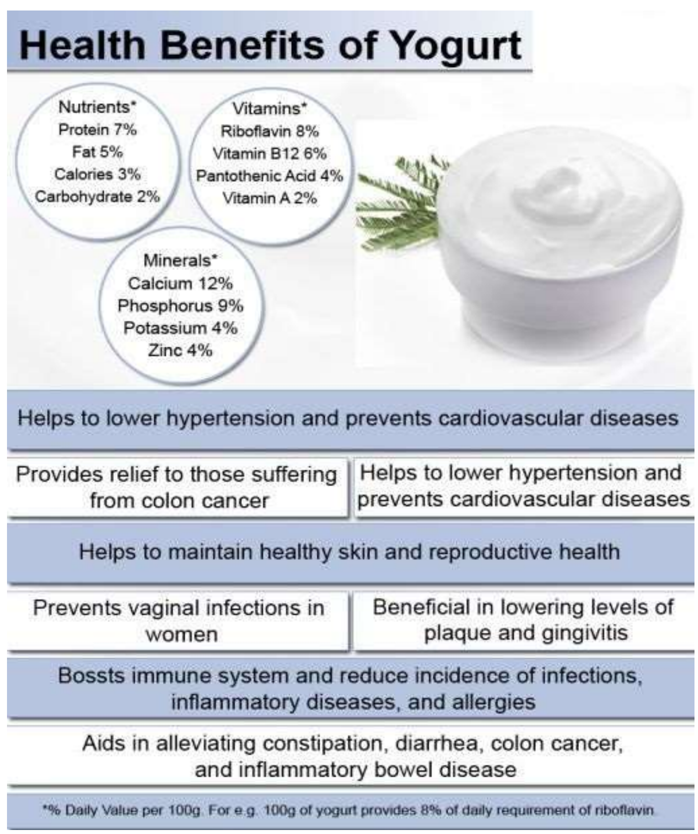
# Chapter-I

# Introduction

Milk is fermented with lactic acid bacteria and a starter culture (*Streptococcus thermophilus and Lactobacillus delbrueckii* ssp. *bulgaricus*) is added to make yogurt. Yogurt has become one of the fastest-growing dairy products due to its usage as a calcium source, but it is now much more than that. Yogurt and other fermented milk products are on their way to becoming key nutraceuticals that can be used to treat a wide range of diseases (Katz, 2001).

Yogurt is high in protein, calcium, phosphorus, riboflavin (vitamin B2), thiamin (vitamin B1), and Vitamin B12, as well as folate, niacin, magnesium, and zinc. Yogurt, especially low-fat variants, are nutrient-dense foods because they include a wide range of vital nutrients in large levels compared to their energy and fat content. Consuming dairy products, such as yogurt, improves the overall quality of one's diet and enhances the likelihood of meeting nutritional guidelines (Mckinley, 2005). Fats are essential macronutrients that keep the body solid. Moderate fat admission is gainful, however higher amounts of fats can decide genuine intricacies (Siraj et al., 2015). In the texture, flavor, and color development of dairy products, milk fat plays a critical role. Some flaws in yogurt, such as a lack of flavor, a weak body, and a bad texture, can be caused by lowering the fat content (Haque and Ji 2003). With the intend to work on the quality attributes of low-fat items are utilized completely or to some extent fat replacers (Akoh, 1998). They are delegated lipid substitutes and as lipid mimetics (Romeih et al. 2002). Fat substitutes can hypothetically



supplant the fat balanced in food items (lipid-or fat-based fat replacers). Fat imitators are substances with comparative properties of fatty oils, yet which can't substitute acurately and they are called fat replacers in light of proteins or starches (Romeih et al. 2002; Akoh, 1998). The link between fat consumption and heart disease like atherosclerosis, coronary illness, hypertension has recently been established, and nutritionists have suggested reducing dietary animal fat (Kucukoner and Haque 2003). In acknowledgement of their health benefits and customers' health problems, use of low-fat dairy products has surged (Haque and Ji 2003; Kucukoner and Haque 2003). Although it has been feasible to make low-fat dairy products for many years, the use of fat replacers in the production of dairy products is still new.

