Development of Improved Quality Yogurt in terms of Texture, Flavor, Food Value and Low Cost

M.S THESIS

A DISSERTATON SUBMITTED TO THE BRAC UNIVERSITY FOR PARTIAL FULFILLMENT OF THE REQUIREMENT FOR THE DEGREE OF MASTER OF SCIENCE (M.S) IN BIOTECHNOLOGY

SUBMITTED BY

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DECLARATION

This is to declare that the research work embodying the results in this thesis entitled "**Development of Improved Quality Yogurt in terms of Texture, Flavor, Food Value and Low Cost"** submitted by Nushrat Hossain, has been carried out by under the joint supervision and guidance of Professor Dr. Naiyyum Choudhury, Coordinator, Biotechnology and Microbiology Program and Associate Professor Dr. Mahboob Hossain, Microbiology Program, BRAC University in partial fulfillment of MS in Biotechnology, at BRAC University, Dhaka. It is further declared that the research work presented here is original, has not been submitted anywhere else for any degree or diploma.

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ABSTRACT

The present study is concerned with how to develop improved quality yogurt in terms of texture, flavor, food value and low cost. Natural flora and traditional flavor in yogurt of indigenous varieties have been continuously changing due to the introduction of imported commercial starter cultures. Because of the necessity to preserve our natural starter cultures and to increase the availability of them for industrial use, these cultures must be isolated from artisanal yogurts, genetically characterized and investigated regarding their desirable properties for commercial use in yogurt production. The aim of this project was to select and isolate the better strains of Lactobacillus (starters) from the locally available yogurt (both branded and non-branded) found in Dhaka, Bangladesh to optimize the yogurt production with an improved quality. Total of 19 different yogurt samples were purchased from different places for this study. Each isolated organism was used individually to produce yogurt and later better starter cultures were selected and combined to find any new or improved quality yogurt. Changes of pH and total titratable acidity were monitored and the viability of probiotic bacteria was evaluated during and after refrigerated storage. One of the most important sensorial attributes for yogurt is texture, which was assessed by sensory analysis. Therefore, chemical, physical, microbial and organoleptic analyses for samples were conducted at predetermined days. It was found yogurt isolates have potential to be used in dairy industry in terms of their high technological and organoleptic characteristics.

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(LIST OF ABBREVIATIONS)

%	percent
α	alpha
β	beta
<	less than
>	more than
≤	less or equal to
≥	more or equal to
ºC	degree celsius
ºF	degree 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.al	and others
etc	et cetra
Fig.	figure
gm/g	gram
mg	milligram
kg	kilogram
ml	millilitre
KJ	kilojoule

Kg/kg	kilogram
MW	molecular weight
IU	International Units
No./no.	number
pH	negative logarithm of hydrogen ion concentration
hr/hrs	hour
yrs	years
i.e.	that is
Ca	Calcium
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
MC	micellar casein
WC	waist circumference
MPa	mega pascal
ppm	parts per million
LAB/LB	Lactic acid bacteria
SMM	skim milk media
TSB	tryptophan soya broth or tryptic soya broth
TTA	total titrable acidity
SPC	standard plate count
TCC	total coliform count
TFC	total fungal count
MRS	Man Rogosa Sharpe agar

SNF	solids-non-fat
DNA	deoxyribonucleic acid
SMP	skim milk powder
LDH	lactic dehydrogenase
EPS	exopolysaccharides
WPCs	whey protein concentrates
MPC	milk protein concentrates
MPI	milk protein isolates
WHO	World Health Organization
CLA	Conjugated linoleic acid
DVS	Direct-vat-set
LBG	locust bean gum
CO_2	carbon di oxide
KOH	potassium hydroxide
NH3	ammonia
F6PPK	fructose-6-phophate phosphoketolase
NH4OH	ammonium hydroxide
Na2CO3	sodium carbonate
AOAC	Association of official Analytical Chemist procedure

CHAPTER 1

INTRODUCTION,

AIMS & OBJECTIVES

1. INTRODUCTION

1.1 Definition and History of Yogurt

Yogurt, is produced when milk or milk products coagulates, causing the lactic acid contained in it to coagulate, via the action of bacterial enzymes lactase provided by the bacteria *Streptococcus thermophilus, Lactobacillus bulgaricus* breaks down the sugar compound glucose and galactose that the lactose is composed of, under anaerobic conditions. The compound sugars are then processed leading to the formation of lactic acid and acetaldehyde, as shown in Figure 1.1. The process, by which milk is acidified, fermented and thus preserved, varies in locality, resulting in a diverse range of similar products such as kefir, yogurt, kumiss, acidophilus milk. It's the lowering of pH, acidification in other words, which gives the yogurt its characteristic sour taste, as well as resulting in the formation as a solid curd, through precipitation and coagulation, forming the solid curd that makes up the yogurt, while forming whey as the leftover liquid (Thapa *et al.*,2000).

Starting Reagents   End Products

Lactase   Glycolysis+ Fermentation

Lactose ------------►Glucose + Galactose -----------------►Lactic acid+Acetaldehyde

Figure 1.1: Enzymatic reaction in yogurt production

The moment milk leaves the cow; it begins to accumulate microbes that contaminate it. Moreover, milk process methods and equipment and bacteria in the air further add to the contamination process. Hence, it is necessary to pasteurize milk, i.e. kill of the microbes preventing it from going bad. According to U.S Public Health Service, heating milk at 62.8°C (145°F) for 30 minutes or 71.7°C (161°F) for 15 seconds meets pasteurization standards at which most of the bacteria, if not all, is eliminated. Principally, yogurt is made from cow's milk by the proto cooperative action of two homo fermentative bacteria *Lactobacillus bulgaricus* and *Streptococcus salivarius* which shows a symbiotic relationship between these two species of bacteria, leads to a rapid acid development in combination culture compared to developments in a single strain culture. However, *Lactobacillus bulgaricus* and *Streptococcus thermophilus* are not the only bacterial agents that enable conversion of lactose into lactic acid. In normal dairy processing industry, various combinations of starter cultures are selected during manufacturing of yoghurt in order to achieve a desirable characteristic of the product and in the process provide the consumers with a wide choice of therapeutic benefits. Based on its activity, manufacturer normally adds 2-4 % yogurt starter culture.

The nature and composition of yogurt with its bacterial cultures determines the quality along with the nature of flavor and the way it appears. The characteristic flavor of a yogurt sample is due to the production of lactic acid, carbon dioxide, acetic acid, diacetyl, acetaldehyde and several other components from the milk fermentation process where the lactose is fermented by the lactic acid bacteria. As a result, high priority is given to maintain good quality yogurt, keeping in mind that even a small contamination can cause health disorders of consumers. Until very recently, yogurts have been made from various sources, including soy milk, grape juice, a combination of mango pulp–soy milk and buffalo milk, and merged with fruits such as natural fruit juice, pulp, dry fruits, and often to serve to increase the aesthetic value (Kumar *et al.,* 2004, Desai *et al.,* 1994; Ghadge *et al.,* 2008, Coisson *et al.,*2005).

The characteristic taste of yogurt is determined by its smooth, yet viscous with a subtle flavor that resembles a walnut. The gel like texture is the primary characteristic and when added with thickening agent such as gelatin or other hydrocolloids, the yogurt texture is shown to stabilize, leading to an effective resistance against syneresis while producing that smooth sensation for the mouth (Fuquay *et al.,* 2011, Kumar *et al.,* 2004; Sodini *et al.,* 2005).

The presence of symbiosis of probiotics and prebiotics in yogurt makes it highly functional food. Probiotics can be defined as "live microbial feed supplements that beneficially affect the host animal by improving its intestinal microbial balance" (Champagne *et al.*, 2005). Prebiotics is a "non-digestible food ingredient that beneficially affects the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon". The symbiosis "beneficially affects the host by improving the survival and the implantation of live microbial dietary supplements in the gastro-intestinal tract by selectively stimulating the growth and/or by activating the metabolism of one or a limited number of health promoting bacteria" (DiRienzo *et al*., 2000).

No concrete record exists on the origin of yogurt, but it's its existence has spanned many years and civilizations, could be approximated to six thousand years. Its production developed along the warmer areas of the Mediterranean and Middle East where animal production, along with its milk, was seasonal and low. Milk was hard to preserve due to microorganisms from air, animal, feeding stuff or hands of milker. It's believed that yogurt was hugely popular amongst the nomadic people of the medieval era. For instance, written in the 11th century,the use of yogurt by ancient Turks is recorded in books, "Divan Lugati't Turk" by "Kasgarl Mahmut" and "Kutadgu Bilig" by "Yusuf Has Hacib." The word "yogurt" is possibly derived from the Turkish word "jugurt" which first appeared in the 8th century (Rasic *et al.*,1978) used to describe any fermented food with an acidic taste (Younus *et al.*,2002) The word "yogurt" is mentioned in different sections and its use by nomadic Turks is described in both books (Anon *et al.*, 2007). These names include Dahi or Dahee in India, Roba in Iraq, and Fiili in Finland (Tamime *et al.*, 1980) and several others. Historically, yogurt was made by fermenting milk with indigenous microorganisms (Rasic *et al.*, 1978, Younus *et al.*, 2002, Anon *et al.*, 2007)

Nomadic people devised the production as an intuitive process to preserve the milk during travel. Packaging was also an issue- they used animal skin to hold yogurt and salted the product and thus made it more stable, texture wise and in preservation.

During the fermentation, they started heating milk over an open fire in order to concentrate it slightly, to modify the properties of the casein, to eradicate any pathogenic microorganisms present in milk, to encourage the fermentation of milk to take place at a slightly higher temperature and also to ensure a gradual selection of lactic acid bacteria capable of tolerating high levels of lactic acid, and of giving the product its distinctive flavor. Similar methods were used by the Turkish, Armenian, and Egyptians as well as other societies. Each society found the best appropriate preservation methods for their needs, for instance, salting and drying, heating for a few hours over low fires of a special type of wood that called smoked yogurt, or keeping salted and dried yogurt in olive oil or tallow. Another method that Turkish, Lebanese, Syrian, Iranian and Iraqi people used was mixing concentrated yogurt with wheat that is called kishk. After the refrigeration became widespread, these traditional methods lost popularity except among certain communities in Middle East.

The production of yogurt has increased due to its popularity as far as nutritional and therapeutic values are concerned. Recently, yogurt was tremendously popularized in Europe for its treatment for diarrhea, under the rule of Emperor Francis I of France. The methods of production over the years have changed bit by bit, for instance the recent trend of fruit yogurts, but the fundamental steps remains the same. The improvements in medical science research have also increased yogurts nutritional effectiveness, resulting in its sustained popularity (Karagul *et al.,* 2004, Tamime *et al.,* 1980).

1.2 Yogurt Manufacturing Process

In dairy industry, no matter which manufacturing process is applied, the fermented dairy product must be appropriate to national and international standard protocol. The large scale yogurt manufacturing process entirely depend on the type of yogurt is being produced as well as the factory conditions (Akın *et al.,*2006).

The flow diagram of manufacturing steps for yogurt production is given in Figure 1.2 and the basic manufacturing steps for any types of yogurts are as follows:

- ***Filtration:***Filtration is needed to separate any cellular matter and other contaminants present in milk.

- ***Checking the presence of antibiotics:***The growth of the starter culture bacteria is inhibited due to the negative effects of antibiotics present in the milk, therefore; presence of antibiotics is checked before fermentation of milk.

- ***Standardization of milk:***The standardization of milk is one of the most important factors obtaining good quality yogurt. For this purpose milk is fortified and often mixed with skim milk and cream to adjust the fat content to the desired level. Sometimes milk powders, including nonfat dry milk, whey protein concentrates can be blended with the milk using a powder dispersion unit. The milk solids content (including the fat content) for yogurt ranges from around 9% for skim milk yogurt to more than 20% for certain types of concentrated yogurt. Many commercial yogurt products have milk solids contents of 14-15%. The minimum milk solids not-fat content required in standards or regulations in many countries ranges from 8.2 to 8.6%. Stabilizers, such as, pectin or gelatin, are often added to the milk to enhance and maintain the appropriate yogurt properties including texture, mouthfeel, appearance, consistency and to the prevention of whey separation (Tamime *et al.*,1999).

- ***Homogenization:*** Milk is homogenized before heat treatment to prevent lipolysis that causes some chemical changes in milk like: fat globule size reduction,casein micelles destructionthus helps to increase in water binding capacity.At the end of the homogenization process, milk become whiter and the yogurt formed from that milk is more viscous and flavor is homogeneously distributed all over the container (Tekinsen *et al.*,2000).

- ***Heat treatment (Pasteurization):***Heat treatment of milk also known as pasteurization can be definedas the eliminating process of pathogens and other unwanted undesirable microorganisms,thus stimulates the starter bacteriaproviding less competition for the starter culture and increasing the solid level of milk. Since heating of milk greatly influences the physical properties and microstructure of yogurt, so thereforethe most commonly used heat treatment in the yogurt industry include 85°C for 30 minutes or 90-95ºC for 5 minutes (Tamime *et al.*, 1999). However, very high temperature short time (100°C to 130°C for 4 to 16 s) or ultra-heat temperature (UHT) (140ºC for 4 to 16 s) are also sometimes used (Sodini *et al.*, 2005). Some advantages of pasteurization are:

  -Heat treatment helps to remove dissolved oxygen promote starter growth.

  -Heat treatment leads to production of some aroma compounds.

  On the other hand, sometimes heat treatment has some disadvantages due to the

  -Formation of some by-products which have an inhibitory effect on the growth of starter culture bacteria.

  - Heat treatment sometime causes reduction of pH, oxygen content of milk and denaturation of serum proteins like β–lactoglobulin and α–lactalbumin thus hydrophilicity of casein increases and syneresis decreases (Akın *et al.*, 2006).

- ***Inoculation:***Soon after pasteurization, milk is allowed to cool to 40-45 °C and inoculated with the fresh starter culture bacteria *Streptococcus thermophilus* and *Lactobacillus bulgaricus* in 1:1ratio in general. Although inoculation level varies between 1-4%, the optimum level is 2%. If inoculation level is less than 2%, the lactic acid production deceleratesleading longer periods of fermentationcauses contamination risk. Apart from that immoderate inoculation levels result in fast and too much acidity production that causes unfavorable conditions likeunwanted aroma formation, break down of yogurt texture. Depending on the capacity of yogurt manufacturing plant, inoculation pattern may vary for example:

- The most common method ofinoculating the starter culture into yogurt vessels is one by one in small-scale plants.

-The second one is the inoculation of starter cultures in large milk tanks and then filled to yogurt vessels which leads to the production of more homogenous yogurts.

-Another method is the direct injection of yogurt starter cultures in sterile milk tanks and immediately filled into vessels (Akın *et al.*, 2006).

- ***Incubation (Fermentation):***Immediate after inoculation, incubation takes place at optimum temperature of 43 ºC in incubation room or cabinet and process ends between 2.5 and 3hours. Incubation time affects the quality of final yogurt production (Tamime *et al.*, 1980).

- ***Cooling and Storage:*** If yogurts are not cooled immediately at the end of the fermentation, starter cultures continue to grow. The acidity continues to decrease and causes syneresis on the surface of yogurts. Yogurts are generally cooled by two different ways. These are:

  -One-phase cooling where yogurts are cooled to 5 or 10 ºC just after fermentation and stored until distribution to the market.

  -Two-phase cooling where the temperature decreases to 37 ºC and then 10 ºC.

  Finally, all yogurts are stored at 4 ºC for 1-2 days before sale due to maturation of viscosity and aroma of yogurt (Akin *et al.*, 2006).

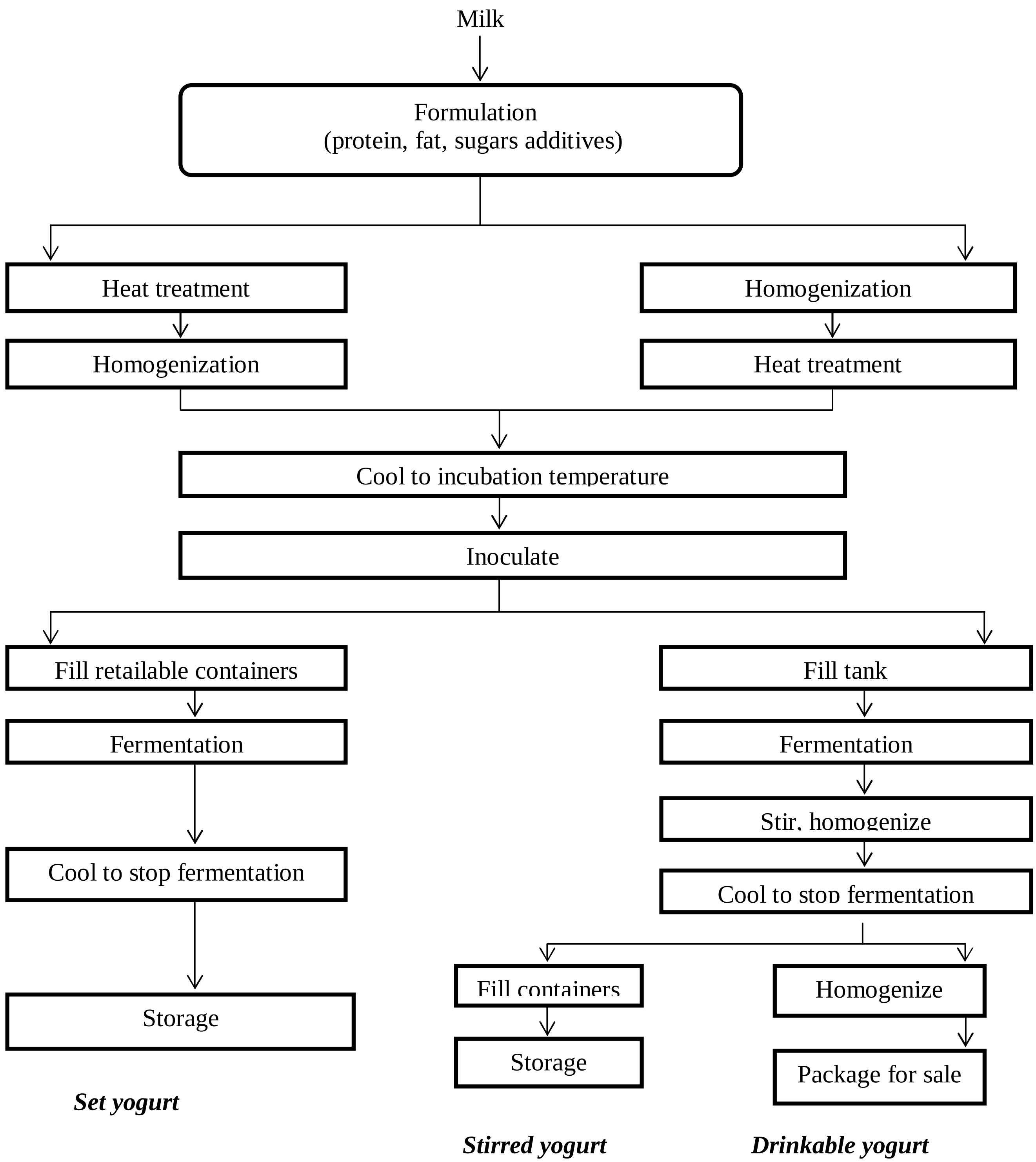

Figure 1.2: Process for yogurt manufacturing. (Source: Duboc et al., 2001)

1.3 Yogurt Types

Yogurt is mainly classified based on its chemical composition, manufacturing type, flavor type or post-incubation process (Shah *et al.,* 2000).

Table 1.1: Types of Yogurt. (Source: Shah *et al.,* 2003)

	Full-fat yogurt	Reduced-fat yogurt	Low-fat yogurt
Fat%	≥ 3	0.5-2	≤ 0.5
Non-fat% milk solids	≥ 8.25	≥ 8.25	≥ 8.25
Titratable acidity %	≥ 0.9	≥ 0.9	≥ 0.9
pH	≤ 4.5	≤ 4.5	≤ 4.5

- According to chemical composition, they are classified as full-fat, reduced-fat or low-fat yoghurt.
- According to the method of production they can be grouped as set-type and stirred-type. Set type of yoghurt is incubated and cooled in the final package and is characterized by a firm jelly like texture. It is fermented in a retail container, filled after milk inoculation and is incubated in an incubation room at a suitable temperature normally 40- 43°C for approximately 2:30 to 4 hrs.For example "Ayran" is the stirred yogurt of low viscosity (Desai *et al.*, 1994). However for stirred yoghurt, milk is incubated in a fermentation tank and the final coagulum is "broken up" by stirring before cooling and packaging stages. Stirred yogurt promotes the growth of *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus salivarius* subsp. *thermophilus* at a mild temperature (between 40°C and 43°C) until a desired acidity level is reached (Tamime *et al.*, 1980). The texture of stirred yogurt will be less firm than a set yogurt.
- According to flavor type, yogurt can be classified into subgroups. Plain yogurt is the known traditional type. The flavors are usually added at or just prior to filling into pots. Common additives are fruit or berries, usually as a puree, fruit particles or as whole fruit in syrup, sweetening and coloring compounds. These

additives often have as much as 50% sugar in them,however for the health conscious customers'manufacturers offer a low sugar and low fat version of their products. Low or no sugar yogurts are often sweetened with saccharin or more commonly aspartame.

