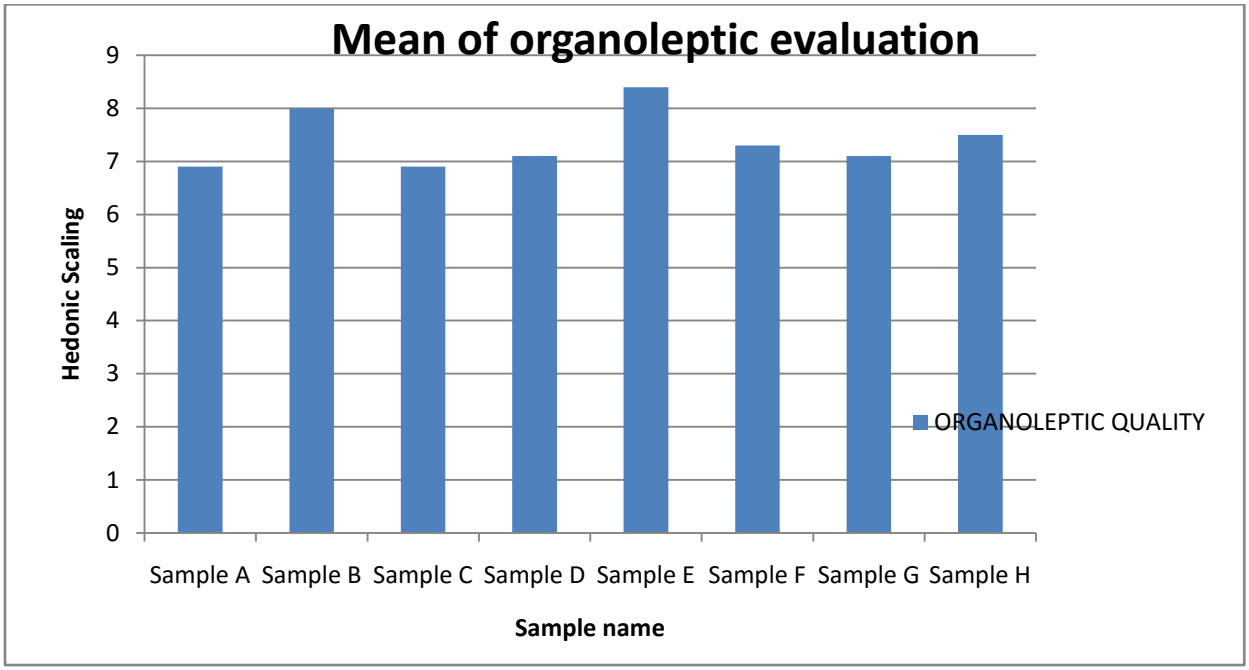



**Fig 4.45: Average mean of quality of second sets yogurts combination chart**

In the fig 4.46, best of two samples, sample B and sample E showed their individual qualities comparison. From here, in terms of texture, appearance and colour, sample E showed higher acceptability. However, in term of taste, both sample B and sample E are shows same scale (8.1).

Therefore it can be said that sample E is the best sample.