Fat replacers, which lower the calorific value of food, can be employed to alleviate various physical and organoleptic issues caused by low fat levels in finished products. Fat replacers are made up of lipid-derived fat substitutes, protein- or carbohydrate-derived fat mimics, or a combination of the two (Huyghebaert et al. 1996). Carbohydrate-based fat replacers such as Inulin can be used in yogurt for improving its functionality, such as binding of water and increasing viscosity (Tamime and Robinson, 1999). Inulin, a carbohydrate-derived fat replacer or dietary fibre, has a gelling capacity with water and is a useful food ingredient (O'Brein et al. 2003). It is not digested in the small intestine, but lactic acid bacteria, such as yogurt starter cultures, ferment it in the colon. As a result, inulin promotes the growth of beneficial bacteria, improves calcium and magnesium absorption and immunological function, and lowers cholesterol and blood lipid levels (Rowland et al. 1998; Staffolo et al. 2004; Ohr 2004). Health benefits of adding Inulin as a Fat Replacer on the low-fat yogurt is shown in **Figure 1.** Furthermore, inulin fermentation may promote the synthesis of short-chain fatty acids such as acetate, propionate, and butyrate, the latter of which is the colonocytes' preferred energy substrate (Kruse et al. 1999). When employed as a fat replacer in water-based meals like dairy products, inulin creates a fat-like tongue feel and texture (Zimeri and Kokini 2003).

**Figure 1. Health benefits of adding Inulin as a Fat Replacer on the low-fat yogurt**

However, very limited work has been done in our country regarding low-fat yogurt development. The findings retrieved from the present study could encourage the people to consume low-fat yogurt more, as it could provide some important knowledge about benefits of consuming low-fat yogurt, which would play an important role for consumption of safe, sound and healthy low-fat yogurt. In addition, the result from this current study could encourage people to manufacture more low-fat yogurt as well as low-fat fermented dairy products, which would boost the national economy as well as improve the health status of the general people of this country. So, the present study was conducted for the production of low-fat yogurt with the fat replacer (inulin) to meet the following objectives -

1. To develop low-fat yogurt using fat replacer (Inulin) and *Streptococcus thermophilus* as starter culture.
2. To select a suitable level of the fat replacers in low-fat yogurt to obtain desired textural characteristics.
3. To evaluate the physicochemical and sensory properties of the developed low-fat yogurt.

# Chapter-II
## Review of Literature

This chapter summarizes the background of Low-fat yogurt, Role of fat replacer (inulin) in low-fat yogurt, low-fat yogurt manufacturing process and yogurt composition with its manufacturing process and also describes the different health benefits of yogurt and future perspective in low-fat yogurt.

### 2.1 Yogurt and Dahi

Yogurt is 'the coagulated milk product obtained by the fermentation of lactic acid via the action of *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophilus* (Krasaekoopt et al., 2005). This dairy starters break 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 development of lactic acid and acetaldehyde.


The 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 various other components from the milk fermentation process. Yogurt contains *Lactobacillus* and *Bifidobacterium* species at $10^6$ viable cells per millilitre at the time of consumption (Arunachalam, 1999). According to the PFA regulations (1976), "Dahi or curd is the product derived from boiled or pasteurized milk by fermenting harmless lactic acid or other bacterial culture". It may contain added cane sugar. It should have same percentage of fat and solids-not-fat as the milk from which it is prepared. Although yogurt and dahi both are cultured or ripened dairy products still there are little differences between those. Yogurt is prepared by using starter organisms *Streptococcus thermophilus* and *lactobacillus bulgaricus* in a proportion of 1:1, whereas dahi is prepared by using mixed culture of *Streptococcus lactis*, *Lactobacillus bulgaricus*, *Streptococcus thermophilus, Streptococcus citrophilus*, *Lactobacillus plantarum* etc. Higher temperature (45°-50°C) and shorter (3-4h) incubation

period is required for yogurt making. On the other hand, lower temperature (37-42°C) and long incubation period (8-15 h) is required for dahi preparation. Comparatively yogurt is softer and dahi curd is reverse of that (Varnam and Sutherland, 1994).