- In drinking yogurt, the agitation used to "break" the coagulum is severe. It also has the coagulum "broken" prior to cooling and so very little reformation of coagulum may occur.
- In concentrated yogurt, it is usually inoculated and fermented in the same manner as stirred yogurt. Following the "breaking" of the coagulum the yogurt is concentrated by boiling off some of the water, this is often done under vacuum to reduce the temperature required. Heating of low pH yoghurt can often lead to protein being totally denatured and producing rough and gritty textures. This is often called strained yogurt due to the fact that the liquid that is released from the coagulum upon heating.
- In frozen yogurt, it is inoculated and incubated in the same manner as stirred yogurt. However cooling is achieved by pumping through a whipper/chiller/freezer. The texture of the finished product is mainly influenced by the whipper/freezer and the size and distribution of the ice crystals produced.
- Other types of yogurts found in the market are lactose hydrolyzed yogurt, carbonated yogurt, dried/instant yogurt, dietetic/therapeutic yogurt and soy-milk yogurt (Tamime *et al.,* 1980).
- According to the type of starter culture used yogurt can be classified after the use of probiotics. Probiotic yogurts are produced by the incorporation of other lactic acid bacteria. The examples are: *Streptococcus thermophilus, Lactobacillusdelbrueckii* ssp. *Bulgaricus, Lb. acidophilus,Lb. casei, Lactobacillus GG, Lb. plantarum, Lb. reuteri, B. bifidum, B. longum, B. reve*. *Lactobacillus acidophilus* and *Bifidobacterium* spp. are most commonly used probiotics in dairy industry (Akın *et al.,* 2006).

1.4 Composition of Yogurt

Nutritional content of yogurt is similar to the nutritional content of milk but varies in the quality of yogurt depend on the type of milk (McKinley *et al.,* 2005). Therefore milk has to be standardized in order to prevent these compositional variations of milk (Tamime *et al.,* 1980).

Table 1.2: Chemical composition of different milk source used in production of yogurt. (Source: Akin *et al.*, 2006)

Milk Source	Fat(%)	Water(%)	Total Solids(%)	Protein(%)	Lactose(%)	Ash(%)
Cow	3.7-3.9	87.4	12.7	3.3-3.4	4.7-4.8	0.7
Goat	4.5	87.0	12.3	2.9-3.3	4.1-4.6	0.6-0.8
Sheep	7.4	81.7	19.3	4.5-5.6	4.4-4.8	0.9-1.0
Buffalo	8.0	82.1	17.9	4.2	4.9	0.8

Table 1.3: Nutritional facts of Yogurt *(www.whfoods.com)*

Components	Value (per 100g)
Energy	257 KJ
Carbohydrates	4.7 gm
Fat	3.3 gm
Protein	3.5 gm
Vitamin A equivalent.	27 µg (3%)
Riboflavin (vitamin. B2)	0.14 mg (12%)

Table 1.4: The average nutritional contents of full-fat, reduced-fat and non-fat yogurts.

(Source: Akin *et al.*, 2006)

Contents	Full-fat Yogurt	Reduced-fat Yogurt	Low-fat Yogurt
Milk solid non fat	-	≥ 8	≥ 8
Energy kcal	70	84	76
KJ	293	351	318
Water (g)	87.0	78.9	80.0
Protein (g)	3.8	4.0	3.5
Fat (g)	3.8	0.9	0.1
Lactose (g)	4.6	6.3	5.5
Other sugars (g)	0	9.0	10.0
Ash (g)	0.8	0.9	0.9
Calcium (Ca) (mg)	120	130	120
Phosphorus (P) (mg)	92	110	100
Iron (Fe) (mg)	0.46	0.2	0.1
Sodium (Na) (mg)	48	60	60
Potassium (K) (mg)	157	150	150
Vitamin A (IU)	100	32	0
Vitamin B_1 (mg)	0.04	0.05	0.03
Vitamin B_2 (mg)	0.18	0.20	0.15
Niacin (mg)	0.1	0.1	0.1

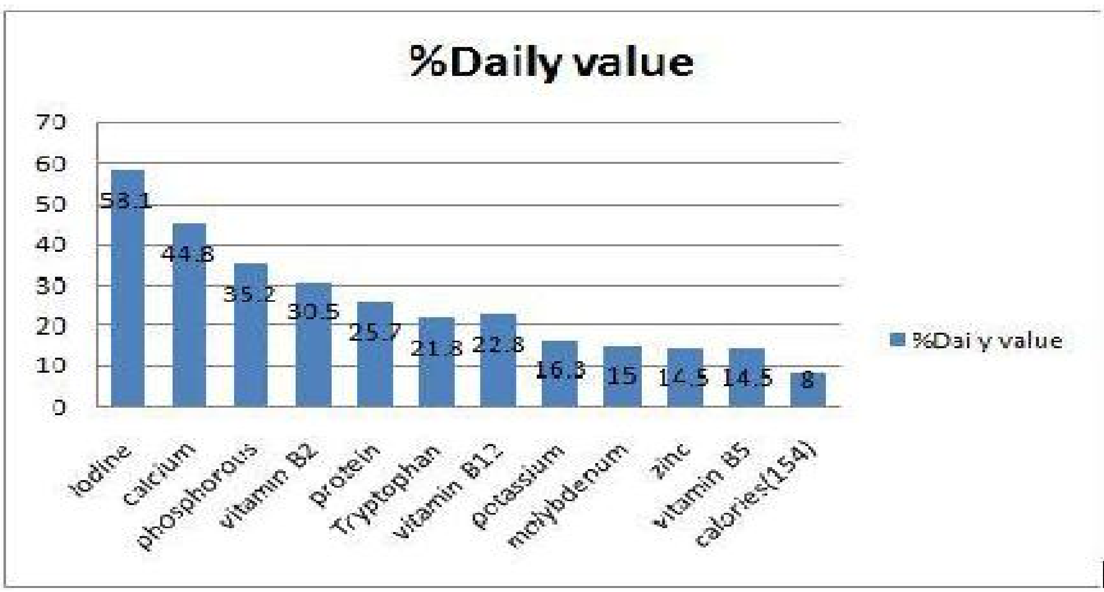

Figure 1.3: Nutrients in yoghurt per cup (245.00gms) (www.whfoods.com)

1.4.1. Total Solids Content

The range of optimum total solids content in yogurt is 15-16% in general. Therefore the viscosity increases if there's a high level of solid contentfoundin the final stage of yogurt production. As a result, fortification of milk plays an integral part before yogurt production which is done by several methods like using skim milk powder (SMP) to enrich the milk before fermentation and also whey protein concentrates (WPCs).The removal of water from the milk under vacuum improves the stability of the coagulum and reduces syneresis during storage. (Tamime *et al.*,1980).

1.4.2 Carbohydrates

The range of carbohydrates content found in yogurt is quite low (lactose content 3-4%) as a few amount of milk lactose is being used during the yogurt fermentation especially by *Streptococcus thermophilus*. Among the carbohydrates, lactose is the dominant disaccharide in milk comparing to other mono- and disaccharides present in yogurt (Tamime *et al.*, 1980).

1.4.3. Lactic Acid

Generally in yogurt production lactic acid is the end product of lactose hydrolyzed by homo-fermentative and hetero fermentative ways depends entirely on the starter culture, milk type, and manufacturing and storage conditions.Other organic acids found in yogurt are mainly citric and acetic acids, fatty acids and hypuric acid which are present in skim milk. Furthermore, lactic acid is produced in D (-) and L (+) forms. L (+) lactic acid is produced during the early fermentation. In contrast, D (-) lactic acid production starts from about the second hour of fermentation and increases continuously (Akin *et.al* 2006).

1.4.4. Protein

The proteins of yogurt are easily digestible than the proteins found in other dairy product like milk although the protein contents of milk and yogurt are quite similar as lactic acid bacteria partially hydrolyze proteins and the amount of free amino acids in fermented dairy products increase. Therefore, this makes yogurt more preferable and safe than liquid milk (Akin *et al.*,2006). Apart from that studies showed that the protein content found in milk plays an essential role for coagulum formation as a result, viscosity of the product is directly proportional to the level of protein present (Tamime *et al.,* 1980, Akın *et al.,* 2006).

1.4.5. Fat

The fat content of yogurt varies from 0.1% to 10% depending on the yogurt standards described by each country in the World. Among the fat content in yogurt conjugated linoleic acid (CLA) is considered one of the essential fatty found exclusively in the fat of dairy products, can be obtained only through the diet because human body cannot produce it (Tamime *et al.*,1980).

Table 1.5: The volatile fatty acid content of raw milk and yogurt (Source: Akin *et al.*,2006)

Volatile Fatty Acids	Raw Milk		Yogurt	
	mg	%	mg	%
Citric acid	229.6	89.0	232.40	28.1
Lactic acid	8.82	3.4	486.45	58.9
Succinic acid	0	0	18.95	2.3
Fumaric acid	1.10	0.4	8.41	1.0
Categlutaric acid	0.74	0.3	0.87	0.1
Pyruvic acid	0.09	0	2.38	0.3
Formic acid	1.33	0.5	19.51	2.4
Acetic acid	8.35	3.2	43.80	5.3
Propionic acid	0.74	0.3	1.78	0.2
n-Butyric acid	0.35	0.1	0.70	0.1
n-Valeric acid	0.20	0.1	-	0
Caproic acid	1.04	0.4	1.32	0.2
Caprylic acid	2.88	1.1	6.63	0.8
Lauric acid	1.72	0.7	2.58	0.3

1.4.6. Vitamins and Minerals

Yogurt is a good source of calcium, magnesium, potassium, phosphorus, iodine, iron, vitamin B2, zinc, selenium, and chloride. It is also a prime source of protein, conjugated linoleic acid (CLA), vitamin B12, folic acid (vitamin B9) tryptophan (an essential amino acid), potassium, vitamin B5, zinc and molybdenum. According to a study published in the British Journal of Nutrition in 2007, vitamins and minerals naturally found in milk are better utilized by the human body when in the form of yogurt due to the fermentation process involving *Lactobacillus bulgaricus, L. acidophilus* and *Streptococcus thermophilus*. The vitamin content of yogurt depends on milk type, animal feeding, manufacturing process, fermentation conditions and starter culture activation (Tamime *et al.*, 1980). The vitamin content of milk and yogurt is given in Table 1.6.

Table 1.6: Vitamin contents of milk and yogurt. (Source: Tamime *et al.*,1980)

Vitamins (Units/100 g)	**Milk**		**Yogurt**	
	Whole	Skim	Full Fat	Low Fat
Vitamin A (IU)	148	-	140	70
Thiamin (B_1) (µg)	37	40	30	42
Riboflavin (B_2) (µg)	160	180	190	200
Pyridoxine (B_6) (µg)	46	42	46	46
Cyanocobalamine (B_{12}) (µg)	0.39	0.4	-	0.23
Vitamin C (IU)	1.5	1.0	-	0.7
Vitamin D (IU)	1.2	-	-	-
Vitamin E (IU)	0.13	-	-	Trace
Folic acid (µg)	0.25	-	-	4.1
Nicotinic acid (µg)	480	-	-	125
Pantothenic acid (µg)	371	370	-	380
Biotin (µg)	3.4	1.6	1.2	2.6
Choline (mg)	12.1	4.8	-	0.6

1.5. Yogurt Starter Cultures

Yogurt starter cultures are the active microorganisms claimed to impart desirable predictable flavor, texture to milk product and considered most crucial component in the manufacture of high-quality fermented products which regarded as harmless, food-grade. At the same time due to their probiotic activities which are able to proliferate or even survive for a long period of time in human gastrointestinal tract, consumers getnutritional and health benefits (Gardini *et al.*,1999). Most commonly used yogurt starter cultures are: Gram-positive rods and cocci were predominant in the past but in recent time lactic acid bacteria used for yogurt production are thermophilic, such as *Streptococcus thermophilus* and *Lactobacillus bulgaricus* (Robinson *et al.*, 2002). These two species of bacteria are very well known starter microorganisms of yogurt fermentation. Some of the key features of yogurt starter cultures are discussed below:

1.5.1 The Role of Starter Culture on Yogurt

The main role of starter culture in the yogurt manufacturing process is:

- The function of yogurt starter culture in the production of yogurt is acidification through the conversion of lactose into lactic acid, coagulation of milk proteins, creation of the viscous texture by the production of exopolysaccharides, and development of the typical yogurt flavor. A starter culture is simply a sample of fermented food that are known as artisanal or undefined cultures may contain historically tested blends of starter culture organisms. The actual identities of the organisms present in a mixed culture which however is not known thus give the disadvantages of inconsistent proportion of different organisms in a mixed culture, inconsistent quality, uncharacterized individual species microbiologically or biochemically (Chaves et al., 2002).
- Precipitation of casein micelles that occurs at pH 4.6 as a result of the increased acidity in order to produce lactic acid homo fermentatively from lactose and lowering the pH below 4.5 in 4 hrs.
- Production of distinct flavor of yogurt by the proto cooperative action of *lactobacilli* and *streptococci*. For example, “acetaldehyde” at levels up to

40mg/kg is the major contributor to the flavor and it comes from *lactobacilli* (Teixeira *et al.*,1999). However, studies have found that the activity of the enzyme “threonine aldolase” produced by *S.thermophilus* decreases drastically when the incubation temperature is above 30°C, while the enzyme from *lactobacilli* remains unaffected (Robinson *et al.*,2002). At the same time “threonine aldolase” which showed almost linear relationship between the level of “acetaldehyde” produced during milk fermentation and activity of this particular enzyme “threonine aldolase” in different *S. thermophilus* strains, turned out to be another potential flavor contributor (Chaves *et al.*,2002).

- Some other flavor contributors work best with starter cultures include: diacetyl, acetone, acetoin, organic acids such as acetic, lactic acid, fatty acids and free amino acids but their effects have not been well understood yet.
- The production of exopolysaccharides (EPS) by starter cultures during fermentation of yogurt contributes special importance especially for the countries where the uses of stabilizers are forbidden. As both strains of *S.thermophilus* and *L. bulgaricus* can produce extracellular polysaccharides (EPS) also known as ropy or slime producers can increases the viscosity by binding free water and preventing the gel fraction and whey synerysis (Pearce *et al.*,1999, (Zirnstein *et al.*,1999).
- The starter culture also improves nutritional value and digestibility of yoghurt as probiotic. As *S. thermophilus* do not colonize the intestine, consumption of viable cells can enhance lactose digestion of lactose intolerant people therefore such individuals are shown well tolerance to yogurt better than other dairy products containing the same amount of lactose (Zirnstein *et al.*,1999).
- The starter culture like *Lb.delbrueckii* ssp. *bulgaricus* has some of the preservative effect on the product as it produces lactic acid, hydrogen peroxide and antimicrobial compound like bacteriocin (Teixeira *et al.*,1999).

1.5.2 Starter Culture Systems Used for Yogurt Production in Dairy Industry

In some countries, there is a legal requirement for *Lactobacillus bulgaricus* to be included in the starter culture of any dairy product labeled as 'yogurt', because its typical flavor of yogurt depends on the presence of *Lactobacillus bulgaricus* where in some parts of the world, *Lactobacillus helveticus* and *Lactobacillus lactis* are sometimes mixed with the starter culture (McKinley et al., 2005). However in other countries, such as Australia, other thermophilic lactic acid bacteria, such as *Lactobacillus helveticus* and *Lactobacillus jugurti* are also permitted. According to the food legislations of some countries, the bacteria also should be abundant and viable during consumption. In literature, starter cultures have been defined as preparations of one or more strains of one or more microbiological species (Wigley et al., 1999). Yogurt is generally inoculated with 1:1 ratio of *Streptococcus thermophilus* and *Lactobacillus bulgaricus* (Tamime et al., 1980). The growthassociation between *Streptococcus thermophilus* and *Lactobacillus bulgaricus* is termedsymbiosis. The rate of acid production in mixed culture is greater than the rate of acidproduction using single strain (Tamime *et al.*, 1980). Yogurt starter cultures are mass-produced in fermenters under aseptic conditions for dairy industry. For large scale industrial production of yogurt, some common systems of using starter culture are done by these following ways:

1. Use of milk or whey based media and molasses and corn-syrup as the basal media are frequently used for commercial production of yogurt.
2. Use of some vitamins like vitamin B and specific amino acids for the optimum growth of starter cultures.
3. Control of pH by the addition of alkaline, usually gaseous NH3, NH4OH, Na2CO3, or KOH.
4. Use of cryoprotectants like glycerol, lactose, sucrose, ascorbate and glutamate for both frozen and lyophilized cultures in order to maintain cell viability (Durso *et al.*,2003).
5. Daily sub culturing of starter cultures which have been propagated and prepared from mother culture(Wigley *et al.*, 1999).

6. Manufacturing of frozen cultures as normal, concentrated or direct set type which has been frozen after inoculation supplied in bottles. For example frozen concentrated cultures should include 10^{10}-10^{11} cfug^{-1} which allows 70ml of culture for 1 ton medium for bulk propagation. Frozen concentrated cultures are transported in insulated aluminum cans containing solid CO_2 to maintain the temperature at -70°C. The shelf-life of culture is usually 3 months at -50°C.
7. Manufacturing of lyophilizedcultures by freeze drying under vacuum for both bulk starter propagation and direct-to vat propagation stored at +4°C and as well stable at room temperature usually packaged in oxygen-impermeable materials.
8. Use of direct-vat-set (DVS) type cultures that reduce the risk of phage contamination and maintain the strain balance in mixed strain cultures (Surono *et al.,* 2002).

1.5.3 The Associative Growth of Yogurt Starter BacteriaAffect the Yogurt Quality:

In large production facilities where precise schedules are essential and consistent product quality is expected, artisanal starters cannot be used. Instead defined cultures have become predominant (Durso *et al.,*2003). Defined cultures contain physiologically, biochemically and genetically characterized strains, which are used individually or as blends. Most of the defined strains have been isolated from wild or artisanal cultures (Hebert *et al.,*2000). They are characterized and screened for the desirable traits. Hence they give consistent quality and flexibility to modify the production according to demands, e.g. high productivity, quality and safety. The associative growth of *S. thermophilus* and *Lb. delbrueckii* ssp. *Bulgaricus*have been used to ferment milk toyogurt together. There are important reasons for this synergistic relationship. It isbased on the metabolic compatibility between the two species. Studies have shown thatcombined culture of starter bacteria produce much higher acidity than the isolatedstrains. While the combined culture produces an acidity of >10g/L within 4 h, the valuesin the isolated strain of *S. thermophilus* is 4g/L and 2g/L for *Lb. delbrueckii* ssp. *bulgaricus* (Robinson *et al.,* 2002). The associative growth of yogurt starter bacteria in Figure 1.4.

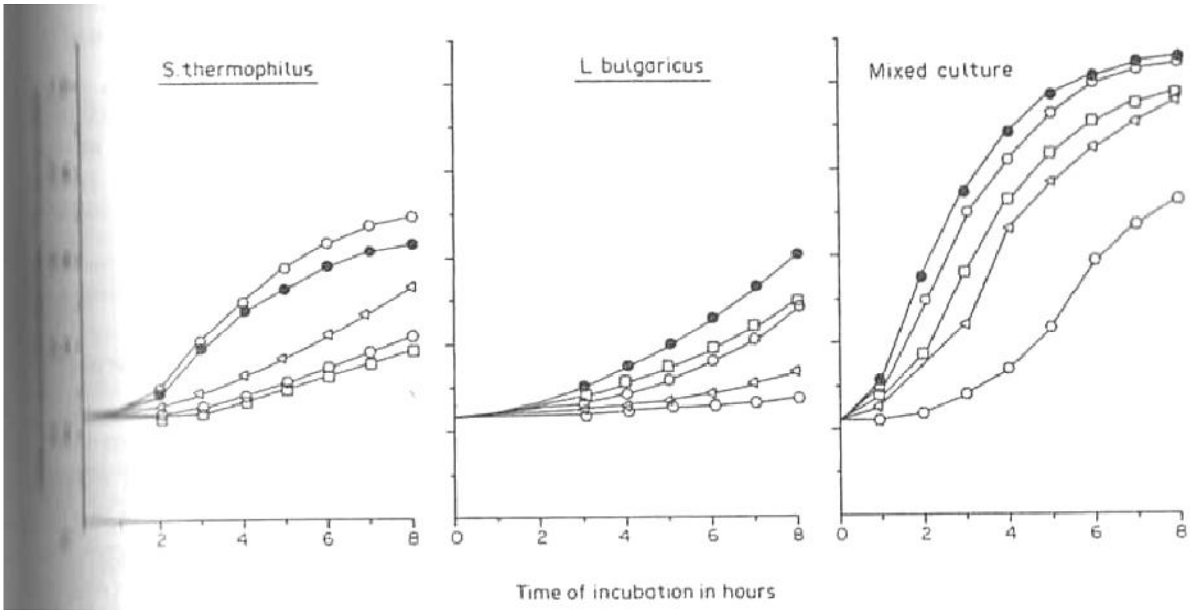

Figure 1.4: The Associative growth of Yogurt Starter Bacteria

(Source: Tamime et al., 1980).

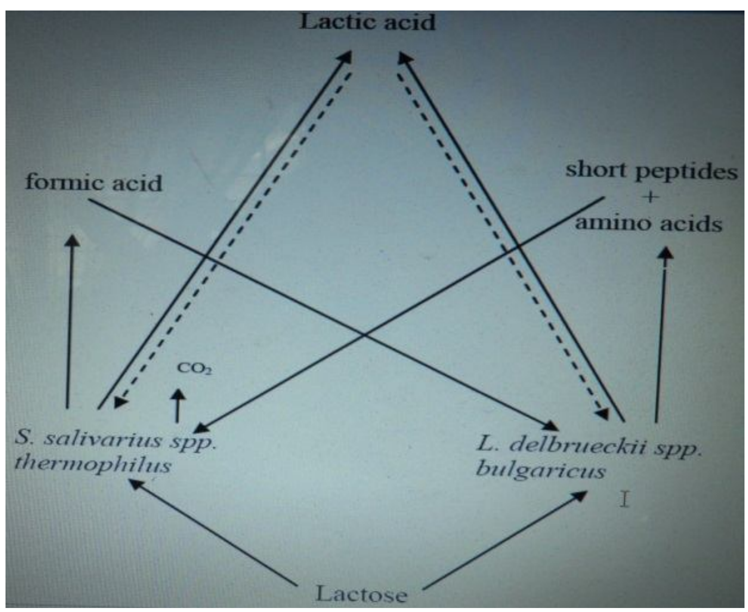

Figure 1.5: Relationship between starter bacteria in milk fermentation.