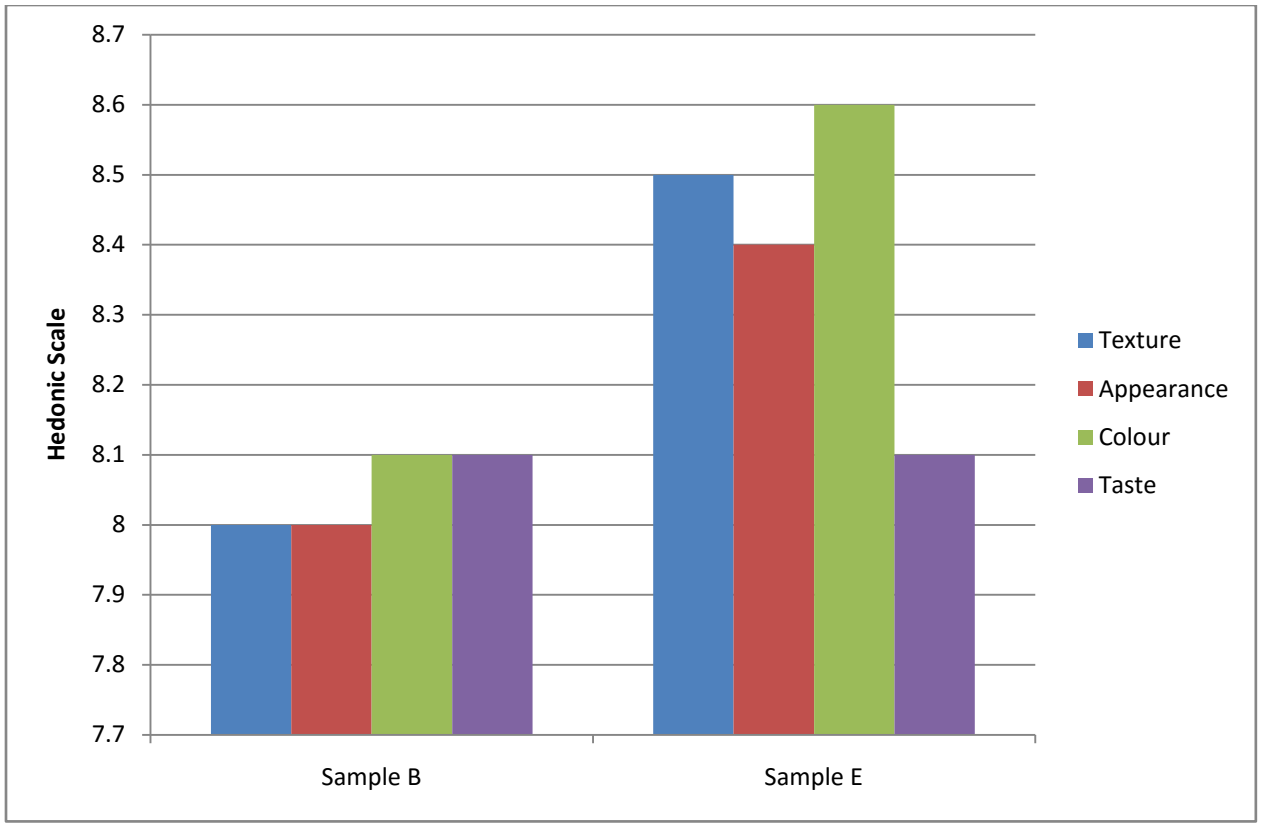



**Fig 4.46: Comparison of best two yogurts samples in terms of texture, appearance, colour and taste chart**

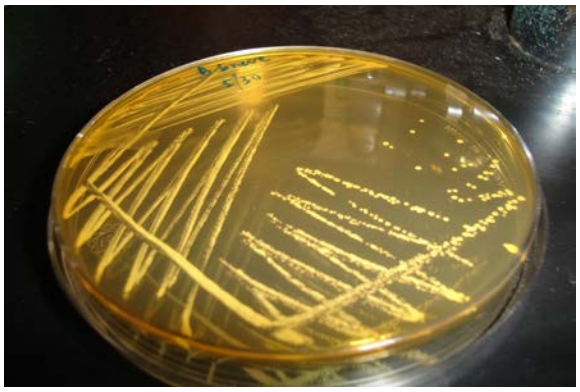
## 4.2 MICROBIOLOGICAL ASSESMENT

### 4.2.1 Culture and Subculture

After finalizing the best two samples, both sample B and sample E were cultured on selected MRS(de Man, Rogosa and Sharpe) and Nutrient Agar media.

MRS agar was selected for *Lactobacillus spp*and nutrient agar is the ideal growth medium for all.

The first phase the samples were cultured on both media. Later on, the microbes from MRS media was again cultured on NA agar as a subculture.



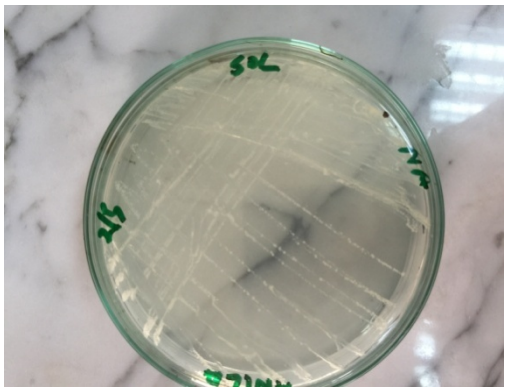
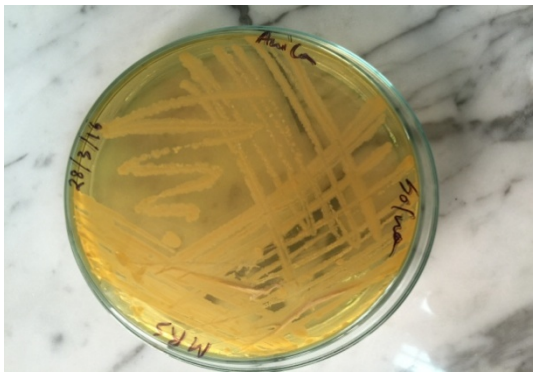