**Reagents** **End Products**

Lactase Glycolysis+ Fermentation

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

**Figure 2. Enzymatic reaction in yogurt production**

**2.2 Composition of Yogurt**

Yogurt is produced via the lactic acid bacterial fermentation of milk. Cow milk is commonly used for the development of yogurt. There may be slight difference in composition of yogurt and the milk from which yogurt is prepared **(Table 3)**.

**Table 1. Chemical composition of different sources of milk used in yogurt production**

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

**(Source: Akin, 2006)**

**Table 2. Nutritional facts of Yogurt**

| Composition (%) | Yogurt | Low-fat yogurt | Non-fat yogurt |
|---|---|---|---|
| **Fat** | >3.25 | >0.5 to <2.0 | <0.5 |
| **Protein** | 4.4 | 5.7 | 5.2 |
| **Carbohydrate** | 7.5 | 7.5 | 6.9 |
| **Milk solids-not-fat** | >8.25 | >8.25 | >8.25 |
| **Titratable acidity** | >0.9 | >0.9 | >0.9 |

**(Source: Surono and Hosono, 2011)**

**Table 3. Vitamin contents of milk and yogurt**

| 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 (B2) (µg)** | 160 | 180 | 190 | 200 |
| **Pyridoxine (B6) (µg)** | 46 | 42 | 46 | 46 |
| **Cyanocobalamine (B12) (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 |
| **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 |

**(Source: Tamime and Deeth, 1980)**

### 2.3 Type of Yogurt

Yogurt is primarily categorized according to its chemical composition, method of manufacture type of flavor or post-incubation process (Shah, 2000).

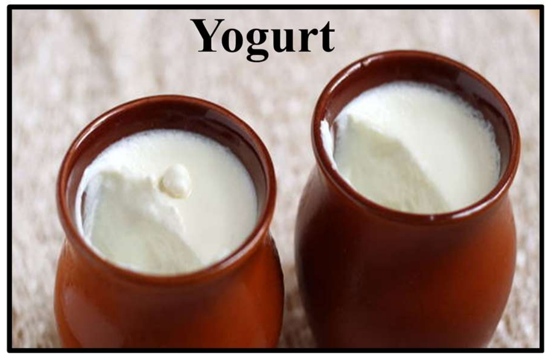
**Table 4. Type of Yogurt (Based on fat content)**

| | Full-fat yogurt | Reduced-fat yogurt | Low-fat yogurt |
|---|---|---|---|
| **Fat%** | ≥ 3 | 0.5-2 | ≤ 0.5 |
| **Solids-not-fat%** | ≥ 8.25 | ≥ 8.25 | ≥ 8.25 |
| **Titratable acidity %** | ≥ 0.9 | ≥ 0.9 | ≥ 0.9 |
| **pH** | ≤ 4.5 | ≤ 4.5 | ≤ 4.5 |

**(Source: Shah, 2003)**

- According to chemical composition, they are classified as full-fat, reduced-fat or low-fat yogurt **(Table 4)**.

- These can be grouped as set-type and stirred-type according to the production method. Set type of yogurt is incubated and then cooled in the final package which is characterized by a firm jelly like texture. It is fermented in a retail container, filled after milk inoculation and is incubated at 37°C for approximately 6 to 8 hours. On the other hand, for stirred-type yogurt, milk is incubated in a fermentation vat and the final coagulum is stirred before cooling and packaging stages. Stirred yogurt promotes the growth of *Lactobacillus bulgaricus* and *Streptococcus thermophilus* at a mild temperature (between 40°C and 43°C) until a desired acidity level is reached (Tamime and Deeth, 1980). Stirred yogurt texture will be less solid than a set yogurt.




- Yogurt can be classified, according to the type of starter culture used. Probiotic yogurts are produced by the incorporation of other lactic acid bacteria such as, *Streptococcus thermophilus, Lactobacillus bulgaricus, Lb. acidophilus, Lb. casei, Lb. plantarum, Lb. reuteri, B. bifidum, B. longum, Lb. acidophilus* and *Bifidobacterium* spp. and these probiotics are most widely used in dairy industry (Akın, 2006).