(Source: Tekinsenet al., 2000)

1.5.4 Factors Leading to Inhibition of Yogurt Starters

Yogurt starter cultures should able to multiply and produce lactic acid very fast for yogurtfermentation. However some major factors that may lead the fermentation to fail are as follows:

1. Growth of pathogenicorganisms found in raw material can grow in the end-products at high pH that providelow acid production or completeinhibition in dairy fermentations. For example both *S. thermophilus* and *L. Bulgaricus*strains are susceptible to virulent phages and problems are observed especially whenthesestarter cultures contain a single strain or when the same culture is re-used over an extendedperiod of time (Josephsen *et al.*,2004)
2. The residues of detergents and disinfectants like quaternaryammonium compounds, iodophors, hypochlorite and hydrogen peroxide used forcleaning of dairy equipment can also decrease the activity of starter cultures thus may hampers the favorable conditions of their growth and survival (Surono *et al.*,2002).
3. Growth of yogurt starter cultures can be inhibited by some natural compounds like lactins andagglutinins found in milk. Since these compounds are heat sensitive so therefore can easily be destroyedduring pasteurization of milk.
4. Environmental pollutants like insecticides have also crucial effect on inhibition (Teixeira *et al.,* 1999).
5. Due to the incompatibility between strains of *S. thermophilus* and *L. Bulgaricus,*some changes in the activity such as the rate of acid production, aroma-flavor production occur during fermentation by this particular cultureleading tocomplete destruction of proto cooperation between these species(Robinson *et al.*, 2003).

Some of the solutions in order to overcome these problems are suggested as follows:

1. Practice goodaseptic techniques, rotating the starter cultures, using phage-resistant starter cultures orusing multiple strain starters, each of which is resistant to different host-specific phages (Teixeira *et al.,* 1999).

2. Phage inhibitory media are also used as a means of phage protection. In 1956, the US Department of Agriculture introduced the idea of using substances tochelate Ca^{++} and Mg^{++} required for successful phage adsorption to the bacterial cell. Forexample, the phosphate salts have buffering capacity for fermentation (Durso *et al.,*2003).
3. *S. thermophilus* is sensitive to antibiotics like penicillin, streptomycin, neomycin and ampicillin. These antibiotics can easily slow down the fermentation process therefore it is very much important to examine the milk forthe presence of antibiotics (Surono *et al.,* 2002).

1.6 Beneficial effects and uses of LAB

Lactic acid bacteria have been used as starter cultures for dairy, meat and vegetable fermentations in many countries. They contribute to flavor development, preservation of foods, influence in intestinal medium because of its enzymes b- galactosidase, glycolases and lactic dehydrogenase (LDH) which produce lactic acid from lactose(Amer *et al.,* 1983). Lactic acid is reported to have some physiological benefits such as:

- Inhibition of *Helicobacter pylori* and intestinal pathogens.
- Reduction of the risks associated with mutagenicity and carcinogenicity. For example bladder cancer
- Prevention of inflammatory bowel disease.
- Improvement of immune system (Metchnikoff *et al.,* 2004)
- Enhancing the digestibility of milk proteins by precipitating them in fine yogurt particles.
- Improving the utilization and absorbtion of calcium, phosphorus and iron.
- Stimulating the secretion of gastric juices.
- To prevent and manage atopic dermatitis (eczema) in children
- Serving as a source of energy in the process of respiration.
- Production of antimicrobial agents, such as organic acids, bacteriocins, diacetyl and hydrogen peroxide serves as preservativesalso inhibit the growth of harmful putrefactive microorganisms (Noordiana *et al.,* 2013).

The antagonistic activities caused by lactic acid bacteria and their mode of action are summarized in table 1.7

Table 1.7: Antagonistic activities caused by lactic acid bacteria. (Source: http://www.medicinenet.com)

Metabolic product	Mode of antagonistic action
1. Carbon dioxide	Inhibits decarboxylation, reduces membrane permeability.
2. Diacetyl	Interacts with arginine-binding proteins.
3. Hydrogen peroxide/ Lactoperoxide	Oxidizes basic proteins.
4. Lactic acid	Un-dissociated lactic acid penetrates the membranes, lowering the intracellular pH. It also interferes with metabolic processes such as oxidative phosphorylation.
5. Bacteriocins	Affect membranes, DNA-synthesis and protein synthesis.

1.7 Health Benefits of Yogurt

Yogurt is considered one of the healthiest foods when it consists of live active bacteria and without any of sugars, artificial sweeteners, thickeners, colorants or preservatives.According to Natasha Trenev, author of "Probiotics: Nature's Internal Healers: “Yogurt, as well as milk, has a perfect balance of proteins, carbohydrates and fats. Yogurt more than holds its own in the nutritional spectrum of foods. It is not a carbohydrate, although it is plentiful in the sugar lactose; it is not strictly a protein but is protein-rich with up to 22 g per cup; and it is not a pure fat, yet it is abundant in healthy fatty acids. While the high amounts of probiotic bacteria in yogurt adds the extra nutrient that make yogurt the healing super food it is becoming praised for. So yogurt without any doubt is a complete food.” There are numerous advantages of consuming fermented dairy products containing probiotic bacteria.

- The probiotic microorganisms found in yogurt provide a good source of protein, calcium, phosphorus, potassium, vitamin B12, riboflavin (vitamin B2), thiamin (vitamin B1), folate, niacin, magnesium and zinc and make sure calcium is efficiently absorbed into the bloodstream so the bones get ample supply of this calcium (Athar, 1986,McKinley *et al.,* 2005).).

- Daily consumption of probiotic organismsfound in natural yogurt ensures good intestinal healthby diminishingworst possible gastrointestinal disorders such as constipation, diarrhea, dysentery, colon cancer andrehabilitating good microorganisms again in the guts and stomach environment.
- It provides immunity, protect us from cold, cough and strengthen body's defense mechanism.
- It strengthens the collagen in the skin and is good for our skin.
- It lowers the blood pressure, bad cholesterol and risk of heart attacks.
- It discourages vaginal infections.
- Consumption of yoghurt can cease the growth of *Helicobaterpylori*; causes most ulcers (Gandhi *et al.,* 1975).

1.8 Texture of Yogurt

As yogurt is a very popular healthy fermented product worldwide,the texture is one of the most claimed distinctive features in general (Britten *et al.,*2001). In yogurt fermentation the starter cultures consisting of *lactobacillus*produce bacteriocins and other antimicrobial substances that facilitate long shelf life of food, optimize its unique texture, flavor and other sensory attributes (Leroy *et al.,*2004). In terms of overall good yogurt texture, the common attributes that has been influenced the stability of yogurts are taken in to consideration are firmness, consistency, adhesiveness, and cohesiveness (Tamime *et al.,* 1980).Normally denaturation of protein during heat treatment of milk is a common way that improves the yogurt texture as it helps to form bonds between water and protein molecules (Tamime *et al.,* 1980). As a result, texture of yogurt improvesa lot of studies and experiments have been done in past few years to improve yogurt texture at its best without hampering its own health benefits. Some of the recent developments that have been applied for improving yogurt texture quality are:

1. Stabilizers and polysacharide-producing cultures have been used (Escalante *et al.,*1998).

2. Addition of milk solids are frequently used to improve the texture of milk products. Apart from that whey protein concentrates (WPCs);polysaccharide such as locust bean gum may provide an increased quality (LBG)(Unal *et al.*,2003).
3. Using EPS-producing starter cultures as they tend to improve the rheological properties of fermented yogurt.

Despite applying these methods in attempts to improve yogurt texture quality, some disadvantages are still there:

1. One of the recent studies showed that the rheological properties of stirred yogurt were affected by the type of EPS producing strains used, suggesting an effect due to the interaction between the polymer and milk proteins (Marshall et al., 1999).

2. Syneresis (whey separation) on the surface of set type yogurt is considered as a defect. Using ropy-EPS (ropy-exopolysaccharide) producing starter cultures, syneresis could be overcome since non-EPS starter cultures had the highest level of syneresis (Amatayakul *et al.,* 2006).

3. Use of some stabilizers are restricted or prohibited in some European countries (Amatayakul *et al.*,2005).

1.9 Storage conditions

Storage conditions are very much important criteria for preservation andviability of probiotic microorganisms in yogurt. It not only avoids possible risk of spoilage from yeasts but alsoprevents further activity by starter culture. The standard storage temperature should be remained within 2- 4ºC (Tamime *et al.,* 1999). Most studies showed that higher survival rates of lactic acid bacteria were obtained at lower storage temperatures (Gilliland *et al* 1988; Foschino *et al.,* 1996). Thus the interesting facts of preserving yogurts in low temperature are not only hinders the excessive growth of the starter culture but also gradually over acidification in general (Kneifel *et al.,* 1993)

1.10 Yogurt Packaging

Yogurt is targeted for a wide range of customers and packaged in a form that is suited to individual groups. Regular packaging involves plastic, glass or terra cotta cups; and also in squeeze tube to make it child-friendly. When sold in drinks, yogurt is packaged in various sizes, ranging from single to family-size containers. For cup yogurt, plastic is the medium of packaging for all types and sizes; containers having plastic inner seal, plastic coated foil inside, as well as plastic lid as cover as optional. With the ongoing advancements of plastic manufacturing technology ensuring clear plastic containers, consumers are presented with the option of buying health-benefit products, often layered with fruits-on-bottom, previously sold on glass pots.

1.11 AIMS AND OBJECTIVES

There's an increasing demand for taste, quality, stability and shelf life of the yogurt from customers' side. Hence the research in the field of quality assessment of yogurt marketed is the basic need to create awareness among common people. The aim of this project was mainly to focus in isolating the better strain of Lactobacillus (starters) from the local brands available and found in Bangladesh to optimize the yogurt production in terms of texture, flavor, food value and health benefits.

Specific objectives

- Analysis of some branded (industrial) and unbranded (locally produced yogurt) samples of yogurt from Dhaka city (the capital city of Bangladesh) for some organoleptic qualities.
- Isolation of fast growing lactic acid bacteria from the collected yogurt samples and their characterization in terms of inoculated yogurt fermentation.
- Optimization of yogurt fermentation with selected isolates of the lactic acid bacteria isolated from natural yogurts collected from the market. So as to have yogurt uniform characteristics in terms of texture, flavor, food value and low cost.

CHAPTER 2
REVIEW OF LITERATURE

2. REVIEW OF LITERATURE

Andreas *et al.*, 2000 studied the sensory investigation of yogurt flavor perception: mutual influence of volatiles and acidity. The sensory properties of traditional acidic and mild, less acidic yogurts were characterized by a trained panel using a descriptive approach. Many of the descriptive attributes varied almost linearly with pH, showing either a positive or negative correlation with increasing acidity. The panel was very sensitive to acidity differences, as demonstrated by the linear relationship between acidity perception and pH. Important flavor differences were found between the two classes of yogurt. They were mainly due to differences in acidity and not to different concentrations of the three impact aroma compounds, acetaldehyde, 2, 3-butanedione, and 2, 3-pentanedione. This emphasizes the importance of acidity in yogurt flavor. Deodorization and impact aroma compound addition had much less influence on yogurt flavor than pH variations.

Shahid *et al.*, 2001 studied the quality evaluation of market yogurt/dahi. This study was planned to evaluate and compare the quality of market yogurt and dahi. Different samples of plant made yogurt and dahi available in local markets of Islamabad and Rawalpindi were randomly collected and analyzed for physico-chemical, microbiological and organoleptic properties. Physicochemical analysis revealed that plant made yogurt samples were consistent and hardly showed any variation as compared to dahi. Microbiological examination showed that total viable count in yogurt brands was less than dahi. The coliform count was nil or ignorable in yogurt brands but dahi contained large number of coliform bacteria. Organoleptically, plant made yogurt was found more suitable as compared to dahi.

Zahoor *et al.*, 2002 studied the viability of *Lactobacillus bulgaricus* as yogurt culture underdifferent preservation methods. In present study, *Lactobacillus bulgaricus* (yogurt starter culture) was isolated from indigenous sources and preserved by three different methods 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's staining 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 best method for maintenance and preservation of starter culture.

Chagarovskii *et al.*,2003 studied the biotechnology of yogurt and kefir production, study of their effect on human health. Biotechnological parameters of bio-yogurt and bio-kefir production have been studied. The impact of the temperature on biological activity of lactic acid bacteria strains have been studied in the consortia of direct vat set (DVS). The phase of milk fermentation and increase of acid production comes within 6 hours at the temperature of 40 ºC and pH in the limits of 4.7-5.1 for thermophilic strains and 30 ºC and pH 5.4-5.7 for mesophilic strains. The concentration of alive cells counts 5 x 10(10) cfu/ml of the product. The probiotic properties of bio-yogurt and bio-kefir have been proved by clinical trials. Positive effect on the health of elder people has been shown.

Nguyen *et al.*, 2004 studied the isolation and identification of Bifidobacteria from yogurt. Bifidobacteria are commonly used for production of fermented milk, alone or in combination with other lactic acid bacteria. This paper presents methods of isolation and determination of bifidobacteria from yogurt. Therein, modified fructose-6-phophate phosphoketolase (F6PPK) assay is effective method for rapid determination of bifidobacteria. Using primers to amplify 16S rDNA gene specific for bifidobacteria is determined and evaluated.

Ozlem *et al.*, 2005 studied the isolation and characterization of *Lactobacillus bulgaricus* and *Lactobacillus casei* from various foods. The aim of this study was to determine *Lactobacillus bulgaricusand Lactobacillus casei* isolated from yoghurt and to determine the antimicrobial activity and antibiotic resistance of these isolates. The identity of the culture was based on characteristics of the strains of *Lactobacillus spp.* carrying out microscopy (morphology), Gram straining, growth at 15 ºC and 45 ºC, and fermentation of different carbon sources and growth in 7.5% NaCl. .

Ana *et al.*, 2006 studied the simultaneous effects of total solids content, milk base, heat treatment temperature and sample temperature on the rheological properties of plain stirred yogurt. Response surface methodology was used to establish a relationship between total solids content, milk base, heat treatment temperature, and sample temperature, and consistency index, flow behavior index, and apparent viscosity of plain stirred yogurts. Statistical treatments resulted in developments of mathematical models. All samples presented 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 significant increase in consistency index and a decrease in flow behavior index. Increase in the sample temperature (1.6-18.4 °C) caused a decrease in consistency index and increase in flow behavior 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 Dairy Company. The stirred yogurt was purchased from the market (sixty samples). They were transported to the Faculty of Animal Production, laboratory to assess the chemical and microbiological content and shelf life of stirred yoghurt. Chemical and microbiological examinations were carried out on 1, 2, 4, 6, 8 and 10 days of manufacturing. Ten samples from six batches were examined for fat, protein, lactose, ash, total solids, solids-non-fat and measurement 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-fat 11.73-13.58, acidity 0.93-1.12, pH 3.81-4.19, and viscosity 61.98-6.95. The highest log counts for *Streptococcusthermophilus* 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 that the storage period had significant ($P > 0.001$) effect on the chemical composition except on the total solids and viscosity. Also there was significant ($P > 0.01$) effect of the storage period on the microbiological tests.

Mihaela*et.al* 2007 studied microbiological evolution of lactic acid bacteria to yogurt storage during shelf-life. Lactic acid bacteria evolution (*Lactobacilus bulgaricus* and *Streptococcus termophilus*) was followed in three stages of storage: in first day of storage after processing; in the middle of storage during shelf-life; in the last day of storage during shelf-life. An important decrease of lactic acid bacteria was observed to storage during shelf-life. This indicates a low stability of starter culture, viable germs being inhibited by other micro-organisms development (first Enterobacteriaceae then yeast and molds).

Reyhan*et al.*, 2008 researches about viable *Lactobacillus bulgaricus* and *Streptococcus thermophilus* numbers in the market yogurts.The industrial production of yogurt is increasingly developed in the world. Yogurt is a fermented milk product obtained from fermentation of *Lactobacillus bulgaricus* and *Streptococcus thermophilus* strains. In Turkey, yoghurt is produced by the two ways; one of them is a traditional method without using starter culture in small dairy plants and the second production method by using industrial starter culture in modern plants. In this study yogurt samples were collected from the local markets which were produced traditional process and produced in modern plants by addition of starter cultures, their viable *L. bulgaricus* and *S.thermophilus* bacteria numbers, coliform, *Escherichia coli*, yeast and mould counts, pH values were determined and compared each other. Yogurts have pH values between 3.95-4.23, viable *S. thermophilus* and *L.bulgaricus* numbers 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 yogurt samples. As the result, yogurts which are produced by starter cultures have high numbers of yogurt bacteria means that yogurts produced by using starter cultures have higher therapeutic and/or antimicrobial properties beside of their organoleptic characteristics.Importance of yogurt production by using starter cultures should be known and advantages of using starter cultures in fermentation products should be stated.

Guliev *et al.*, 2009 studied the isolation and identification of lactic acid bacteria from some Azerbaijani yogurts. Isolation of lactic acid bacteria (LAB) was performed from three types of yogurts (Ka-rabakh, Ganja, Baku). These products were produced in the territory of Azerbaijan. Karabakh and Baku yogurts are made from cow milk and Ganja yogurt - from buffalo milk. All types of yogurts were produced without the addition of lactic acid bacteria starter cultures. From these dairy products overall 178 isolates were isolated and after catalase test, Gram staining and microscopic observation, 115 were chosen for further analyses. The selection of LAB isolates was based on their proteolytic and antimicrobial activity. Based on the identification of LAB isolates by biochemical tests and molecular methods it was determined that four strains of LAB were primarily present in three yogurt types: *Lactobacillus delbrueckii* subsp. *lactis, Lactobacillus delbrueckii subsp.bulgaricus* and *cocci* representatives to species *Streptococcus thermophillus* and *Enterococcus faecium*. It was determined that 5 enterococci out of 115 tested isolates, were antimicrobial compounds producers.

Istikhar *et al.*, 2009discussed thequality comparison of probiotic and natural yogurt. The study 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 organoleptic properties. Physico-chemical analysis showed that probiotic yogurts have more pH, fat and solid 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 overall acceptability of the yogurt. Similarly, an increase in the TA of the natural yogurt might affect the quality of the product. Microbiological analysis found no significant variation in total viable count between probiotic and natural yogurt.

Oyeleke *et al.*, 2009 studied the microbial assessment of some commercially prepared yogurt retailed in Minna, Niger State. Five samples each of twenty brands of commercially produced yogurt were purchased randomly from different provision stores within Minna. The results showed that the total bacterial count ranged from 1.0 x 10^7 to 9.4 x 10^7cfu/ml. The organisms isolated included species of *Staphylococcus, Lactobacillus,Enterobacter* and *Bacillus*, for bacteria, and species of *Aspergillus, Fusarium, Candida, Penicillium,Cephalosporium* and *Mucor* for fungi. However, species of *Bacillus* and *Aspergillus* were isolated the most frequently. The result revealed that yogurt commercially produce in Minna are of high quality. All effort should be geared toward sustaining it.

Ashraf *et al.*,2010 studied some technological and compositional aspects of set yogurt from reconstituted whole and mixed milk powder**.** This study was based on collection of ten batches of milk and set yogurt samples from a modern dairy plant in Khartoum State to estimate the compositional quality of set yogurt. The milk samples used for the manufacturing of set yogurt were of reconstituted whole milk powder or a mixture of reconstituted whole milk and skim milk powder. The set yogurt samples were collected immediately after processing then they were stored at refrigerator. Results indicated that the mean level of total solids (T.S.), ash content and acidity in set yogurt made from reconstituted whole milk powder + skim milk powder samples were found to be 14.00± 0.87 %, 0.769± 0.10 % and 0.847± 0.127%, respectively. Similarly in set yoghurt from reconstituted whole milk powder the mean level of T.S., ash content and acidity were 15.29± 0.524%, 0.736± 0.037% and 0.770± 0.081%, respectively. It was concluded that the values of set yogurt agreed with international standards for total solids, ash and titratable acidity. However, due to the uneven levels of these measurements, standardization of the compositional quality should be adopted in the country.

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

Lee *et al.*,2010 studied the formation and physical properties of yogurt. Yogurt gels are a type of soft solid, and these networks are relatively dynamic systems that are prone to structural rearrangements. The physical properties of yogurt gels can be qualitatively explained 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) and repulsive (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 of yogurts. Various processing variables are discussed which influence the textural properties of yogurts, such as total solids content, heat treatment, and incubation temperatures. A better understanding of factors contributing to the physical and structural attributes may allow manufacturers to improve the quality of yogurt.

Dey *et al.*,2011 studied the evaluation of the quality of Dahi available in Sylhet Metropolitan City. The study was undertaken to evaluate the quality of Dahi available in Sylhet Metropolitan City. Dahi samples from five different Sweetmeat Shops namely Fulkoli, Banaful, Mohanlal, Modhuban and Shad were collected and analyzed. Significant difference in chemical (protein, fat, total solids, ash, acidity and pH) and microbiological status was found among different Dahi samples. Of the five Dahi Brands examined, Fulkoli Brand Dhai had the highest protein content (4.58 ± 0.24) and Shad Brand Dahi had the lowest protein content (4.01 ± 0.17). Fat content was highest in Fulkoli Brand (4.02 ± 0.13) and lowest in Shad Brand (2.10 ± 0.21). The highest total solids content was found in Dahi of Shad Brand (38.00 ± 2.23) and lowest total solids content was found in Banaful Brand Dahi (32.02±1.50). Highest Total Viable Count (log cfu/ml.) was recorded in the Dahi of Shad Brand (5.92±0.09) and lowest Total Viable Count was recorded in Mohanlal Brand Dahi (5.84±0.06). Coliform bacteria, Yeast and Mould were present in all the samples. From this experiment, it was found that Fulkoli Brand Dahi was the best in quality. Dahi of Banaful and Mohanlal were in 2nd and 3rd position in quality.