**Fig4.47: Sample B on MRS media** **Fig4.48: Sample B on NA**



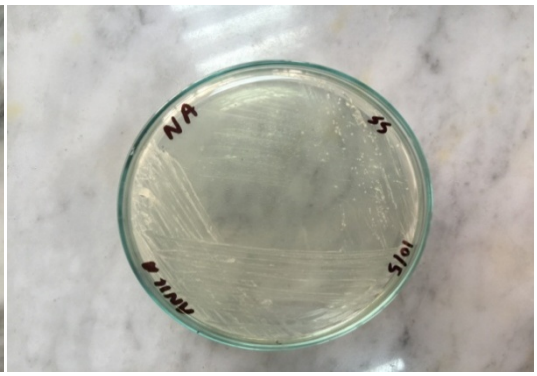


**Fig4.49: Sample E on MRS media** **Fig4.50: Sample E on NA**

Fig 4.47 and fig 4.49 shows sample B and sample E on MRS media respectively. Colonies on Sample B showed the characteristics of yeast and colonies of sample E showed the characteristics like *Lactobacillus*. Fig 4.47showed as small coccoid, white colored colonies with smooth surface. Whereas, fig 4.49 show large, overgrowth and scattered colonies, had white in colour and smooth surface. Fig 4.48 and fig 4.50 are showed respectively sample B and sample E on NA media. There, in fig 4.48, colonies were numerous and clearly seen. However in fig 4.50, colonies were scattered and hazy in shape.

### 4.2.2 Gram Staining:

To determine specific microbial observation under microscope, gram staining was conducted. Gram staining is the first step in the preliminary identification of a bacterial organism. It is used to differentiate bacterial species into two large groups (gram-positive and gram-negative) based on the physical properties of their cell walls. The gram positive bacteria stain violet as a result of the presence of a thick peptidoglycan layer in the walls of their cell, the gram negative bacteria stain red, due to the thinner peptidoglycan layer in their cell wall (a thicker peptidoglycan layer allows for the retention of the stain, but a thinner layer does not).

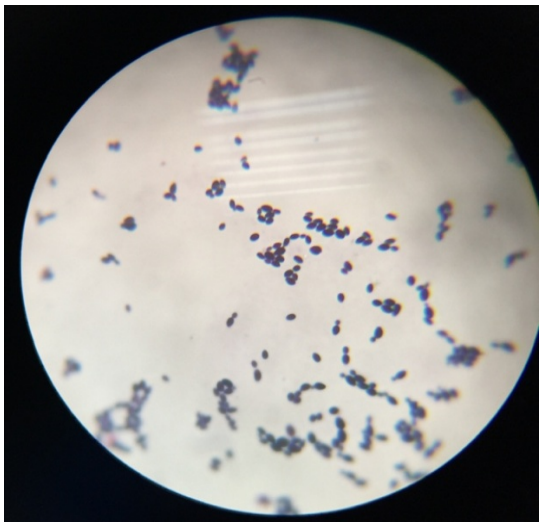


**Fig4.51: Gram staining of Sample B**

Fig 4.51 shows the gram staining of sample B. It shows oval shaped, grouped colonies. It was also dark purple in colour. These non-motile colonies had the similarities with yeast colonies. It could be said that sample B had yeast type of organisms.