### 2.3.1. Low-fat yogurt

Right now, the inclination to devour low fat dairy products has grown up, regardless of whether the fat has critical effect over their physical, rheological and textural properties, microbiological solidness. Consequently, for this reason, to create subjective dairy items, are utilized fat mimetics, which bring a lower caloric worth, guarantee similar physical and sensorial attributes in the eventual outcome (Frost et al, 2001). The development of low-fat dairy items is certifiably not a simple undertaking. For instance, fermented milk items with low fat substance are more enthusiastically acknowledged by purchasers.

### 2.3.2. Fat replacer (Inulin)

The modern extraction of inulin-type fructans is regularly done from plants that have a place with the Compositae family, i.e., chicory. The level of polymerization (DP) of chicory inulin shifts from 2 to ~ 60 units. High sub-atomic weight inulin, otherwise called elite execution (inulin HP), is ready by applying actual detachment strategies to dispose of all oligomers with DP < 10. Inulin HP has a normal DP of 25 and a sub-atomic circulation range from 11 to 60 (Roberfroid, 2007).

Inulin is utilized either as enhancements to food sources or as macronutrient substitutes in food sources. As enhancements to food sources, it is added predominantly for its dietary properties. Such augmentations are generally in the scope of 3-6g per segment, not surpassing 10g. As macronutrient substitutes, it is utilized essentially as a fat replacer. Normally 1 g of fat is supplanted by 0.25g of inulin which will prompt inulin groupings of ~ 6g per segment. Since inulin has been a characteristic part of numerous food varieties ate securely by people over centuries, it is thusly commonly perceived as protected (Coussement, 1999).

The usefulness of inulin relies upon its impact on water arrangements at different strong levels. At lower fixations it tends to be utilized as a rheology modifier since it causes huge expansion in thickness while at a centralization of 40-45% an inulin gel or crème is shaped which is firm however with a greasy velvety feel. In this structure inulin is steady in acidic conditions and at high temperatures (Murphy, 2001).


**Figure 3.** A) What is Inulin?, B) Natural Source of Inulin, C) & D) Chicory root E), F) & G) Inulin powder

### 2.3.3 Role of fat replacer (inulin) in low-fat yogurt

The limited work was done over the few years that studied the role of inulin on the physicochemical and textural properties of low-fat yogurt has been outlined below. The use of inulin as a fat replacer did not affect the pH values but negatively affect on some physical properties such as whey separation, consistency and organoleptic properties on low-fat yogurt (Guven et al., 2005). They observed that the consistency was highest in yogurt containing 1% inulin. Akin et al. (2005) found that the addition of inulin (0.5%) reduced proteolysis in low-fat yogurt samples.

It additionally caused a significant expansion in the cell counts of *B. bifidum* BB-02, being around 4.6 and 7.5 crease for the inulin added tests at levels of 0.5% and 1.0%, individually. They inferred that supplementation of milk with inulin as development advertisers for *B. bifidum* BB-02 and *L. acidophilus* LA-5 could be a good method of keeping the quantity of reasonable probiotic cells in AB yogurt over the proposed restorative least (~107 CFU/g) during cold storage. Kip et al. (2006) tracked down that inulin (DP 23, 3%) added to a worked on rich mouthfeel by upgrading the characteristic vaporous and positively affecting thickness and stickiness. Aryana et al. (2007) showed that the chain length of inulin (little, medium or since quite a while ago) impacted the pH and syneresis in yogurt and that inulin helped the endurance of *L. acidophilus* in the item.

### 2.4 Low-fat Yogurt Manufacturing Process

In the dairy industry, no matter which manufacturing process is applied, the fermented dairy product must be appropriate to national and international standard protocol. The flow diagram of manufacturing steps for low-fat yogurt production is given in **Figure 4** and the basic manufacturing steps for any types of yogurts are as follows:

#### 2.4.1 Filtration

Filtration should be done to separate any cellular matter and other extraneous matter present in milk.