Sudeep *et al.*, 2012 studied the effect of heat treatment of milk on the sensory and rheological quality of dahi prepared from cow. Milk Dahi is a popular fermented Indian dairy product prepared by fermenting the milk by lactic acid bacteria. The texture of dahi depends mainly on the heat treatment given to milk. Cow milk (3.5% fat and 8.5% SNF) was subjected to two separate treatments: (1) heating at 63ºC for 30 min and (2) boiling treatment without holding period. The milk was cooled to about 40 ºC and inoculated with *Lactococcus lactis* culture and incubated at 30 ºC for about 12 hours. The dahi formed was chilled to 5 ºC and evaluated for quality. Firmness, consistency and index of viscosity as measured by Texture Analyser increased with increased heat treatment and the highest values were observed in dahi prepared from boiled milk. Boiling treatment of milk resulted in least syneresis of whey in the curd. Based on the results, it was recommended that milk be subjected to boiling treatment to produce best quality dahi.

Ahmad*et al.*, 2013 studied the quality assessment of yogurt produced at large (industrial) and small scale. The quality of fermented dairy products is a delicate subject. In addition to processing conditions, it largely depends on pre and post process handling. The present study is concerned with the physicochemical and microbiological quality of commercially available yogurt in Faisalabad, Pakistan. For this purpose, two branded (produced at large scale) and three unbranded (produced at small scale) yogurt samples were collected from the city and were analyzed in triplicate. The data was analyzed by complete randomized design and comparison of means was done by Duncan's multiple range tests. The coliform count in branded samples was nil or ignorable. However, unbranded samples contained a higher count of coliforms. The branded samples were mono cultured (*S. thermophilus*), which does not fulfill the quality criteria as *L. bulgaricus* is also needed for good quality yoghurt. In contrast, in unbranded samples, both the bacteria were present but their growth was uncontrolled. The fat, lactose and total solid contents were low in unbranded yogurt samples than in branded samples showing lack of standardization. However, acidity and syneresis value of branded samples were low as compared to unbranded samples.

Karam*et al.*,2013 reviewed the effect of dairy powders fortification on yogurt textural and sensorial properties.Yogurts are important dairy products that have known a rapid market growth over the past few decades. Industrial yogurt manufacture involves different processing steps. Among them, protein fortification of the milk base is elemental. It greatly enhances yogurt nutritional and functional properties and prevents syneresis, an undesirable yogurt textural defect. Protein enrichment can be achieved by either concentration process (evaporation under vacuum and membrane processing: reverse osmosis and/or ultrafiltration) or by addition of dairy ingredients. Traditionally, skim milk powder (SMP) is used to enrich the milk base before fermentation. However, increased quality and availability of other dairy ingredients such as milk protein isolates (MPI), milk protein concentrates (MPC) whey protein isolates (WPI) and concentrates (WPC), micellar casein (MC) and caseinates have promoted their use as alternatives to SMP. Substituting different dry ingredients for skim milk powder in yogurt making affects the yogurt mix protein composition and subsequent textural and sensorial properties. This review focuses on various type of milk protein used for fortification purposes and their influence on these properties.

Donovan*et al.*, 2014 discussed on the health effects of yogurt. Yogurt has been part of the human diet for thousands of years, and during that time a number of health benefits have been associated with its consumption. The goal of the First Global Summit on the Health Effects of Yogurt was to review and evaluate the strength of current scientific knowledge with regard to the health benefits of yogurt and to identify areas where further research is needed. The evidence base for the benefits of yogurt in promoting bone health, maintaining health throughout the life cycle, improving diet quality, and reducing the incidence of chronic diseases, such as obesity, metabolic syndrome, and cardiovascular disease, was presented. When assessing a complex food matrix, rather than specific nutrients, scientists and consumers are faced with new challenges as to how a food item's quality or necessity would be judged as part of an individual's whole diet. To tackle this challenge, speakers described 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 products in reducing health care expenses.

CHAPTER 3

MATERIALS & METHODS

3. MATERIALS & METHODS

3.1 Place of Experiment: This experiment was conducted at the Biotechnology laboratory of the Mathematics & Natural Sciences department of BRAC University, Mohakhali, Dhaka-Bangladesh.

3.2 Collection of Milk and Market Yogurt Sample:NIDO powder milk was obtained from Nestle, Switzerland consisted of 26% protein, 28% fat and 37.4% lactose and was used in all the experiments presented here.

3.3 Collection of Market Yogurt Sample:All the yogurt samples were fresh and purchased from the available local market from different places of Dhaka like Uttara, Mohakhali, Dhanmondi, New market, Badda, Gazipur and Sirajganj with a proper expiry date. From collection to analysis, cold chain (4 ºC) was maintained for all the samples. Care was taken as much as possible to avoid contamination or spoilage of the yogurt from any external sources (Nurul *et a.,* 2011).

3.4 Preservation of The Sample: After collection, yogurt samples were brought to the laboratory and carefully preserved in the refrigerator at 4ºC before and after microbial analysis.

3.5 Preparation of Milk and Yogurt: The milk was prepared from powder and in all cases; the corresponding amount of powder i.e. 40 gm of Nido Milk powder (4%), was taken and mixed with 0.5% (0.5 gm) NaCl was dissolved in and made up to 400 ml by adding purified water taking in a 500 ml beaker and pasteurized it. The solution was stirred at moderate speed for about 5 mins and then left to cool to 45 ºC. About 30 gm of each yogurt sample was added to the pasteurized milk sample. A negative control was taken for this set-up. Theyogurt was stored at 4 ºC for3 days.

- ***<u>Use of selected isolates (Streptococcus S5 isolate) for making yogurt (Inoculation fermentation)</u> :***

  Forty gram (4%) of powdered milk was taken and made up to 400 ml by adding tap water in a 500 ml beaker and pasteurized it. Initial milk pH was 6.5 measured by pH paper.30 gm fermented yogurt was mixed with 400 ml milk (4% powdered milk) in a 500ml beaker as here the temperature of the pasteurized milk was 45 ºC. This process has been performed twice to see the reproducibility in the respect of same parameter like pH and texture keeping every condition exactly the same (Here 3 beaker for each and every parameter were taken for cross check).

- ***<u>Effect of milk concentration of streptococcus S5 isolate</u>***

  Thirty gram of yogurt mixed with 2%, 4%, 6% and 8% powdered milk (20 gm, 40 gm, 60 gm and 80 gm) that is 200 ml, 400 ml, 600 ml and 800 ml of pasteurized milk respectively in a 1000 ml beaker while the temperature of the milk was checked before adding the yogurt sample and it was around 44-45 ºC. The incubation was carried out at 45 ºC. Initial milk pH was 6.5 measured by pH paper.

- ***<u>Inoculated fermentation of yogurt with mix culture of LAB Set One</u>***

  The following two sets of combination of LAB we used here for inoculated fermentation of yogurt under “set one” given as: (b+c) combination, where b = S3 c = S1 and (b+c+d) combination where b=S3 c=S1 d=S2. 300 ml of pasteurized milk (6% milk conc and mixed with 0.5 gm NaCl) was taken and aliquoted in a 500 ml beaker. The initial milk pH was 6.5 checking by pH paper. The Incubation was carried out at 45ºC.The first set of the combination here we used as: (b+c) combination, where b = S3 c = S1 And for (b+c) combination we had taken 0.5 ml of liquid culture broth of each sample and mixed it with pasteurized milk and added 1 ml of distilled water to each sample. The second set of the combination here used as: (b+c+d) combination, where b = S3 c = S1 d=S2. And for b+c+d combination we had taken 0.5 ml of liquid culture broth of each sample and mixed it with pasteurized milk and added 0.5 ml of distill water to each sample. For the negative control of this experiment, we added 2 ml of distilled water in 300 ml plain pasteurized milk (6% milk conc).

- ***Inoculated fermentation of yogurt with mix culture of LAB Set Two***

The combination here used as: (A+B+C) combination, where A = S3 B = S13 C=S1. Around 300 ml of milk (6%) was taken in a beaker with 0.5 gm NaCl and initial milk pH was 6.5. incubation temperature was set at 45 ºC.

- ***Effect of inoculums concentration (1%,2%,3%) at constant 6% milk concentration***

Stock culture or starter culture was added in the following way:

-In beaker one, 1% inoculum concentration was taken i.e. liquid broth culture was 9 ml mixed with 291 ml of pasteurized milk made the volume up to 300 ml.

-In beaker two, 2% inoculum concentration was taken i.e. liquid broth culture was18 ml mixed with 282 ml of pasteurized milk made the volume up to 300 ml.

-In beaker two, 3% inoculum concentration was taken i.e. liquid broth culture was 27 ml mixed with 273 ml of pasteurized milk made the volume up to 300 ml.

3.6 Quality Assessment by Sensory & Organoleptic Evaluation or Physical Test (Hedonic Scale): Various methods have been used to measure food preferences. The most common method is the hedonic scale which is used to rate the degree of liking. Hedonic scale is an organoleptic quality rating scale where the judge expresses his or her degree of liking. Although this test has been used by experts and untrained consumers, but it is felt to be more applicable to the latter. Sensory properties of yogurt samples were evaluated by the faculties of MNS department of BRAC University (ranging from 30-65 yrs) and its color and appearance, flavor, body and texture and overall acceptance were evaluated on 9- point Hedonic scale (Amerine *et al.*, 1965).The yogurt samples were served at 7 to 10 °C in plastic cups with coded numbers. Order of presentation of the samples was randomized. A test form comprising four sensory attributes, namely, appearance, flavor, texture and overall acceptability, was given to each assessor. A standard 9-point scale was used for evaluation of sensory characteristics of samples, in which 1 was equal to the worst and 9 was equal to the best (YANG *et al.*, 2010).

3.7 *Physico-Chemical Analysis or Chemical Analysis (pH & Acidity Determination of the Samples):*

3.7.1 *Determination of the pH:* The yogurt samples were analyzed for pH and acidity using standard Association of official Analytical Chemist procedure (AOAC, 2000) (Mamoona *et al.*, 2013). The pH of pasteurized milk and yogurt samples was measured by potentiometric method i.e. by potential difference between the sample and electrolyte solution present inside the electrode of pH meter (Sudeep *et al.*, 2012), using through electronic digital pH meter (E 201-C,Shanghai RuosuaaTechnology company,China).The electrode of the pH meter was directly dipped in the set yogurt samples and the pH was recorded every two hours interval immediately after taking out the samples from the incubator. The pH meter was calibrated routinely with fresh pH 4.0 and 7.0 standard buffers (Behrad *et al.*, 2009). But before that the initial milk pH was checked with a pH paper and found 6.5.

3.7.2 *Determination of the TTA:* Acidity of yogurt samples was assayed by simple titration methodalso known as Total Titrable acidity determination test (AOAC method.) As the natural yogurt have higher total titrable acidities, so the analysis of acidity & syneresis or technological evaluation of lactic acid bacteria is required. Total Titrable Acidity Determination test is determined by titration with sodium hydroxide to a pH end point of 8.2. Tritable acidity is measured as free and bound hydrogen ions by titration with NaOH, expressed in gm/L. Titrable acidity is used to express an acidity mostly tartaric acid(Wu *et al.*, 2001).TTA was determined by titrating yogurt sample and distilled water (1:9) mixture with 0.1NNaoH using a 0.1% Phenolphetalein as color indicator. The amount of acid produced during fermentation was calculated as follows:

TTA% = Dilution factor (10) x V NaoH x 0.1N x 0.009 x100%

Where, V is volume of NaoH required to neutralize the acid. Titrable acidity is expressed as % lactic acid, (CH3-CHOH-COOH, MW=90). Reproducibility is±0.01% lactic acid (Shah *et al.*, 2000).

3.8 Microbiological Test (Microbial analysis, observation of the samples, Isolation of culture & preservation of the isolates):

3.8.1 Determination of the Bacterial Load

3.8.1.1 Media Used: MRS agar, nutrient agar (NA) medium were used for enumeration and isolation of bacteria from the local yogurt sample.

3.8.1.2 Techniques Employed: Pour plate technique was applied for enumeration and isolation of bacteria. Enumeration of bacteria was carried out by aseptically mixing yogurt sample (1 ml) with 9 ml of sterilized 0.9 % physiological saline. The sample was thoroughly mixed and serial dilutions were performed using physiological saline as the diluents (Behrad *et al.,* 2009). The sample was diluted up to 10^{-5}. Then 50 µl, 100 µl, 100 µl, 150 µl & 200 µl amount of sample from 1 to 5 dilution tube were transferred respectively to the sterile petri-plates filled with melted, cooled MRS agar medium. Initially 15 ml of melted (45 °C) MRS agar medium was placed into a sterile petri dish followed by cooling of agar to temperature to allow solidification. The plates were rotated clockwise and anti-clockwise several times to spread the sample evenly on to the medium. 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 (Benson *et al.,* 2002).

3.8.1.3 Enumeration of Bacterial Load*:* After incubation, the plates having well-spaced colonies 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 sample:

cfu/ml = cfu/plate x dilution factor, where, cfu is the colony forming unit (Behrad *et al.*, 2009).

3.8.1.4 Purification of the Isolates: The selected isolates were then streaked on both MRS agar and nutrient agar (NA) medium and incubated at 37 ºC for 24 hours.

After incubation isolation of well separated discrete bacterial colonies was done. Characteristics of the colonies were recorded 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.

3.8.2 Microscopic Observation or Characterization of the Isolates

3.8.2.1 Morphological and Cultural Studies of Selected Isolates: Morphological characters of selected isolates can be observed by cultural and microscopic methods. 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:

3.8.2.1.i 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, umbonate etc), opacity, surface (smooth, rough etc) and color of the colony (mostly white, off white, yellowish).

3.8.2.1.ii Agar Slant: The modes of bacterial growth on MRS agar slants such as spreading, echinutlate, filiform, arborescent and adherent or slimy etc. were studied.

***3.8.2.1.iii Broth Culture*:** MRS broth media were inoculated with selected isolates. The characteristics of growth into broth was observed visually and noted. Production of turbidity, sedimentation and surface growth (flocculant, ring, pellicle and membranous) in MRS broth were observed and recorded.

3.8.2.1.iv 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. They are:

  -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 (Hucker and Conn's 1923): Here Hucker and Conn's (1923) modified method was also followed. 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.

3.8.3Maintenance& 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.

3.8.4 Coding of the Isolates:

The selected isolates were coded according to the sample source and the serial of the sample used. The code numbers were maintained and followed till the end of this study. The isolates from the yogurt samples were coded as S1 to S19.

CHAPTER 4
RESULTS

4. RESULT

4.1 Spontaneous fermentation of S1, S2, S3, S4, S5, S6, S7, S8, S9, S10, S11, S12, S13, S14, S15, S16, S17, S18 and S19 in terms of pH and texture:

Results presented in Table 4.1, 4.2, 4.3 and 4.4 demonstrate that among these samples, the best one was sample S5 showed the quality of fermented yogurt with good texture, thick solid portion, less water content after 4 hours of fermentation with low acidic pH 3.85 incubated set at 45 ºC and still maintained that quality texture even after 8 hours of fermentation with the pH of 3.62. While sample S8 showed moderate quality with more water content after 4 hours of fermentation but failed to maintain it after 8 hours.

Table 4.1: Spontaneous fermentation of S1, S2, S3, S4 and S5 in terms of pH and texture

Sample	Time	pH	Texture/Appearance
S1	2hr	5.17	Still liquid
	4hr	4.88	Semi solid
	6hr	4.0	Same as previous
	8hr	3.77	Same as previous
S2	2hr	4.90	Heavy content, less water
	4hr	4.20	Clotted portion, didn't coagulate properly, more water content
	6hr	3.85	Very bad texture, solid portions were broken and more water content increases
	8hr	3.31	Very bad texture, more water content increases
S3	2hr	4.48	Heavy content, less water
	4hr	3.88	More solid portions, more water content
	6hr	3.70	Semi solid texture
	8hr	3.46	More water content increases
S4	2hr	5.62	Liquid milk
	4hr	4.02	More water portions and less solid contents
	6hr	3.88	Solid content were broken now, more water content
	8hr	3.72	More water content increases, solid portions are now breaking, very bad texture
S5	2hr	4.41	Heavy content, less water
	4hr	3.85	Good texture, thick portion, less water content
	6hr	3.74	Very good solid and thick texture, less water content
	8hr	3.62	Very good texture, not breaking apart yet, moderate water Content

Table 4.2: Spontaneous fermentation of S6, S7, S8, S9 and S10 in terms of pH and texture

Sample	Time	pH	Texture/Appearance
S6	2hr	5.16	Semi solid, nice smell
	4hr	4.88	Semi solid texture
	6hr	3.94	Same as previous with a nice smell
	8hr	3.77	Same as previous
S7	2hr	5.60	Liquid milk
	4hr	4.44	Thick texture, more water content
	6hr	3.88	Solid content is now breaking, same water portions.
	8hr	3.52	Very bad texture, more water content
S8	2hr	5.26	Less heavy content, less water
	4hr	3.89	Good texture but more water content
	6hr	3.56	Water content increases, medium texture
	8hr	3.30	More water content, medium texture
S9	2hr	5.70	Cloudy liquid
	4hr	5.20	Semisolid texture, more water content
	6hr	4.77	More water content increases, bad texture
	8hr	4.54	Same as previous
S10	2hr	5.88	Cloudy liquid
	4hr	5.64	Semisolid texture, more water content
	6hr	4.81	More water content increases, bad texture
	8hr	4.67	Same as previous

Table 4.3: Spontaneous fermentation of S11, S12, S13, S14 and S15 in terms of pH and texture

Sample	Time	pH	Texture/Appearance
S11	2hr	5.88	Liquid texture
	4hr	5.64	Semisolid texture, more water content
	6hr	4.81	More water content increases, bad texture
	8hr	4.67	Same as previous
S12	2hr	5.34	Still liquid
	4hr	4.44	Semi solid
	6hr	3.88	Same as previous with nice smell
	8hr	3.64	Same as previous
S13	2hr	4.46	Heavy content, less water
	4hr	3.77	More solid portions, more water content
	6hr	3.58	Solid content were broken now, more water content than previous
	8hr	3.2	More water content increases
S14	2hr	5.60	Liquid milk
	4hr	4.00	Less water portions and solid contents
	6hr	3.77	Solid content were broken now, more water content
	8hr	3.67	More water content increases, solid portions are now breaking
S15	2hr	4.95	Liquid milk
	4hr	4.21	Semi solid texture
	6hr	3.83	More water increases, nice smell
	8hr	4.06	Same as previous

Table 4.4: Spontaneous fermentation of S16, S17, S18 and S19 in terms of pH and texture

Sample	Time	pH	Texture/Appearance
S16	2hr	5.26	Still liquid
	4hr	4.40	Semi solid
	6hr	3.98	Same as previous with nice smell
	8hr	4.10	Same as previous and more water content
S17	2hr	5.13	Semi solid, nice smell
	4hr	4.00	Semi solid texture
	6hr	3.55	Same as previous with a nice smell
	8hr	3.62	Same as previous
S18	2hr	5.61	Cloudy liquid
	4hr	5.20	Same as previous
	6hr	4.73	Semi solid texture
	8hr	4.47	Same as previous
S19	2hr	4.40	Semi solid, nice smell
	4hr	3.88	Same as previous
	6hr	3.74	Same as previous but with a nice smell
	8hr	3.68	More water content increases.

S1 2hrs S1 4hrs S1 6hrs S1 8hrs

Figure 4.1: Experiment of spontaneous fermentation of S1 in terms of pH and texture

S2 2hrs S2 4hrs S2 6hrs S2 8hrs

Figure 4.2: Experiment of spontaneous fermentation of S2 in terms of pH and texture

S3 2hrs S3 4hrs S3 6hrs S3 8hrs

Figure 4.3: Experiment of spontaneous fermentation of S3 in terms of pH and texture

S4 2hrs S4 4hrs S4 6hrs S4 8hrs

Figure 4.4: Experiment of spontaneous fermentation of S4 in terms of pH and texture

S5 2hrs S5 4hrs S5 6hrs S5 8hrs

Figure 4.5: Experiment of spontaneous fermentation of S5 in terms of pH and texture

S6 2hrs S6 4hrs S6 6hrs S6 8hrs

Figure 4.6: Experiment of spontaneous fermentation of S6 in terms of pH and texture

S7 2hrs S7 4hrs S7 6hrs S7 8hrs

Figure 4.7: Experiment of spontaneous fermentation of S7 in terms of pH and texture

S8 2hrs S8 4hrs S8 6hrs S8 8hrs

Figure 4.8: Experiment of spontaneous fermentation of S8 in terms of pH and texture

S9 2hrs S9 4hrs S9 6hrs S9 8hrs

Figure 4.9: Experiment of spontaneous fermentation of S9 in terms of pH and texture

S10 2hrs S10 4hrs S10 6hrs S10 8hrs

Figure 4.10: Experiment of spontaneous fermentation of S10 in terms of pH and texture

S11 2hrs S11 4hrs S11 6hrs S11 8hrs

Figure 4.11: Experiment of spontaneous fermentation of S11 in terms of pH and texture

S12 2hrs S12 4hrs S12 6hrs S12 8hrs

Figure 4.12: Experiment of spontaneous fermentation of S12 in terms of pH and texture

S13 2hrs S13 4hrs S13 6hrs S13 8hrs

Figure 4.13: Experiment of spontaneous fermentation of S13 in terms of pH and texture

S14 2hrs S14 4hrs S14 6hrs S14 8hrs

Figure 4.14: Experiment of spontaneous fermentation of S14 in terms of pH and texture

S15 2hrs S15 4hrs S15 6hrs S15 6hrs

Figure 4.15: Experiment of spontaneous fermentation of S15 in terms of pH and texture

S16 2hrs S16 4hrs S16 6hrs S16 8hrs

Figure 4.16: Experiment of spontaneous fermentation of S16 in terms of pH and texture

S17 2hrs S17 4hrs S17 6hrs S17 8hrs

Figure 4.17: Experiment of spontaneous fermentation of S17 in terms of pH and texture

S18 2hrs S18 4hrs S18 6hrs S18 8hrs

Figure 4.18: Experiment of spontaneous fermentation of S18 in terms of pH and texture

S19 2hrs S19 4hrs S19 6hrs S19 8hrs

Figure 4.19: Experiment of spontaneous fermentation of S19 in terms of pH and texture

4.2 Organoleptic Quality Assessment:

4.2.1 Texture Acceptability

The texture mean score of yogurt S1,S2, S3, S4, S5, S6, S7, S8, S9, S10, S11, S12, S13, S14, S15, S16, S17, S18 and S19 were 5.4 ,4.7, 7.1, 3.9 ,8.4,7.1, 5.2, 5.9, 4.7, 3.7, 4.6, 7.1, 4.4, 5.3, 5.1, 6.5, 6.1, 6.6 and 6.4 respectively. In the texture acceptability test, Hedonic scale showed that the yogurt sample S5 was excellent, S3, S6 and S12 considered very good S16, S17, S18 and S19 considered acceptable texture quality.