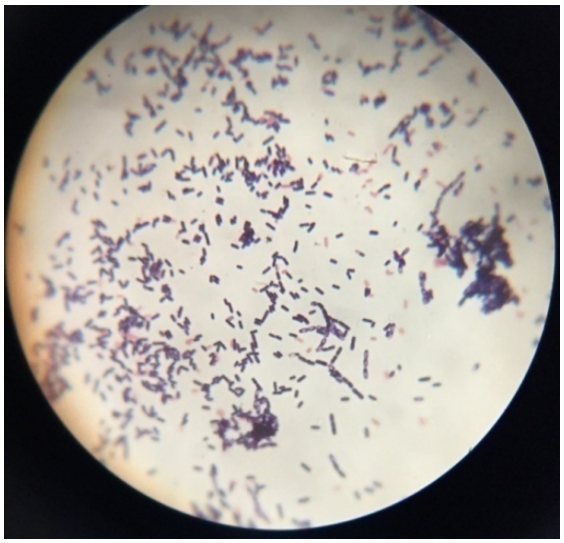


**Fig4.52: Gram staining of Sample E**

Fig 4.52 showed the gram staining of sample E. Large and rod shaped numerable amount of scattered and motile colonies showed the characteristics of *Lactobacillus spp.*

It could be said that sample B had *Lactobacillus* (LAB) type of organisms.

**Table4.5: Microscopic observation from sample B and sample E isolates**

| Sample name | Colony shape on medium | Gram stain; shape | Genera | Growth duration | Media used |
|---|---|---|---|---|---|
| Sample B | Small colony, white in colour and smooth surface | Small, Oval colony and dark purple in colour | Yeast | 24 hrs | MRS, NA |
| Sample E | Large colony, white in colour and smooth surface | Large , rod shaped and moving | *Lactobacillus spp* | 24 hrs | MRS, NA |

Table 4.5 showed the microscopic observations of sample B and sample E isolates, from where it could be clearly said that sample B had yeast colonies and sample E had *Lactobacillus spp* colonies.

### 4.3 Final yogurt: Inoculated sample

After comparing the microbes from sample B and sample E, *Lactobacillus acidophilus* as sample E, was taken after considering all those previous results.

Small amount of microbes from sample E, *Lactobacillus* (LAB) was taken and inoculated in milk sample. It was mixed well and kept in incubator at 37°C for 24 hour. No sugar, flavor and colour was added. The milk sample was taken from a local store of branded company.

**Fig4.53: yogurt from experimental LAB**

Fig 4.53 showed the yogurt sample after 24 hours incubation. It was again tasted by several other people and the result was similar to the sample E.

### 4.4 Preservation of isolates

The isolates of both sample B and sample E were preserved for the further study. It was preserved in Tryptone Soya Agar (TSA). This agar is the growth media for the culturing of bacteria. It is a non-selective media providing enough nutrients to allow for a wide variety of microorganisms to grow. They are used for a wide range of applications, including culture storage, enumeration (counting), isolation of pure cultures, or simply general culture.

Fig 4.54 showed the fresh 24 hours sub culture from sample E which is *Lactobacillus* (LAB) was inoculated in freshly made Tryptophan Soya agar (TSA) and was sealed properly for long term preservation and use

### 4.5 Chemical Analysis: percentage of fat of milk and yogurt.

Percentage of fat is an important nutritional fact for milk and milk derivative products. In the present study, a new type of yogurt was produced from a new combined strain of *Lactobacillus* (LAB). To determine the fat percentage and other nutritional value of both yogurt producing milk and of yogurt, a chemical analysis was done. This analysis was done from Bangladesh Council for Scientific and Industrial Research (BCSIR).

Here's given the compared nutritional values of both whole some milk and final yogurt given here.

**Table4.6: Nutritional value of milk (full fat)**

| | % Daily Value* |
|---|---|
| **Total Fat** 3.3 g | 5% |
| Saturated fat 1.9 g | 9% |
| Polyunsaturated fat 0.2 g | |
| Monounsaturated fat 0.8 g | |
| **Cholesterol** 10 mg | 3% |
| **Sodium** 43 mg | 1% |
| **Potassium** 132 mg | 3% |
| **Total Carbohydrate** 4.8 g | 1% |
| Dietary fiber 0 g | 0% |
| Sugar 5 g | |
| **Protein** 3.2 g | 6% |

| | | | |
|---|---|---|---|
| Vitamin A | 3% | Vitamin C | 0% |
| Calcium | 11% | Iron | 0% |
| Vitamin D | 12% | Vitamin B-6 | 0% |
| Vitamin B-12 | 8% | Magnesium | 2% |