#### 2.4.2 Standardization of milk

Standardization of milk refers to the adjustment of fat and solids not fat levels of milk by raising or lowering the levels. Standardization of milk is usually done in cases of market milk supply and also in the case of manufacture of milk products. The standardization of milk is one of the most important factors in obtaining good quality yogurt. Milk is fortified and mixed with skim milk and cream to adjust the fat content to the desired level. The minimum amount of milk solids not-fat specified in the requirements or regulations ranges from 8.2 to 8.6% in many countries. Also, stabilizers are applied to the milk to improve the correct yogurt properties including texture, mouth feel, appearance, consistency and prevention of whey separation (Tamime and Robinson, 1999).

### 2.4.3 Homogenization

Homogenization is a process of reducing a substance, such as the fat globules in milk, to extremely small particles and distributing it uniformly throughout milk. The cream will not rise to the top if milk is properly homogenized. Milk become whiter at the end of the homogenization process, and the yogurt formed from that milk is more viscous and also the flavor is homogeneously distributed all over the container (Tekinsen, 2000).

### 2.4.4 Heat treatment (Pasteurization)

The most commonly used heat treatment in the yogurt industry include 85°C for 30 minutes or 90-95ºC for 5 minutes (Tamime and Robinson, 1999) as heating of milk greatly influences the physical properties and microstructure of yogurt. Nevertheless, sometimes 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 used (Sodini et al., 2005).

There are several advantages of pasteurization such as:

- It helps to remove dissolved oxygen which promotes starter growth
- It leads to production of some aroma compounds

On the other hand, heat treatment also has some disadvantages because of the-

- Formation of certain by-products that have an inhibitory effect on the growth of bacteria in starter culture.
- Sometimes it causes reduction of pH, oxygen content of milk and denaturation of serum proteins, thus hydrophilicity of casein increases and syneresis decreases.

### 2.4.5 Effect of heat treatment on quality of yogurt

Shekhar et al., (2012) have shown that, there is an effect of heat treatment of milk on the sensory and rheological quality of yogurt. The texture depends primarily on the milk heat treatment. Among the other heat treatment, boiling treatment of milk resulted in least syneresis of whey in the yogurt. Yogurt prepared from boiled milk, showed the highest value in cases of firmness, consistency and index of viscosity. The study recommended that milk should be subjected to boiling treatment to produce best quality yogurt. High heat treatment of milk increases gel firmness and reduces syneresis in the final product (Lucey et al., 1998; Vasbinder et al., 2004). Another study showed that better rate of gelation can be obtained via high heat treatment (Xu et al., 2008).

### 2.4.6 Inoculation

Following pasteurization, milk is allowed to cool to 40-45°C and generally inoculated in 1:1 ratio with the fresh starter culture bacteria *Streptococcus thermophilus* and *Lactobacillus bulgaricus*. Although inoculation level varies between 1-4%, the optimum level is 2%.

### 2.4.7 Incubation (Fermentation)

The quality of final yogurt production can be affected by the incubation time (Tamime and Deeth, 1980). After inoculation, incubation takes place at 37ºC until the curd formation in the incubator (Vijayendra and Gupta, 2012) or may be incubated at 37ºC for 6 hours (Anukam and Olise, 2012).

### 2.4.8 Cooling and Storage conditions

Starter cultures continue to grow, if yogurts are not cooled immediately at the end of the fermentation. As a result, it causes syneresis on the surface of yogurts.

Studies showed that higher survival rates of lactic acid bacteria can be obtained at lower storage temperatures (Gilliland and Lara, 1988; Foschino et al., 1996). Preservation at low temperature not only hinders the excessive growth of the starter culture, but also gradually over acidification in general (Kneifel et al., 1993).

**Procedure of yogurt preparation**

Milk

↓

Formulation

(Protein, fat, sugars additives)

↓

Heat treatment

↓

Homogenization

↓

Cool to incubation temperature

↓

Inoculate

↓

Fill retailable containers

↓

Fermentation

↓

Cool to stop fermentation

↓

Storage

**Figure 4. Process flow chart for the preparation of set yogurt**

**(Source: Duboc and Mollet, 2001)**

### 2.5 Health benefits of low-fat yogurt

The fundamental medical advantages of normal utilization of low-fat yogurt incorporate the improvement of the digestive microbial equilibrium; mitigating the indications of lactose intolerance through the development of lactase; improving the immune system; decreasing the danger of colon cancer in human and prevention against breast cancer; lessening a few types of food sensitivities; bringing down the blood cholesterol levels; reducing of blood pressure of hypertensive people; play a good role in the counteraction of loose bowels; and repressing the development of a few pathogenic microorganisms (Alhaj et al., 2007).