4.2.2 Color Acceptability

It appears that S5 yogurt obtained the highest score for its color (mean is 8.3). The mean for others were 7, 6.8, 7.1, 5.4, 8.3, 6.7, 6.9, 6.7, 7.5, 4.4, 7, 7.6, 5.2, 6.7, 6.9, 7.1, 7.1, 7 and 7.4 for S1, S2, S3, S4, S5, S6, S7, S8, S9, S10, S11, S12, S13, S14, S15, S16, S17, S18 and for S19 and so on. In the color acceptability test, Hedonic scale showed that the yogurt sample S5 was excellent, S1, S3, S9, S11, S12, S16, S17, S18 and S19 considered as very good, Sample S2, S6,S7, S8, S14 and S15 considered as acceptable and S10 had poor color quality.

4.2.3 Flavor Acceptability

The flavors mean score were 6.4, 6.4, 6.2, 5.1, 8.4, 7.1, 7, 5.8, 6.5, 5.8, 6.9, 7.8, 6.2, 6, 7.4, 7.1, 7.5, 6.4 and 7.4 respectively. In the flavor acceptability test, Hedonic scale showed that the yogurt sample S5 was excellent (mean score is 8.4), S6, S7, S12, S15, S16, S17 and S19 considered as very good, S1, S2, S3, S9, S11, S13, S14 and S18 considered as acceptable and S4 had poor taste quality.

4.2.4 Taste Acceptability

Taste is influenced by the quality of the raw milk and added materials to it. The taste score of S1, S2, S3, S4, S5, S6, S7, S8, S9, S10, S11, S12, S13, S14, S15, S16, S17, S18 and S19 were 6.4, 5.3, 5.5, 4.9, 8,6.9, 6.2, 5.5, 4.9, 3.9, 5.9, 5.5, 4.7, 5.3, 5.5, 5.7, 5.7, 5.5 and 5.7 respectively. In the taste acceptability test, Hedonic scale showed that the yogurt sample S5 was excellent (mean score 8), S1, S6 and S7 considered was very good, S2, S3, S8, S11, S12, S14, S15, S16, S17, S18 and S19 considered as acceptable and S10 had poor taste quality.

Development of Improved Quality Yogurt in terms of Texture, Flavor, Food Value and Low Cost

Scale Point Description	Assigned Value	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	S11	S12	S13	S14	S15	S16	S17	S18	S19
		Frequency of Responses																		
Like Extremely	9	0	0	2	0	15	1	0	0	1	0	0	4	0	0	0	1	1	1	1
Like Very Much	8	10	3	8	0	21	8	6	2	4	1	5	10	0	3	7	8	10	7	10
Like Moderately	7	16	12	13	3	4	20	13	12	7	0	12	14	5	11	12	14	12	11	13
Like Slightly	6	6	11	6	12	0	10	13	14	13	7	12	7	10	13	10	11	9	13	11
Neither Like nor Dislike	5	4	6	7	9	0	1	5	7	5	12	5	4	13	5	5	3	5	3	3
Dislike Slightly	4	0	6	3	8	0	0	4	5	5	9	5	1	9	5	6	3	3	5	2
Dislike Moderately	3	0	0	1	7	0	0	3	0	3	10	1	0	3	3	0	0	0	0	0
Dislike Very Much	2	4	2	0	1	0	0	2	0	0	1	0	0	0	0	0	0	0	0	0
Dislike Extremely	1	1	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
Total Responses-------------------------		40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40
Mean Rating																				
• Texture------------------------		5.4	4.7	7.1	3.9	8.4	7.1	5.2	5.9	4.7	3.7	4.6	7.1	4.4	5.3	5.1	6.5	6.1	6.6	6.4
• Color----------------------------		7	6.8	7.1	5.4	5.3	6.7	6.9	6.7	7.5	4.4	7	7.6	5.2	6.7	6.9	7.1	7.1	7	7.4
• Aroma--------------------------		6.4	6.4	6.2	5.1	8.4	7.1	7	5.8	6.5	5.8	6.9	7.8	6.2	6	7.4	7.1	7.5	6.4	7.4
• Taste----------------------------		6.4	5.3	5.5	4.9	8	6.9	6.2	5.5	4.9	3.9	5.9	5.5	4.7	5.3	5.5	5.7	5.7	5.5	5.7
Standard Deviation																				
• Texture------------------------		1.71	1.77	1.29	1.10	0.70	0.88	1.23	0.74	1.16	0.67	0.97	0.99	0.97	1.05	0.88	0.85	0.99	0.97	0.34
• Color----------------------------		0.82	0.79	0.99	0.70	0.67	0.82	0.88	1.16	0.85	1.17	0.82	0.70	1.03	1.16	1.20	1.37	1.37	1.41	0.84
• Aroma--------------------------		1.90	0.97	1.55	1.37	0.51	0.74	0.94	1.23	0.53	0.92	0.74	0.79	0.79	1.41	0.52	0.73	0.53	1.43	0.70
• Taste----------------------------		2.37	1.25	1.43	1.60	0.67	0.88	0.79	0.97	1.10	1.10	0.99	0.85	0.95	1.49	0.97	1.25	1.25	0.97	1.25
Percentage Dislike Responses------		12.5	22.5	10	40	0	0	25	12.5	20	50	15	2.5	27.5	20	15	7.5	7.5	12.5	5

Source: *http://www.pk-research.com/services/media/paperandpublications/theninepointhedonicscale-papers.pdf*

Table 4.5: Distribution of Responses on Hedonic Scale, With Resulting Statistical Indices for Various Yogurt Sample

4.3 Chemical Quality Assessment

- ***Changes in pH of yogurts with S5 isolates (Inoculated fermentation with S5 isolates)***

  Results presented in Figure 4.20 demonstrate the inoculated fermentation of S5 isolates that showed fermentation process after 2 hours of incubation set at 45 ºC with a solid texture along with water content, still maintained that texture even after 6 hours of fermentation process and after 8 hrs of fermentation more broken pieces increased while the pH was 3.44. After repeating the experiment with Streptococcus S5 isolates (three repetitions) maintaining the exact conditions, it showed quite similar result i.e. pH 3.91 with a semisolid texture of fermented yogurt compared to that of pH 3.84

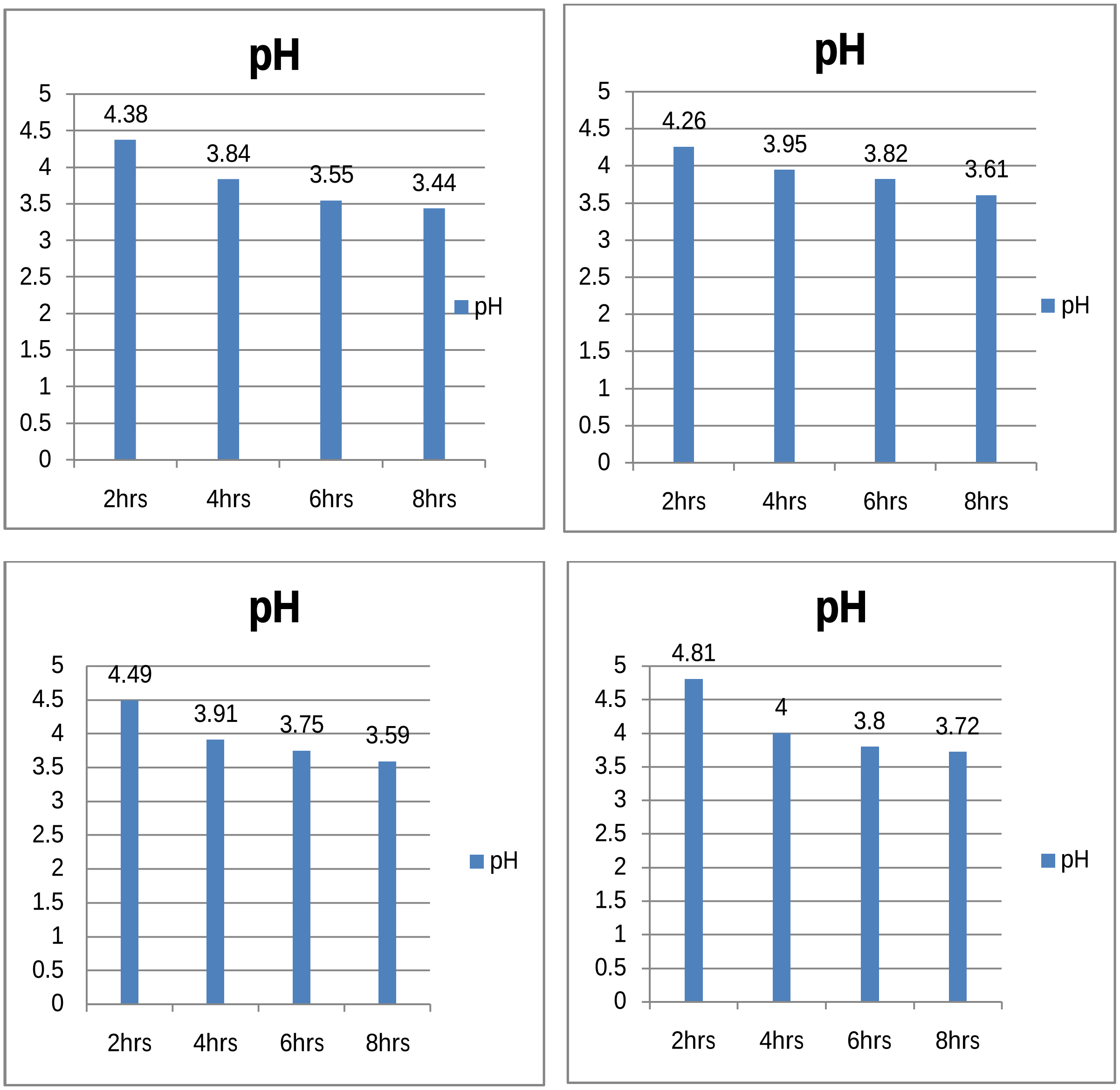

Figure 4.20: Changes in pH of yogurts with S5 isolates with incubation time

- ***Changes in pH of yogurts with S5 isolate during storage:***

  Figure 4.21 showed an overall decline of pH of yogurts (including the three repetitions) occurred during refrigerated storage at 4 ºC. The pH for all yogurts reduced from the initial values of 3.35 to between 3.18 and 3.17 by day 3 of storage at 4 ºC. During the storage for three days, (after 72 hours) the texture of fermented yogurt changed a bit but maintained a good aroma**.**

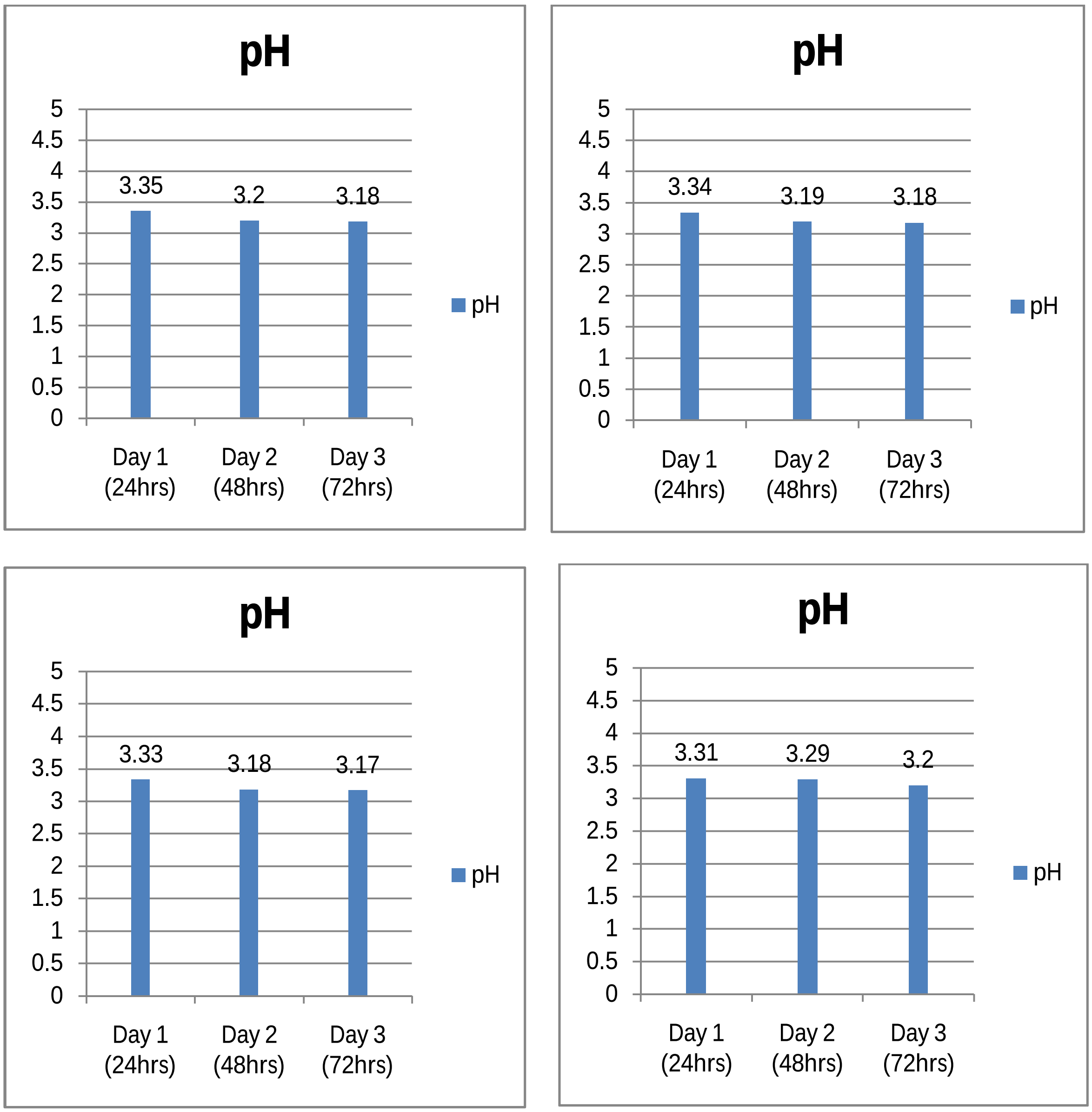

Figure 4.21: Changes in pH of yogurts with S5 isolates during storage

- ***Effect of milk concentration of Streptococcus S5 isolates***

  Results presented in Figure 4.22, 4.23, 4.24 and 4.25 demonstrate that the effect of milk concentration on Streptococcus S5 isolates which showed good fermented yogurt at 6% milk concentration i.e. 60 gm milk powder with a low acidic pH 3.91 and still maintained this texture even after 8 hours of fermentation process. While S5 isolate also showed comparatively good texture at 8% milk concentration i.e. 80 gm milk powder and pH value was 4.07, although maintained the consistency till the end of fermentation process and even during the storage after 72 hours.

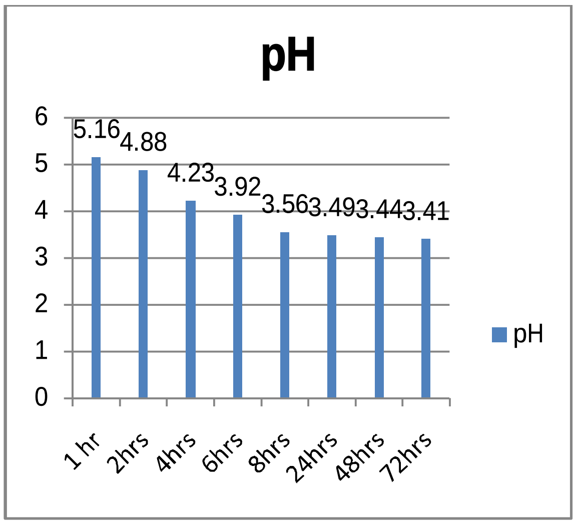

Figure 4.22: Effect of milk concentration (2%) of S5 isolate

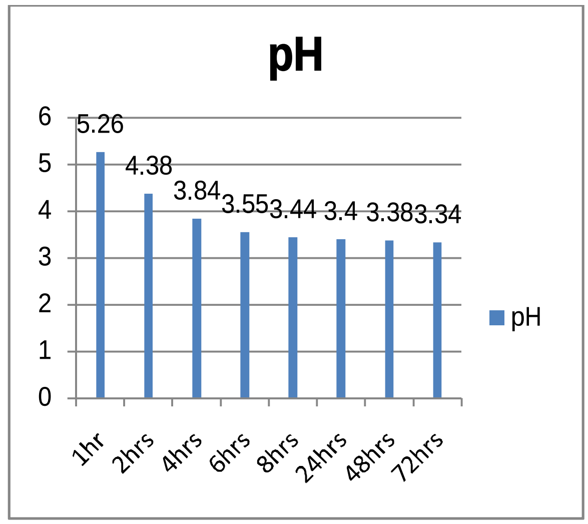

Figure 4.23: Effect of milk concentration (4%) of S5 isolates

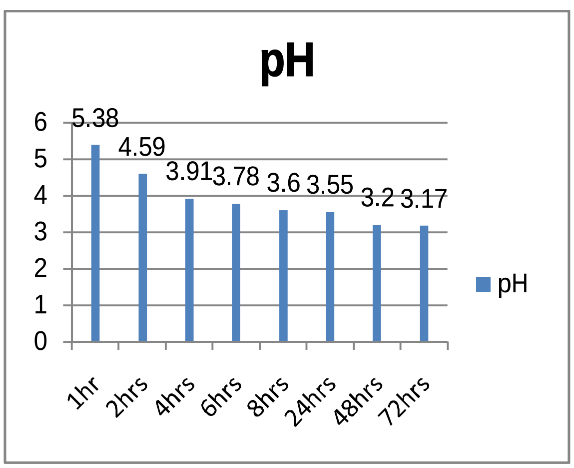

Figure 4.24: Effect of milk concentration (6%) of S5 isolates

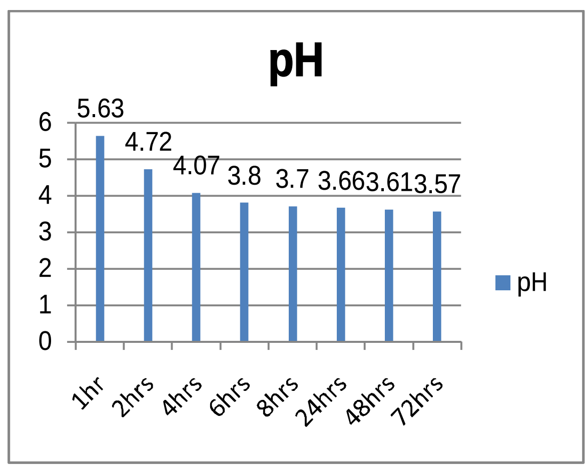

Figure 4.25: Effect of milk concentration (8%) of S5 isolates

Figure 4.26: Inoculated fermentation of yogurt of S5 isolates after 24hrs with 2% milk conc.

Figure 4.27: Inoculated fermentation of yogurt of S5 isolates after 24hrs with 4% milk conc.

Figure 4.28: Inoculated fermentation of yogurt of S5 isolates after 24hrs with 6% milk conc.

Figure 4.29: Inoculated fermentation of yogurt of S5 isolates after 24hrs with 8% milk conc.

- ***Inoculated fermentation of yogurt with mix culture of LAB (First set combination)***
  Results presented in Figure 4.30demonstrate that the inoculated fermentation of yogurt with mix culture of lactobacillus as a combination of b & c which were S3 as *L. plantarum* & S1 as yeast was used in yogurt making process but came up with an extreme bad texture after 4 hrs, 6 hrs, 8 hrs of fermentation but after 24 hours showed a semi solid texture with pH of 3.20.