**Table4.7: Nutritional value of yogurt**

| | % Daily Value* |
|---|---|
| **Total Fat** 4 g | 6% |
| Saturated fat 2.2 g | 11% |
| Polyunsaturated fat 0.2 g | |
| Monounsaturated fat 1 g | |
| **Cholesterol** 0 mg | 0% |
| **Sodium** 286 mg | 11% |
| **Potassium** 714 mg | 20% |
| **Total Carbohydrate** 71 g | 23% |
| Dietary fiber 2.3 g | 9% |
| Sugar 57 g | |
| **Protein** 14 g | 28% |

| | | | |
|---|---|---|---|
| Vitamin A | 27% | Vitamin C | 95% |
| Calcium | 45% | Iron | 0% |
| Vitamin D | 0% | Vitamin B-6 | 10% |
| Vitamin B-12 | 28% | Magnesium | 13% |

Table 4.6 and table 4.7 showed the nutritional value of whole milk and yogurt, respectively. The milk contains 3.3 gm or 5% of fat and yogurt contained 4g or 6% of fat. It could be said that yogurt contained more fat than the milk.

# CHAPTER FIVE:

# DISCUSSION

# Discussion

For developing the improved quality of yogurt, all samples were collected from renowned brands of Dhaka city. Out of total 40 samples, a strain of *Lactobacillus* (LAB) was isolated to make the improved quality of yogurt. Hashemi et al. (2015) says that compared to milk, yogurt is more nutritious and is an excellent source of protein, calcium, phosphorus, riboflavin, thiamin, vitamin B12, folate, niacin, magnesium and zinc. Since lactose in milk is converted to lactic acid during fermentation and due to the presence of lactose fermenting bacteria in yogurt, lactose intolerant people can consume yogurt without any adverse effect. Hatting et al. (2001) also claims that certain *L. acidophilus* are able to lower cholesterol levels within intestine. As a result, yogurt significantly reduces serum cholesterol

At the first phase 32 samples were taken. Table 4.1 shows the recombined samples name of first phase. The results of table 4.2 shows the hedonic scaling of first 32 samples and fig 4.1-fig 4.32 shows those samples 24 hrs after the outcome. After the first phase, selected eight high quality yogurts were mixed in different combinations and yogurts produced from them were evaluated. Table4.3 shows the name of recombined 8 samples after screening and fig 4.33-fig 4.40 shows their outcomes. In the table 4.4, Hedonic data of second set of yogurts are presented. All of were tasted from pH range from 3-4. Tambekar et al., 2010 found that *Lactobacillus brevis, Lactobacillus bulgaricus, Lactobacillus plantarum, Lactobacillus rhamnosus, Lactobacillus fermentum*can tolerate pH up to 2.0. The final pH measurement also indicates that slight pH change (lowered) also occurs after incubation of the sample.

Fig 4.41to fig 4.45 shows the average hedonic data of eight samples in terms of texture, appearance, colour, taste and average quality respectively. Even though that, all the sample was mean collectively. Each parameters mean helped to look for the highest level of acceptance. From these screenings it can be said that sample E scored the highest in terms of texture, appearance, colour, taste and average mean of quality.

As the sample B was found to be the second the highest scorer, so a comparing test was conducted to find out the best. Fig 4.46 shows the comparison of best two yogurts samples in terms of texture, appearance, color and taste. Here the sample E scores higher than the sample B in terms of texture, appearance and colour. Monzur et al., 2004 described that the colour of the yogurt depends on the colour of milk or caramelized colour obtained during heating of the milk or added colouring materials. Both sample B and sample E scored same in taste (8).

After finalizing the best two samples both sample B and sample E were cultured on selected MRS media and Nutrient Agar media. Gram staining was performed of the strains isolated from the two samples. In sample B like oval shaped yeasts were observed and from sample E, rod shaped, gram positive *Lactobacillusspp*was found. Suriyarachchi(1981) et al., says that the growth of yeasts in yogurts was related to the ability of the yeasts to grow at refrigeration temperatures, to ferment lactose and sucrose, and to hydrolyze milk casein. Most yeast isolates grew in the presence of 100 μg of sorbate and benzoate preservatives per ml. Of the 128 samples examined, he found that 45% exhibited yeast counts above $10^3$ cells per g. A total of 73 yeast strains were isolated and identified as belonging to the genera *Torulopsis, Kluyveromyces, Saccharomyces, Candida, Rhodotorula, Pichia, Debaryomyces,* and *Sporobolomyces. Torulopsis candida* and *Kluyveromycesfragilis* were the most frequently isolated species. *Lactobacillus spp*found from sample E were then inoculated in the milk sample and incubated for overnight

growth contains the same texture, flavor, colour and taste as it was in sample E. Panesar et al., 2011 stated that the *Lactobacillus*(LAB) are profound to keep the pH and colour as same from the sub cultured species. Roberts et al., 2016 says that real yogurt, without thickeners, is usually formulated with milk, milk solids and cream, it is more dense in protein, calories and fat than milk.