Low-fat yogurt has additionally been accounted for to contain a few substances that lower serum cholesterol. St-Onge et al. (2000) revealed the impacts and components of activity of low-fat yogurt on serum cholesterol focuses and recommended a moderate cholesterol-bringing down activity by low-fat yogurt. Also, the survey of creature and human investigations by Pereira and Gibson (2002) recommended moderate cholesterol-bringing down activity by low-fat yogurt fermented with appropriate strains of LAB and bifidobacteria. They additionally revealed that prebiotics, FOS, inulin and oligofructose, showed persuading lipid-bringing down impacts but only at high portion levels (50-200 g/kg) in animals. Kiessling et al. (2002) inferred that drawn out day by day utilization of yogurt, containing *S. thermophilus*, *L. lactis*, *L. acidophilus* 145, *B. longum* 913 and 1%

oligofructose didn't bring down the total and LDL cholesterol in healthy women but increased the serum concentration of HDL-cholesterol that led to the ideal improvement of LDL/HDL proportion.

### 2.6 Health benefits of adding Inulin as a Fat Replacer on the low-fat yogurt

Inulin-type fructans are named utilitarian food fixings. They are accepted to target gastrointestinal capacities and furthermore, undoubtedly through their consequences for the stomach and the stomach microflora, foundational capacities that are known to be connected with wellbeing and prosperity (Roberfroid, 2007). Inulin has an impartial taste, is boring and has insignificant effect on the organoleptic attributes of an item (Niness, 1999). Dissolvability of inulin increments fundamentally with temperature, coming to 34% (w/v) at 90 °C (Kim et al., 2001).

**Table 5. Nutritional effects and potential health benefits of inulin-type fructans.**

| | |
|---|---|
| Enhanced colonic functions | Composition and activities of the gut microflora |
| | Stool production |
| | Absorption of minerals |
| | Production of gastrointestinal endocrine peptides |
| | Immunity and resistance to infections |
| | Digestion of high protein diets |
| Enhanced systemic functions | Lipid homeostasis |
| Reduction of disease risks | Intestinal infections |
| | Irritable bowel disease |
| | Colon cancer |
| | Osteoporosis |
| | Obesity Diabetes |

(Source: Niness, 1999; Roberfroid, 2007)

### 2.7 Future Perspective in low-fat yogurt

Low-fat yogurt items were initially produced for diabetics and individuals with explicit medical conditions and they were extensively costly. These days, consumer's interest for low-fat yogurt has fundamentally increased in an endeavor to restrict medical issues, to lose or balance out their weight, and to work within the frame of a better eating routine.

The food business has been faced with another test to fulfill purchasers; development of low-fat yogurt with acceptable organoleptic taste and competitive cost, by ideally utilizing the traditional processing equipment and in concurrence with current strict regulation. The role of fat replacers and sugar substitutes in the fruitful assembling of these items is essential (Sandrou and Arvanitoyannis, 2000)

### 2.8 Significance of the research work

The findings from the present study are expected to encourage the people to consume more fermented low-fat dairy food, as it will provide some important knowledge about influence of adding inulin as a fat replacer on the characteristics of yogurt, which play a major role for beneficial health effects of consumers. From the obtained results, it could be concluded that the yogurt of acceptable quality could be made from skim cow's milk fortified with inulin. Addition of 1-2% inulin to skim milk resulted in yoghurt with comparable results to full fat cow's milk. In addition, the study would assist to produce functional fermented dairy products which will have impacts on the national GDP, along with the improvement of the health status of the people of this country.

From this section of review, it is clear that various scientists around the globe have tried to find out the health effects of adding Inulin as a Fat Replacer on the yogurt/low-fat yogurt. Inulin can have a promising application to supplement dairy products, such as yogurt.