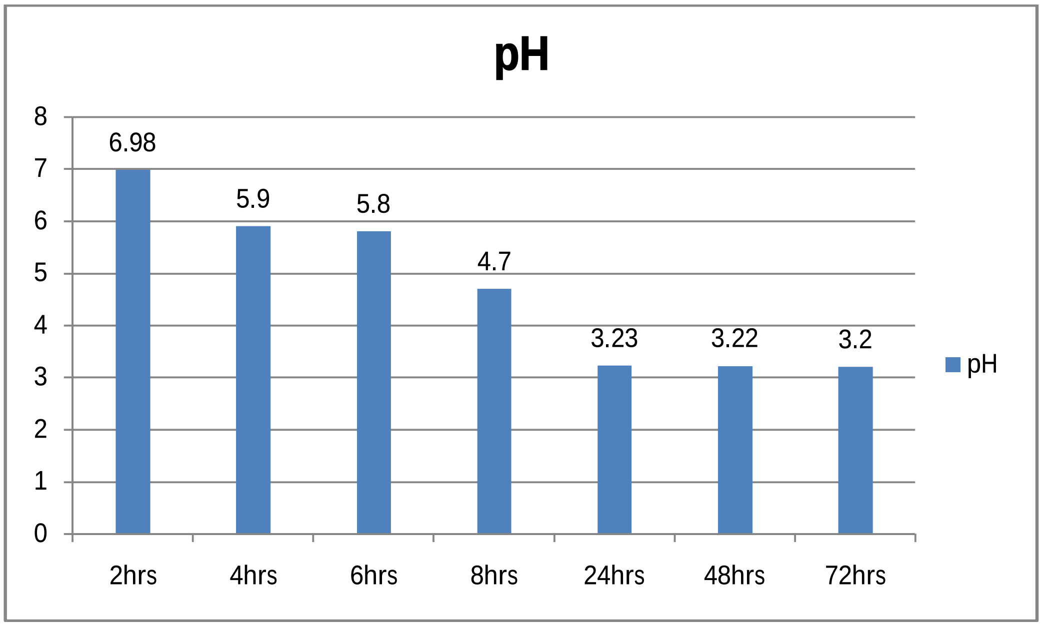

Figure 4.30: Changes in pH in Inoculated fermentation of yogurt with mix culture of LAB (First set combination)

Figure 4.31: Inoculated fermentation of yogurt with mix culture of LAB after 24hrs (First set combination)

- ***Inoculated fermentation of yogurt with mix culture of LAB (Second set combination)***
  The results presented inFigure 4.32 demonstrate that the inoculated fermentation of yogurt with second set of mix culture of lactobacillus as a combination of b, c & d which were S3 as *lactobacillus plantarum*, S1 as yeast & S2 was used in yogurt making process but came up with a texture that is semi solid, more acidic and better than First set combination (b+c) after 24 hours fermentation process with a pH of 3.16.

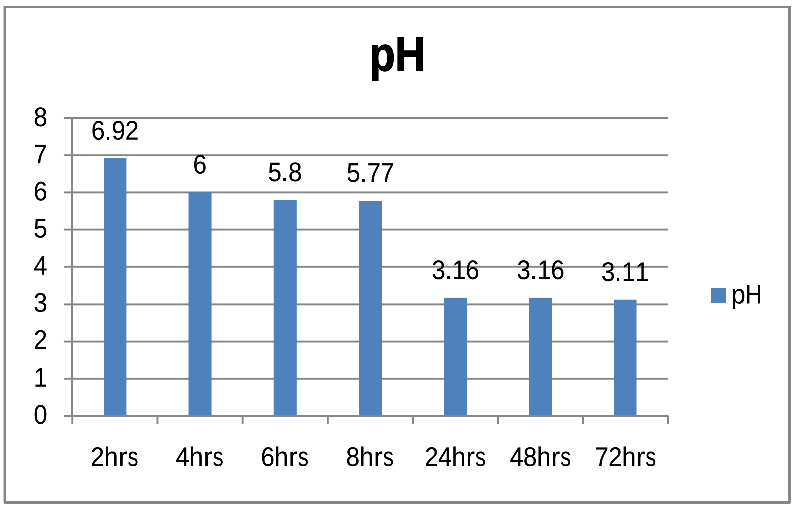

Figure 4.32: Changes in pH in Inoculated fermentation of yogurt with mix culture of LAB (Second set combination)

Figure 4.33: Inoculated fermentation of yogurt with mix culture of LAB after 24hrs (Second set combination)

- ***Inoculated fermentation of yogurt with mix culture of LAB or Second set combination (in terms of the effect of 1%, 2% & 3% inoculums concentration at constant 6% milk concentration)***

  The results in Figure 4.34, 4.35 and 4.36 demonstrate that the inoculated fermentation of yogurt with mix culture of LAB set two where at constant milk concentration 6%, beaker 3 containing 3% inoculums concentration showed the best result of fermented yogurt which showed good texture of yogurt with a nice smell and moderate pH value of 4.72 after 4 hours of fermentation compared to others and maintained this texture with less water content even after 24 hours of incubation period.

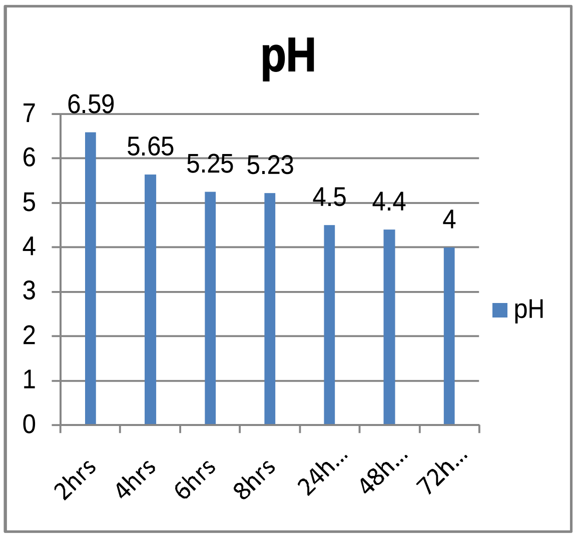

Figure 4.34: Changes in pH of yogurt (1% inoculums)

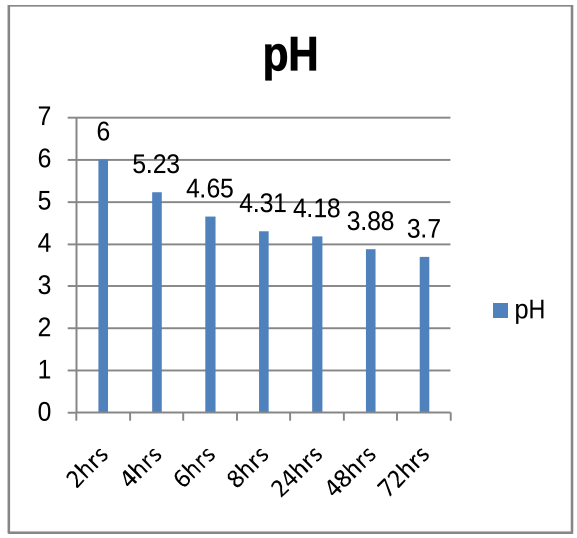

Figure 4.35: Changes in pH of yogurt (2% inoculums)

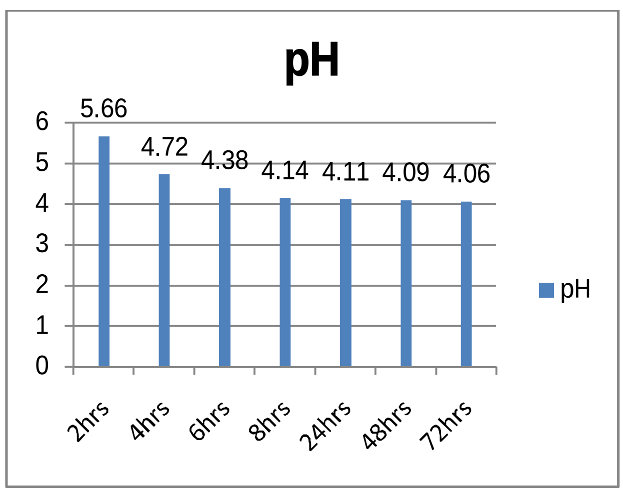

Figure 4.36: Changes in pH of yogurt (3% inoculums)

Figure 4.37: Inoculated fermentation of yogurt with Second set combination after 24hrs (1% inoculums concentration)

Figure 4.38: Inoculated fermentation of yogurt with Second set combination after 24hrs (2% inoculums concentration)

Figure 4.39: Inoculated fermentation of yogurt with Second set combination after 24hrs (3% inoculums concentration)

4.4 TTA Determination of the Samples

- ***Effect of milk concentration of Streptococcus S5 isolates***

  Results presented in Figure 4.40, 4.41, 4.42 and 4.43 demonstrate the effect of milk concentration on Streptococcus S5 isolates which showed good fermented yogurt in 6% milk concentration i.e. 60 gm milk powder with a high TTA value of 2.25% and still maintained this texture even after 8 hours of fermentation process with the decreased TTA value of 1.45% . While S5 isolate also showed comparatively similar results but better texture in 8% milk concentration i.e. 80 gm milk powder but with a high TTA value of2.25% and after 8 hours of fermentation it showed decreased TTA value of 1.45%. Although maintained the consistency till the end of fermentation process and even during the storage after 72 hours.

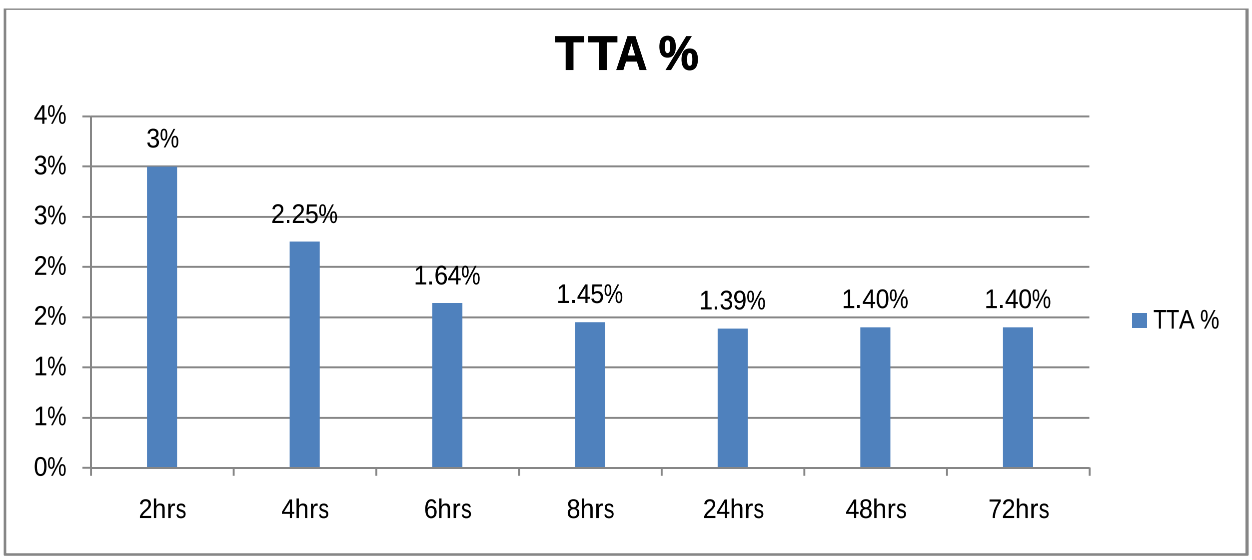

Figure 4.40: Changes in TTA of yogurt with 2% milk concentration

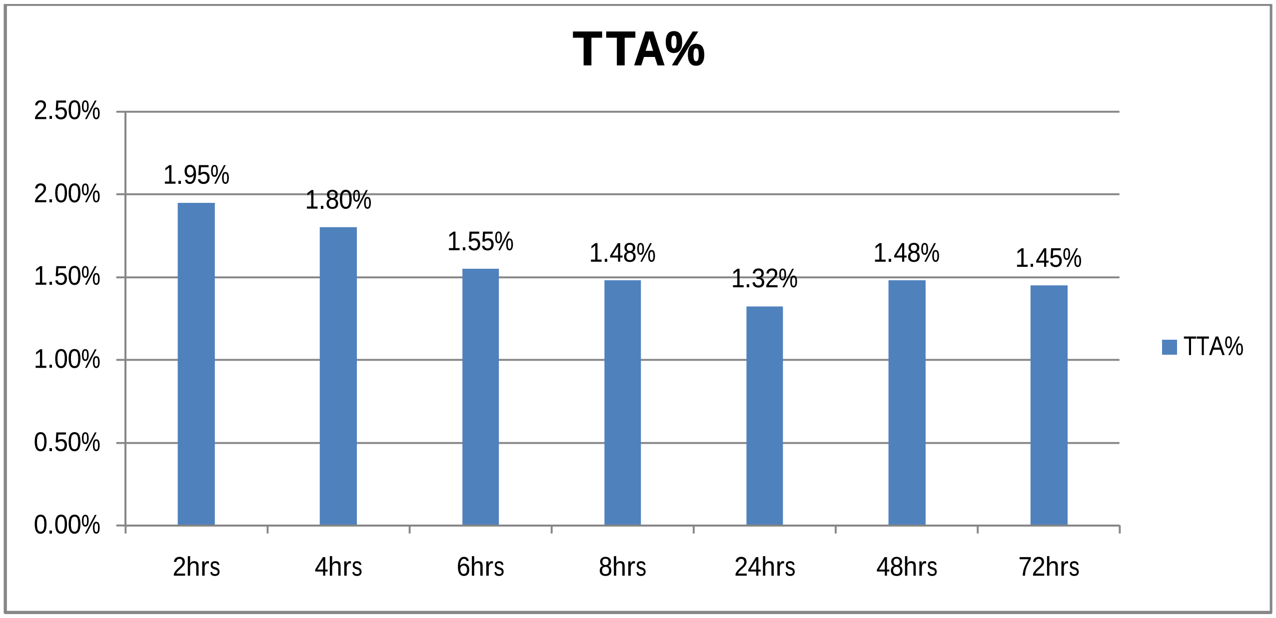

Figure 4.41: Changes in TTA of yogurt with 4% milk concentration

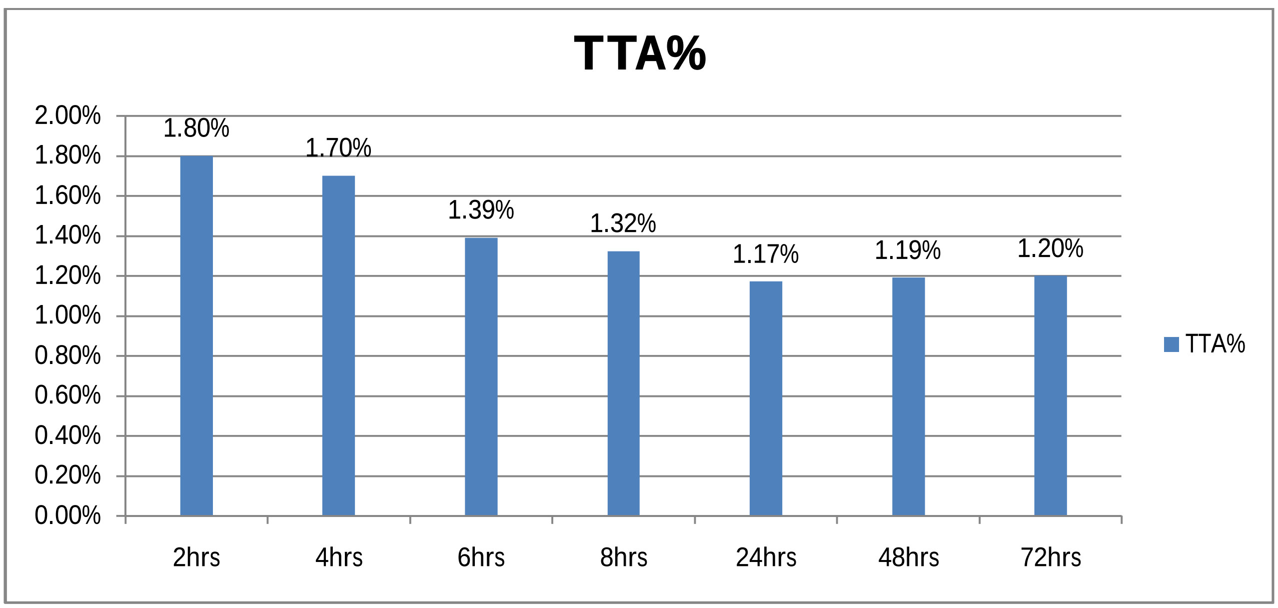

Figure 4.42: Changes in TTA of yogurt with 6% milk concentration

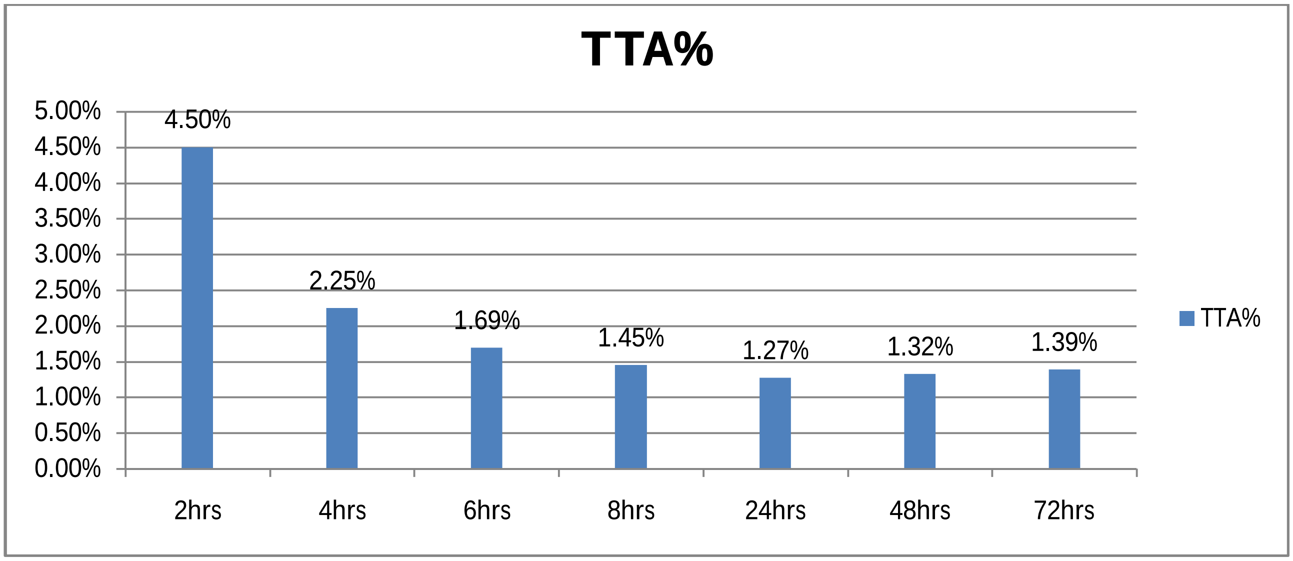

Figure 4.43: Changes in TTA of yogurt with 8% milk concentration

- ***Inoculated fermentation of yogurt with mix culture of LAB (First set combination)***

Results presented in Figure 4.44 demonstrate the inoculated fermentation of yogurt with mix culture of lactobacillus as a combination of b & c which were S3 as *lactobacillus plantarum* & S1 as yeast was used in yogurt making process but came up with an extreme bad texture after 4hrs, 6hrs, 8hrs of fermentation but even after 24 hours showed a semi solid texture with the TTA% value of 1.80%.

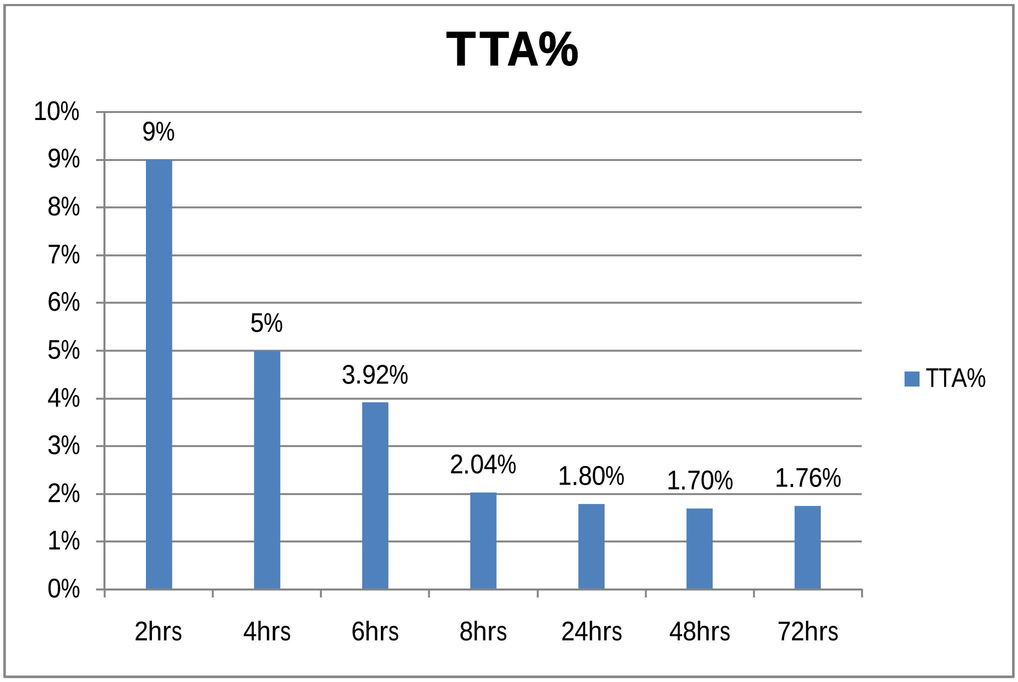

Figure 4.44: Changes in TTA in Inoculated fermentation of yogurt with mix culture of LAB (First set combination)

- ***Inoculated fermentation of yogurt with mix culture of LAB (Second set combination)***

The results presented in Figure 4.45 demonstrate the inoculated fermentation of yogurt with another set of mix culture of lactobacillus as a combination of b, c & d which were S3 as *lactobacillus plantarum,* S1 as yeast & S2 used in yogurt making process but came up with a texture that is semi solid, more acidic and better than (b+c) combination after 24 hours fermentation process with a TTA% value of 1.55%.