Finally it can be said that isolates from bacterial sample E showed the best yogurt compared to other branded yogurts of Dhaka in terms of overall yogurt texture quality

# CHAPTER SIX:

# CONCLUSION

# Conclusion

The present study has been carried to find the best strains of *Lactobacillus* to determine better quality of yogurts in terms of texture, appearance, colour and taste. Out of 40 recombined samples, eight were better quality yogurt. All samples were handled appropriately with utmost precautions and standard protocol before lab testing to obtain accurate microbial quality and further investigative analysis. In the present investigation it was observed that quality of the most of the samples was average. Only sample E showed best and consistent quality in terms of texture, appearance, colour and taste in the quality assessment test. Also the distribution of lactic acid bacteria was not uniform in different samples. The fat percentage of yogurt (6%) is slightly higher than the milk fat (5.0%), from which it was developed. In term of accurate fat percentage, yogurt has higher fat content than milk. One of the most promising isolate was obtained from sample E. Finally, it can be said that an overall good quality of yogurt in terms of texture, appearance, colour and taste was produced by inoculated fermentation of from *Lactobacillus spp* (LAB) obtained from sample E.

# CHAPTER SEVEN:

# REFERENCES

# REFERENCES

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# APPENDICES

**APPENDIX-I**

**(Composition of some of the media used in this course of work)**

**□ MRS broth**

| | |
|---|---|
| Peptone | 10.0 gm |
| Beef extract | 10.0 gm |
| Yeast extract | 5.0 gm |
| D-glucose | 20.0 gm |
| Polysorvate | 801.0 gm |
| K211P04 | 2.0 gm |
| Sodium acetate | 5.0 gm |
| Triammonium citrate | 2.0 gm |
| MgSO 4 .7H 2 O | 0.2 gm |
| MnSO 4 .4H 2 O | 0.05 gm |
| Distilled Water | 1000 ml |

**□ MRS Agar**

MRS broth+2% agar

**□ Nutrient agar**

| | |
|---|---|
| Beef Extract | 3.0 gm |

| | |
|---|---|
| Soluble starch | 2.0 gm |
| Agar | 20.0 gm |
| Distilled water | 1000 ml |

**□ Nutrient broth**

| | |
|---|---|
| Beef Extract | 3.0 gm |

Peptone 5.0 gm

Gelatin 8.0 gm

Agar 15.0 gm

Distilled Water 1000 ml

**□ Tryptophan Soya broth medium**

Tryptone (Pancreatic Digest of Casein) 17.0 gm

Soytone (Peptic Digest of Soybean Meal) 3.0 gm

Glucose 2.5 gm

Sodium Chloride 5.0 gm

Dipotassium Hydrogen Phosphate 2.5 gm

**□ Skimmed Milk medium**

Skim Milk Powder 100.0 gm

# APPENDIX-II

## Reagents

 **Physiological saline**

NaCl 0.9 gm

Distilled water 100 ml

 **Phenalphthale in indicator**

Phenolphthalein 1 %

Ethanol 99 %

 **Methylene blue**

Methylene blue 3.0 gm

Ethyle alcohol 30.0 ml

Dilute KOH (1:10000) 1000 ml

Ethyle alcohol 97.0 ml

Conc. HCl **3.0 ml**

 **Ammonium oxalate crystal violet**

Crystal violet 2.0 gm

Ethyle alcohol 20.0 gm

Ammonium oxalate 0.8 gm

Distilled water 80.0 ml

 **Iodine solution**

Beef extract 3.0 gm

Peptone 5.0 gm

KNO 3 1.0 gm (Nitrate free)

Distilled water 1000 ml

pH 7.0

 **Safranine**

 **Crystal violet**

 **Ethanol solution (95%)**

 **0.1% NaOH solution**

0.1 N NaOH solution was made by taking 2 gmNaOH and mixed with 500 ml distilled water.