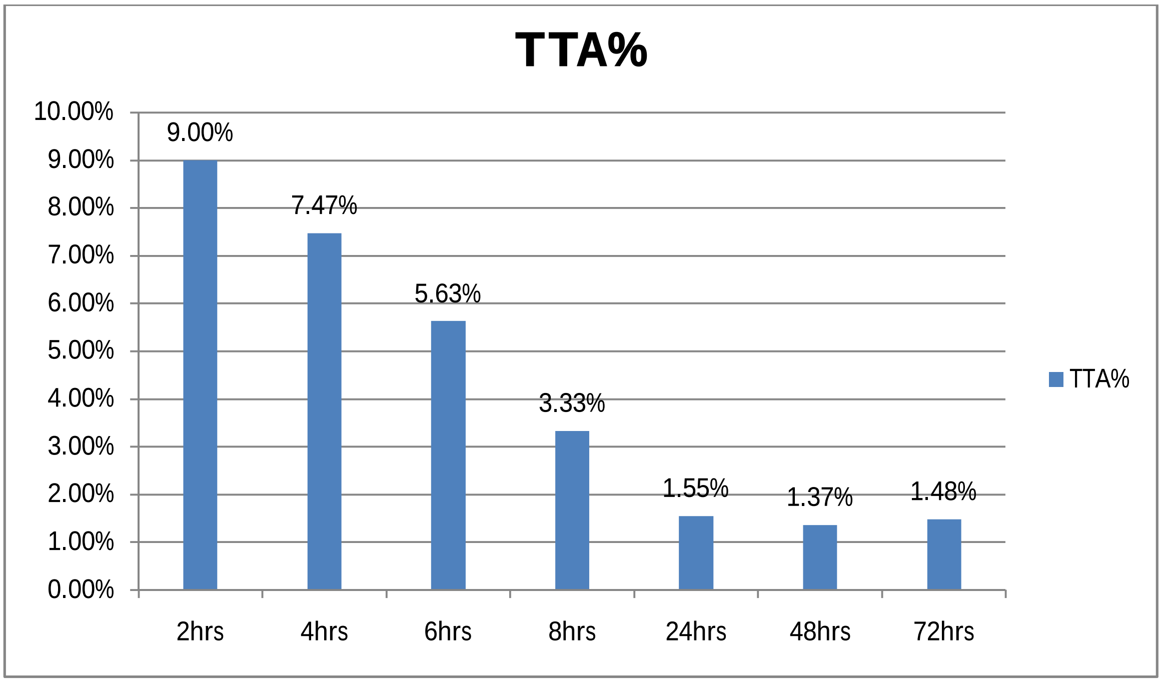

Figure 4.45: Changes in TTA in Inoculated fermentation of yogurt with mix culture of LAB (Second set combination)

- ***Inoculated fermentation of yogurt with mix culture of LAB or Second set combination in terms of the effect of 1%, 2% and 3% inoculums concentration at 6% constant milk concentration***

The results in Figure 4.46, 4.47 and 4.48 demonstrate the inoculated fermentation of yogurt with mix culture of LAB or in Second set combination where at constant milk concentration 6%, beaker 3 containing 3% inoculums concentration showed the best result of fermented yogurt which showed good texture of yogurt with a nice smell and TTA% value of 2.14% compared to others and maintained this texture with less water content even after 24 hours of incubation period with the decreased TTA% value of 1.50%.

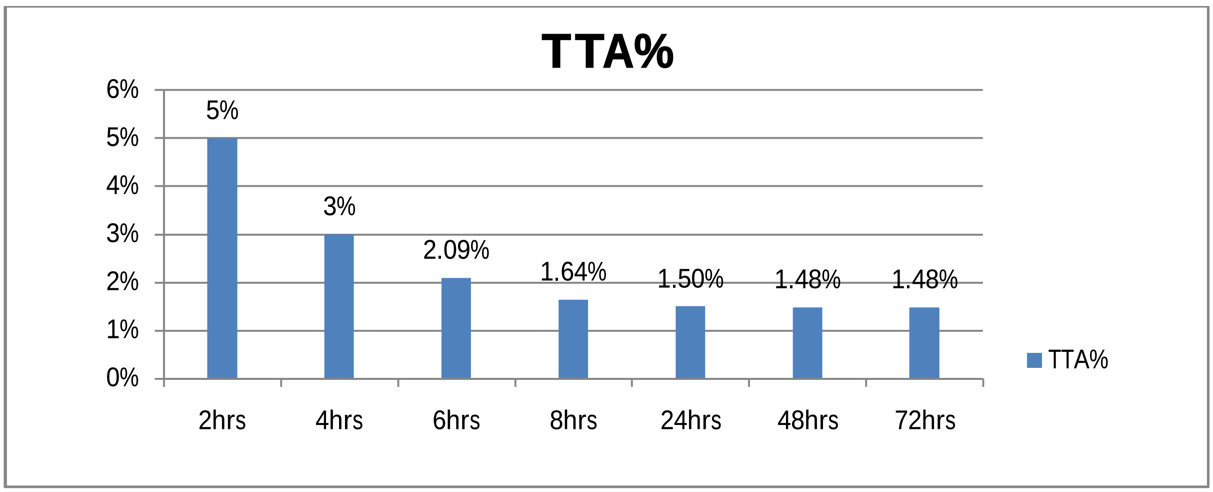

Figure 4.46: Changes in TTA% of yogurt (1% inoculums)

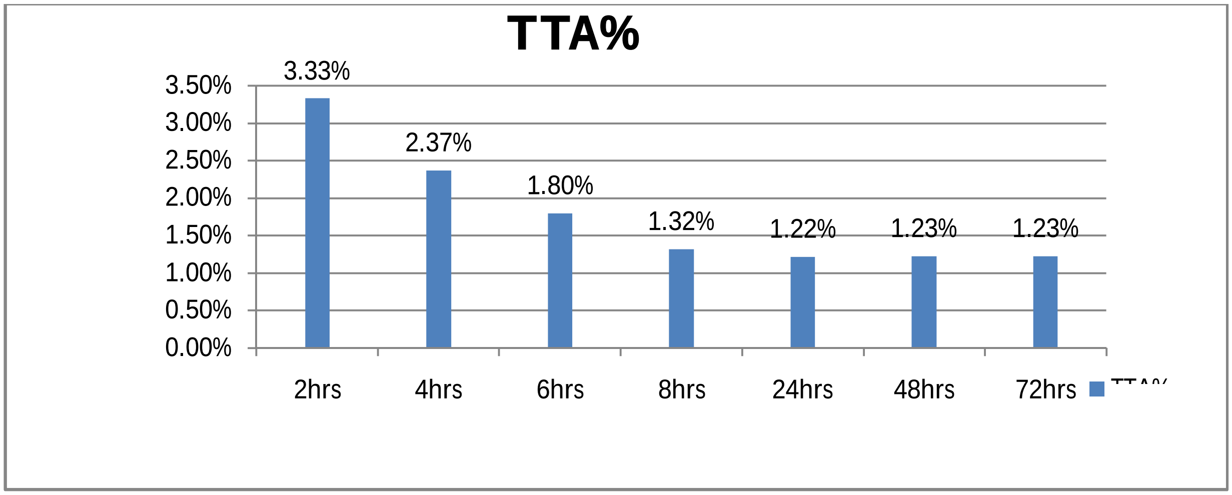

Figure 4.47: Changes in TTA% of yogurt (2% inoculums)

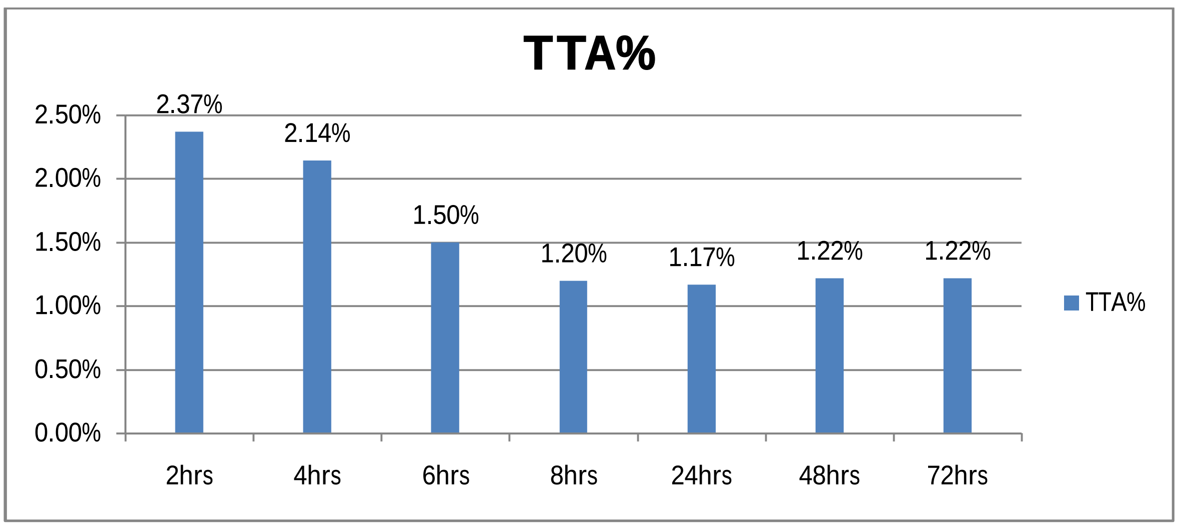

Figure 4.48: Changes in TTA% of yogurt (3% inoculums)

Figure 4.49: Total tritable acidity (TTA) test

- ***Comparisons of TTA% between yogurt samples***

The results showed in the Figure 4.50 demonstrate that among the yogurt samples, the best result showed in S5 isolate (8% milk concentration) with a thick texture of yogurt, nice smell along with a TTA% value of 2.25%. Although this TTA% value which found in S5 isolates (8% milk concentration) is quite close to that found in the yogurt with 3% inoculums (TTA% value is 2.14%) but in terms of overall yogurt texture S5 isolates with 8% milk concentration showed the best result compared to other yogurt samples i.e. mix culture of LAB or First set combination mix culture of LAB or Second set combination, yogurt with 1% inoculums concentration, yogurt with 2% inoculums concentration along with TTA% values are 5%, 7.47%, 3% and2.37% respectively.

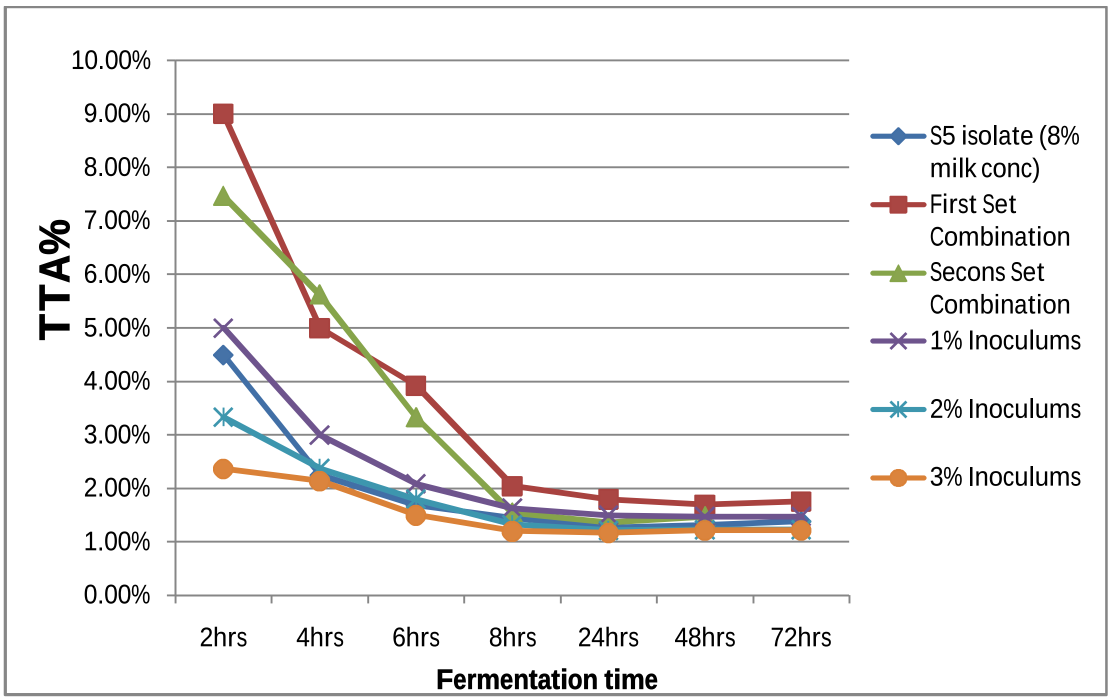

Figure 4.50: Comparisons of TTA% between yogurt samples

- ***The relationship between pH and titrable acidity of experimental yogurt***

  The results presented in the Table 4.6 demonstrate the relationship between pH and TTA in all the experimental yogurt samples. Although it's been found that pH is not related to TTA but it can somehow influences TTA in a sample (http://www.crcv.com.au). In terms yogurt texture, the sample obtained the best texture with good aroma is S5 isolates with 8% milk concentration and showed a low pH value of 3.20 with a high TTA% value of 0.71%. Also showed quite good quality of yogurt texture were mix culture of LAB with 3% inoculums concentration and Second set combination with a pH value of 4.11, 3.16 and TTA% value of 0.77%, 0.58% respectively.

Table 4.6: The relationship between pH and titrable acidity of experimental yogurt (after 24hrs of fermentation)

Yogurt Sample	pH	TTA (percent lactic acid)	Comments
S5 isolates (8% milk conc)	3.40	0.71 N	Better texture
First set combination	3.23	0.50 N	Bad texture
Second set combination	3.16	0.58 N	Good texture
1% inoculums	4.50	0.60 N	Bad texture
2% inoculums	4.18	0.74 N	Bad texture
3% inoculums	**4.11**	**0.77 N**	**Good texture**

4.5 Microbiological Quality Assessment

4.5.1 Standard Plate Count (SPC): The SPC of S3, S4, S5, S6, S8, S9, S11 and S13 were$1.6x10^7$, $1.89x10^7$, $1.96x10^7$, $1.09x10^7$, $1.13x10^7$, $3.1x10^6$, $1.9x10^7$, $1.4x10^7$cfu/ml respectively. The highest SPC was recorded in the sample S5 ($1.96x10^7$) and the lowest SPC was recorded in the sample S6 ($1.09x10^7$ cfu/ml).Count of other samples remained in the acceptable range and thus these yogurt samples were considered as safe for consumption.

Table 4.7: Total bacterial count

Name of Sample	**No. of Bacteria (cfu/ml)**	**Species**
S1	$4.9x10^5$	Yeast
S2	$7x10^5$	Yeast
S3	$1.6x10^7$	*Lactobacillus*
S4	$1.89x10^7$	*Lactobacillus*
S5	$1.96x10^7$	*Lactobacillus*
S6	$1.09x10^7$	*Lactobacillus*
S7	$1.15x10^6$	Yeast
S8	$1.13x10^7$	*Lactobacillus*
S9	$3.1x10^6$	*Lactobacillus*
S11	$1.9x10^7$	*Lactobacillus*
S12	$2.9x10^5$	Yeast
S13	$1.4x10^7$	*Lactobacillus*
S14	$4.2x10^5$	Yeast

4.5.2 Total Coliform Count (TCC): The total coliform count of a yogurt sample gave an indication of the total number of coliform bacteria present in the yogurt. TCC was nil in all yogurt samples. Count of all these samples remained in the acceptable range and thus these yogurt samples were considered as safe for consumption.

4.5.3 Total Fungal count (TFC): The total fungal count procedure selects mainly for yeast that are most commonly associated with poor starter culture contamination. The TFC was recorded in the sample S1, S2, S7, S12 and S14 were $4.9x10^5$, $7x10^5$,$1.15x10^6$, $2.9x10^5$ and $4.2x10^5$ cfu/ml respectively. The highest TFC was recorded in the sample S2 ($7x10^5$ cfu/ml) and the lowest TFC was recorded in the sample S7 ($1.15x10^6$ cfu/ml).

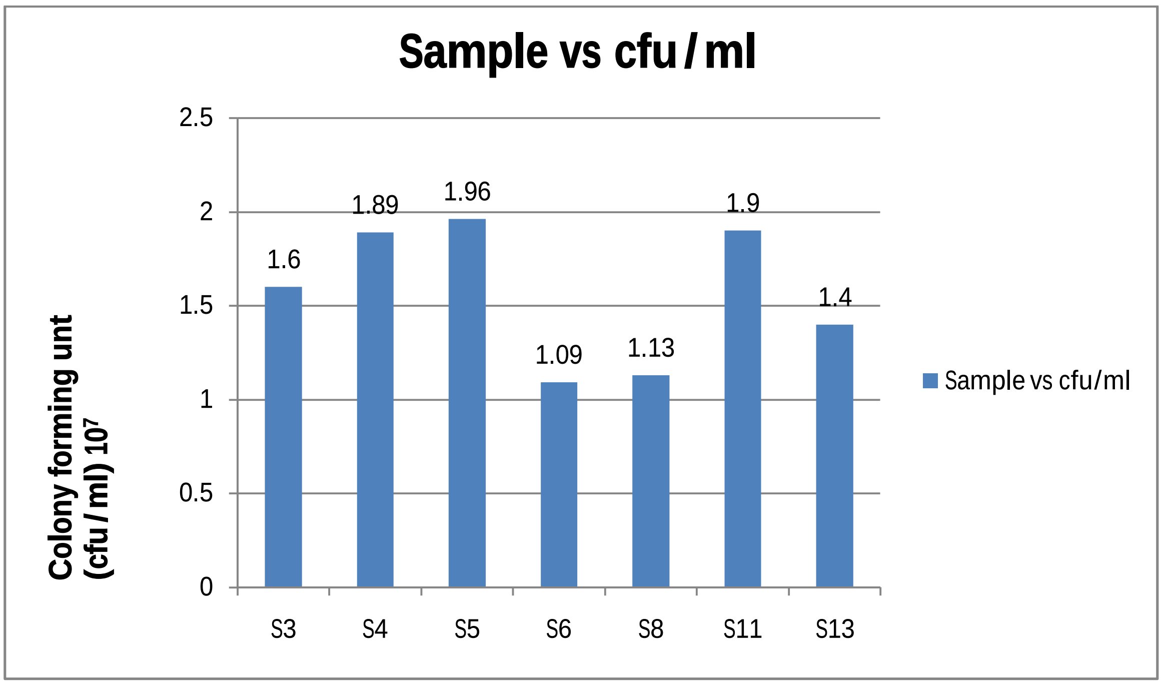

Figure 4.51: Viable Lactobacillus in yogurt samples after 24hrs of fermentation

Table 4.8: Microscopic observation of S1, S2, S3, S4, S5 & S6 isolates from local yogurt samples

Sample	Colony shape on medium	Gram stain; shape	Genera	Growth duration	Medium used
S1	-Small colony: round, white smooth surface, -Large colony: round, white smooth surface	Moving and typical budding yeast with round or oval cells.	Organism	24hrs	MRS
S2	Large colony: round, cream white, smooth surface	Mixed culture of yeast with round or oval cells and bacteria.	Organism	24hrs	MRS, NA
S3	Small colony: round, white smooth surface	*Lactobacillusplanterum* +; rod	Lactobacillus	24hrs	MRS
S4	Medium colony: cream color, round, smooth surface. Small colony: round, white smooth surface	Moving streptococcus or diplococcic, chain shape, +; cocci	Steptococcus	24hrs	MRS, NA
S5	-Medium colony: cream color, round, rough surface, irregular shape. - Small colony: round, cream color, smooth surface	Moving streptococcus or diplococcic, chain shape, +; cocci	Steptococcus	24hrs	MRS, NA
S6	Medium colony: cream color, round, smooth surface. Small colony: round, white smooth surface with zone of inhibition	Moving streptococcus or diplococcic, chain shape, +; cocci	Steptococcus	24hrs	MRS, NA

Table 4.9: Microscopic observation of S7, S8, S9, S10, S11, S12 & S13 isolates from local yogurt samples

Sample	Colony shape on medium	Gram stain; shape	Genera	Growth duration	Medium used
S7	Large colony: Round, white, smooth surface.	Yeast with round or oval cells	Organism	24hrs	MRS
S8	Small colony: round shape, white, smooth surface.	Moving vigorously chain shaped+; cocci	Steptococcus	24hrs	NA
S9	- Large colony: Round, white, smooth surface. -Small colony: round, white smooth surface.	Moving streptococcus or diplococcic, chain shape +; cocci	Steptococcus	24hrs	MRS, NA
S10	- Large colony: Round, white, smooth surface. - Medium colony: cream color, round, smooth surface.	Yeast with round or oval cells, Small rods, chain shape, moving,+; rod	Organism & Steptococcus	24hrs	MRS, NA
S11	Large colony 1: red in color, rough surface, irregular shape.	Moving lactococcus +; cocci	Steptococcus	48hrs	MRS, NA
	Large colony 2: off whitish, irregular shape, smooth surface.	Moving, +; cocci			
	Small colony: cream white, round shape.	Moving vigorously chain shaped,+; cocci			
S12	-Large colony: white in color, smooth and round surface. -Small colony: cream white, round shape.	Yeast with round or oval cells	Organism	24hrs	MRS, NA
S13	Medium & small colony: round, smooth surface, white or pale color.	Small rods, chain shape, moving,+; rod	Steptococcus	24hrs	MRS, NA

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Table 4.10: Microscopic observation of S14, S15, S16, S17, S18 & S19 isolates from local yogurt samples

Sample	Colony shape on medium	Gram stain; shape	Genera	Growth duration	Medium used
S14	Medium and small colony: round, white smooth surface.	Moving and Typical budding yeast with round or oval cells	Organism	24hrs	MRS, NA
S15	Large colony: white in color, smooth and round surface.	Mixed population of yeast and bacteria	Organism , +; cocci	24hrs	MRS, NA
	Medium colony: Round, smooth surface.	Mixed population of yeast and bacteria	Organism Yeast, +; cocci		
	Small colony: round, white smooth surface.	Few rod shaped bacteria +; rod	+; cocci		
S16	Large colony: Round white, smooth and round surface	Mostly yeast with round or oval cells	Organism	24hrs	MRS
	Medium colony: Round, white, smooth surface.	Mixed population of yeast and bacteria	Organism , +; cocci		
	Small colony: Round, white smooth surface.	Mixed population of yeast and bacteria	Organism , +; cocci		
S17	Medium colony: Round, cream white, smooth surface.	Mixed population of yeast and bacteria	Organism , +; cocci	48hrs	MRS
S18	-Large colony: white in color, smooth and round surface -Medium colony: Round, white, smooth surface.	Mixed population of yeast and bacteria	Organism , +; cocci	24hrs	MRS
S19	Medium colony: Round, white, smooth surface.	Mixed population of yeast and bacteria	Organism , +; cocci	24hrs	MRS

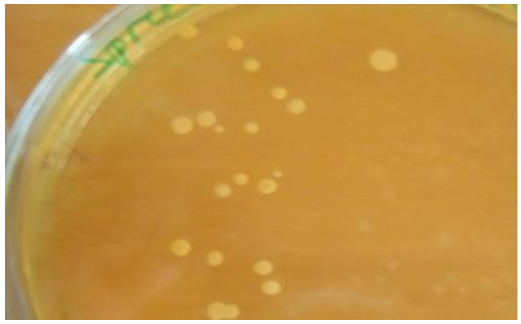

Figure 4.52: Growth of Yeast on MRS medium

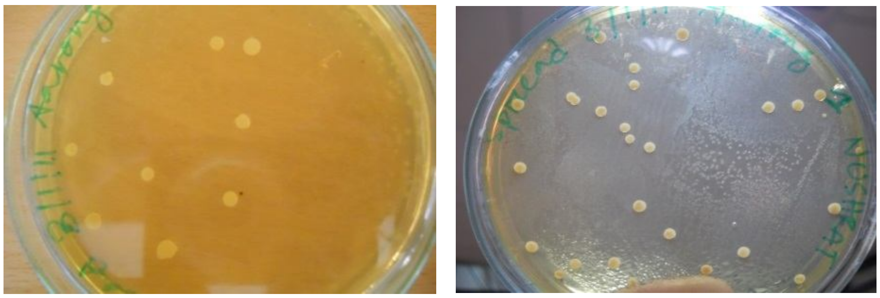

Figure 4.53: Growth of Yeast and bacteria on MRS & NA medium

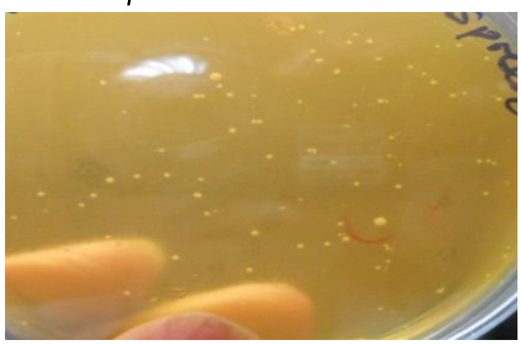

Figure 4.54: Growth of Lactobacillus on MRS medium

Figure 4.55: Growth of Yeast on MRS medium

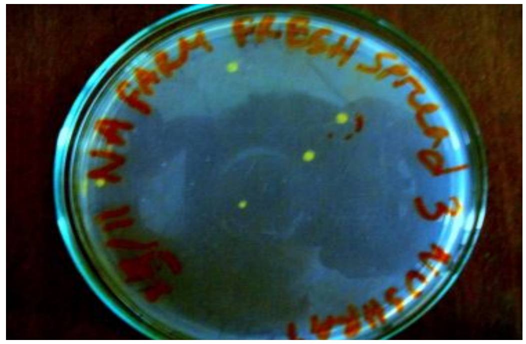

Figure 4.56: Growth of Steptococcus on NA medium

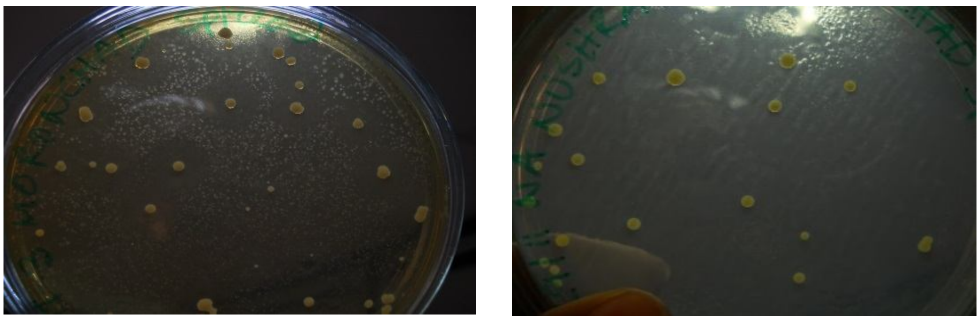

Figure 4.57: Growth of Steptococcus on MRS & NA medium

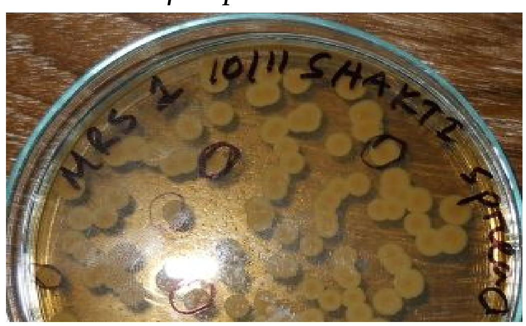

Figure 4.58: Growth of Yeast on NA medium

Figure 4.59: Growth of Steptococcus on NA medium

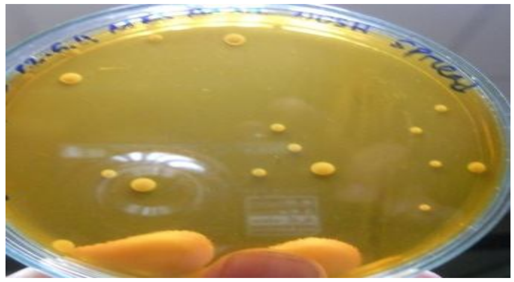

Figure 4.60: Growth of Steptococcus on MRS medium

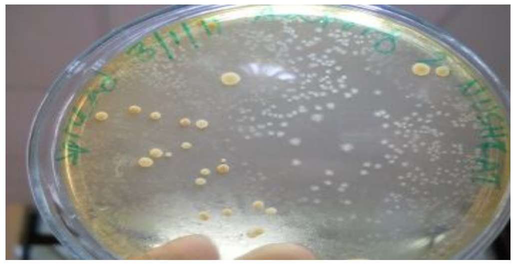

Figure 4.61: Growth of Yeast and bacteria on MRS medium

Figure 4.62: Growth of Steptococcus on MRS medium

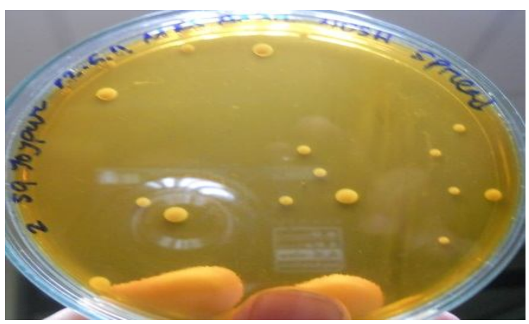

Figure 4.63: Growth of Yeast on MRS medium

Figure 4.64: Growth of Steptococcus on MRS medium

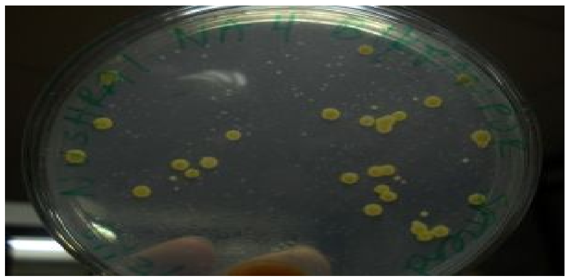

Figure 4.65: Growth of Yeast and bacteria on NA medium

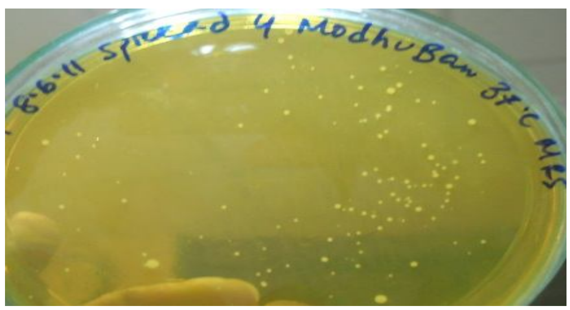

Figure 4.66: Growth of Yeast and bacteria on MRS medium

Figure 4.67: Growth of Yeast and bacteria on MRS medium

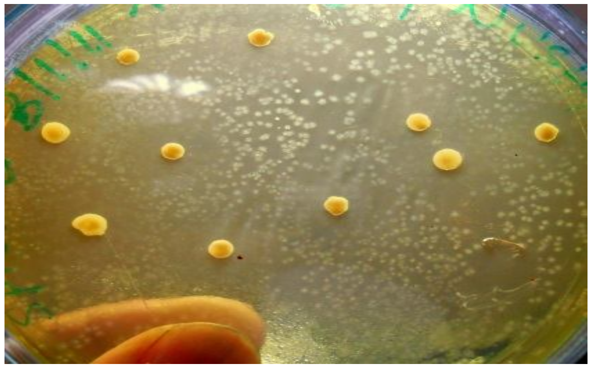

Figure 4.68: Growth of Yeast and bacteria on NA medium

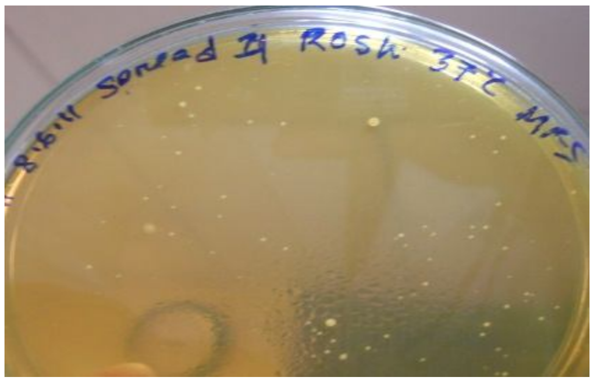

Figure 4.69: Growth of Yeast and bacteria on NA medium

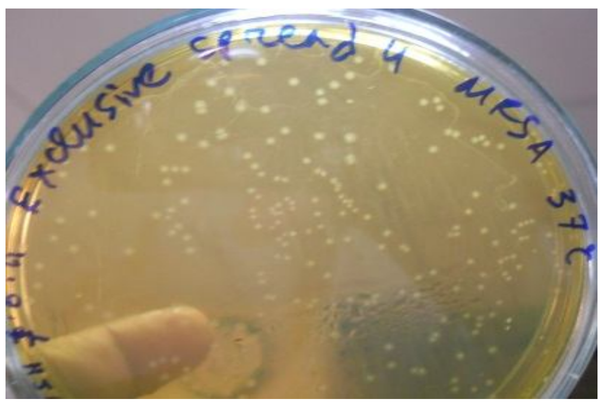

Figure 4.70: Growth of Yeast and bacteria on NA medium

Figure 4.71: Coding and preservation of the isolates in TSB and SM media (S1 to S19)

CHAPTER 5
DISCUSSIONS

5. DISCUSSIONS

For the development of improved quality yogurt, all the samples were collected from different places of Dhaka city regardless of branded (industrial) and unbranded (locally produced) yogurt. In this study efforts were made to isolate the better strains of *Lactobacillus* which were used in this study as starters or raw material for yogurt production. For this purpose 19 different samples of yogurt mostly as sour yogurt were selected finally.

Spontaneous fermentation of S1, S2, S3, S4, S5, S6, S7, S8, S9, S10, S11, S12, S13, S14, S15, S16, S17, S18 and S19 were done and observed their pH and texture. The results presented in Table 4.1, 4.2, 4.3 and 4.4 demonstrate that among these samples, the best one was sample S5 showed the quality of fermented yogurt with good texture, thick solid portion, less water content after 4 hours of fermentation with low acidic pH 3.85 and still maintained that quality texture even after 8 hours of fermentation with the pH of 3.62. While sample S8 showed moderate quality with more water content after 4 hours of fermentation with the pH of 3.89 but failed to maintain that texture after 8 hours of fermentation with a pH of 3.30. Growth increases with the increasing of pH from pH-1 to pH-6 but growth decreases when it exceeds the neutral pH range. Tambekar *et al.*, 2010 found that *lactobacillus brevis, lactobacillus bulgaricus, lactobacillus plantarum, lactobacillus rhamnosus, lactobacillus helviticus, lactobacillus casei* and *lactobacillus fermentum* can tolerate pH up to 2.0. The final pH measurement also indicates that slight pH change also occurs after incubation of the sample. It may be due to higher growth of those yogurt cultures of the yogurt samples resulting in the production of greater amount of lactic acid.

In the Table 4.5 shows the distribution of responses on Hedonic Scale for quality assessment of these 19 yogurt sample soon after the spontaneous fermentation study. Gupta *et al.,* 2000 described that the texture of the yogurt depends mainly upon the rate of development of the acidity i.e. type of organisms present in the starter culture. The wide variation in the quality parameter of yogurt can be attributed to the manufacturing conditions and type of organism used. The texture mean score of yogurt S1,S2, S3, S4, S5, S6, S7, S8, S9, S10, S11, S12, S13, S14, S15, S16, S17, S18 and S19 were 5.4, 4.7, 7.1, 3.9 ,8.4,7.1, 5.2, 5.9, 4.7, 3.7, 4.6, 7.1, 4.4, 5.3, 5.1, 6.5, 6.1, 6.6 and 6.4 respectively. In the texture acceptability test, Hedonic scale showed that the yogurt sample S5 was excellent, S3, S6 and S12 considered very good S16, S17, S18 and S19 considered acceptable texture quality. Munzur *et al.,* 2004 described that the color of the yogurt depends on the color of milk or caramelized color obtained during heating of the milk or added coloring materials. In Table 4.5 Hedonic scale showed that the yogurt sample S5 was excellent, S1, S3, S9, S11, S12, S16, S17, S18 and S19 considered as very good, Sample S2, S6, S7, S8, S14 and S15 considered as acceptable and S10 had poor color quality. The flavor of the product depends on the volatile constituents of milk and also influence by the quality of the raw milk and fermentation pattern of the product. In Table 4.5 Hedonic scale showed that the yogurt sample S5 was excellent (mean score is 8.4), S6, S7, S12, S15, S16, S17 and S19 considered as very good, S1, S2, S3, S9, S11, S13, S14 and S18 considered as acceptable and S4 had poor flavor quality. Taste is influenced by the quality of the raw milk and added materials to it. In Table 4.5 , Hedonic scale showed that the yogurt sample S5 was excellent (mean score 8), S1, S6 and S7 considered as very good, S2, S3, S8, S11, S12, S14, S15, S16, S17, S18 and S19 considered as acceptable and S10 had poor taste quality.

Fadela *et al.,* 2009 in their research data show that the time needed for a successful fermentation and storage of yogurt, significantly affects the decrease in pH. As acidity is important for flavor balance and a low pH leads to more stable color and inhibits microbial spoilage. Since the pH value is in correlation with the acidity, as can be seen from the Table 4.6 in terms yogurt texture, the sample obtained the best texture with good aroma is S5 isolates with 8% milk concentration and showed a low pH value of 3.20 with a high TTA% value of 0.71%. Also showed quite good quality of yogurt texture were mix culture of LAB with 3% inoculums concentration and Second set combination with a pH value of 4.11, 3.16 and TTA% value of 0.77%, 0.58% respectively.

In Figure 4.21 shows the changes in pH of yogurts with S5 isolates during storage for 3 days. The pH of the yogurt samples decrease during storage. The pH for S5 isolates reduced from the initial values of 3.35 to between 3.18 and 3.17 by day 3 of storage at 4 ºC. The similar results are reported by Anjum *et al.,* 2007 and Wofschoon *et al.,* 1983. This decrease in pH is due to the consumption of lactose by microbial culture that ultimately results in the formation of lactic acid, formic acid and small quantity of CO2 (Panesar *et al.*, 2011).

Wong *et al.*, 1983 stated that under the protocooperative stimulations during combined growth of the yogurt bacteria, lactic acid is produced at a must faster rate than by that of individual pure cultures. So here the result presented in Figure 4.31 and 4.33 showed that organism concentration enhanced the quality of yogurt as compared to control sample of yogurt i.e. in second set combination or combination of three different isolates gave good yogurt texture than first set combination where used only two isolates. In similar way the inoculated fermentation of yogurt with mix culture of LAB or 3% inoculums at 6% milk concentration showed the best quality yogurt than that of 2% and 1% inoculums concentration which presented in the Figure 4.37, 4.38 and 4.39.

Kroger, 1976 stated that the addition of milk solids to the yogurt milk or mix is much simpler, and the nutritional value is better than when made from plain milk. Milk fat also contributes to the body and texture if the mix is homogenized. So here the results presented in Figure 4.26, 4.27, 4.28 and 4.29 showed that the effect of milk concentration on Streptococcus S5 isolates enhanced the quality of yogurt as compared to control sample and showed good fermented yogurt in 6% milk concentration while comparatively better texture in 8% milk concentration.

Finally in the conclusion it can be said that S5 isolate with 8% milk concentration showed the best yogurt compared to that with 3% inoculums concentration with 6% milk concentration in terms of overall yogurt texture quality.

CHAPTER 6
CONCLUSION

6. CONCLUSION

The present research study has been carried to find the best quality strains of *lactobacillus* from the local variety of yogurt for the development of improved quality yogurt in terms of texture, flavor, food value and low cost. Total 19 collected samples of yogurt were handled appropriately with utmost precaution and standard protocol before lab tasting to obtain accurate microbial quality and further investigative analysis. Based on the results reported here, it can be said that, most of the samples failed to maintain their overall quality texture and out of 19 collected yogurt samples, only S5 showed best and consistent quality in terms of texture in the quality assessment test. Also the distribution of lactic acid bacteria was not uniform in different samples and the major type of isolates obtained from the natural yogurt was mainly *lactobacillus.* Among them the most promising isolates obtained were the *streptococcus* and *lactobacillus planterum.* Apart from that, an overall good yogurt texture quality was produced by inoculated fermentation of 3% inocula concentration with 6% milk concentration.

Recommendations for future work:

Following points should be strongly observed and maintained for future work and further studies to improve the quality of yogurt, based on this research study:

1. Inoculated fermentation with the most promising isolates obtained from the natural yogurt and their combination
2. Optimization of yogurt fermentation with selected isolates of the lactic acid bacteria isolated from natural yogurts collected from the market. So as to have yogurt uniform characteristics in terms of texture, flavor, food value and low cost.

Some parameters should have been taken into consideration which could not be done during this study are:

1. Identification of lactic acid bacteria as starter culture that is to be used for yogurt production, at optimized pH, temperature and milk concentration.
2. Kinetics of growth of selected potential lactic acid bacteria at different environmental conditions so as to optimize yogurt fermentation.
3. The composition of yogurt in terms of its taste and food value.
4. Firmness of set yogurt in terms of its texture by Texture Analyzer

CHAPTER 7
BIBLIOGRAPHY

7. BIBLIOGRAPHY

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CHAPTER 8
APPENDICES

8. 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
$MgSO_4.7H_2O$	0.2 gm
$MnSO_4.4H_2O$	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

- **Phenalphthalein 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 gm NaOH and mixed with 500 ml distilled water.

APPENDIX-III

Instruments

Table 8.1: Equipment with brand name

Name of the equipment	Brand name
Autoclave	WiseClave
Dry heat sterilizer	Lab tech, Daihan Labtech Co. LTD.
Incubator	SAARC
pH meter	E 201-C,Shanghai RuosuaaTechnology company,China
Weighing balance	Axis
Laminar air flow	SAARC Engineeing
Vortex	Digi system
Microscope	Olympus: CX21FS1 (Japan)

APPENDIX-IV

Collected yogurt samples

Table 8.2: Coding of the yogurt sample

Serial No	**Name of Yogurt Sample**
S1	premium sweets
S2	Aarong batch one
S3	*lactobacillus plantarum*
S4	Homemade yogurt from shop n' save
S5	Farm fresh
S6	Moronchad
S7	Shakti doi
S8	Bismillah
S9	Alibaba
S10	Aarong batch two
S11	Jobadoighar (sirajgonj)
S12	Joypur
S13	Ekushe
S14	Bikrampur
S15	Modhuban
S16	Rajbhog
S17	King's
S18	Rosh
S19	Exclusive