# Chapter-III
# Materials and Methods

## 3.1 Statement of the experiment

The experiment was completed at the Department of Dairy and Poultry Science (DDPS), Chattogram Veterinary and Animal Sciences University (CVASU) to develop low-fat yogurt using fat replacer (inulin) and *Streptococcus thermophilus* cultures from preserved stock solution, previously isolated by supervisor research group from locally available yogurt (Amin et al., 2022). Analytical process was performed at the PRTC and DDPS laboratories of CVASU during July to November, 2021.

## 3.2 Development of low-fat yogurt using fat replacer (inulin)

### 3.2.1 Preparation of *Streptococcus thermophilus* culture

*Streptococcus thermophilus* cultures were reconstituted from frozen stock cultures (300 µl 50% glycerol in 700 µl BHI broth) by plating out in blood agar. The plates were incubated 24 hours at 37°C. After incubation the bacterial growth was observed. Thereafter a loopful colony was inoculated in 10 ml BHI broth. The inoculums were incubated at 37°C for 24 hours to get sample approximately close to the concentration level of $10^6$ cfu susceptibility testing.

### 3.2.2 Preparation of mother culture

The *Streptococcus thermophilus* cultures grown in BHI broth was centrifuged (4000 rpm for 5 minutes) to get bacterial pellets. Then the supernatant fluid was carefully discarded by pipetting. The bacterial pellets were washed twice with sterile PBS (phosphate buffered saline) to remove MRS broth. Finally, the pellets were reconstituted in 2 ml of PBS. Out of this, a 200 µl aliquot containing 5 x $10^8$ cfu/ml was added to 10 ml of the heat-treated milk and incubated for 18 h at 37°C.

### 3.2.3 Preparation of low-fat yogurt using inulin

Whole fresh raw cow milk was used for preparation of yogurt. After collection milk was filtrated by a strainer to remove any extraneous matter. Skim milk was prepared by separating raw whole milk using a cream separator at 40°C.

**Table 6. Experimental design**

| Sample | Milk fat (%) | Inulin (%) | Skim milk powder (%) |
|---|---|---|---|
| **Control ($T_0$)** | 3.5 | 0 | 1.9 |
| **$T_1$** | 0.2 | 1 | 4.2 |
| **$T_2$** | 0.2 | 2 | 3.2 |
| **$T_3$** | 0.2 | 3 | 2.2 |

Three replicate trials were conducted in the manufacture of yogurt with low-fat milk (0.2%) and whole fat milk (3.5%) as a control. Experimental groups were divided into three parts and inulin was added in different ratios as shown in **Table 6**. After blending the milk with inulin and skim milk powder in varying ratios, the mixtures were separately homogenized with a blender at 14000 r.p.m. until all ingredients were dissolved in the milk. The homogenates were then pasteurized at 90°C for 5 min and cooled to 47°C, inoculated with 2% of *Streptococcus thermophilus* culture and starter culture (w/v), dispersed into plastic cups, ca. 200 g, and incubated at 37°C. Following incubation, all samples were kept at room temperature (21°C) for 30 min and moved to the refrigerator. The yogurt samples were stored at 4°C for 7 days and sampled after 1 and 7 days of storage.

The whole procedure of low-fat yogurt preparation is presented below in **Figure 5**.

Receiving of milk (fresh and good quality)

↓

Filtration

↓

Preparation of skim milk (0.2%)

↓

Adding inulin and skim milk powder

↓

Pasteurization at 90°C for 5 min

↓

Cooling (47°C)

↓

Inoculation (2% prepared culture)

↓

Filling in the container at rearing capacity

↓

Incubation (37°C for 6-8 hours)

↓

Cooling and storage (4°C)

**Figure 5. Process flow chart of development of low-fat yogurt using fat replacer (inulin)**

### 3.3 Physicochemical analysis of developed yogurt

The developed yogurt was analyzed for pH, titratable acidity, moisture, ash and protein. All the determinants were done in triplicate and the results were expressed as the average. The pH and titratable acidity were done on day 1 and 7.

#### 3.3.1 Determination of titratable acidity

Acidity percentage was determined as per the method as followed by Aggarwala and Sharma., (1961). Acidity percentage was determined as per (Aggarwala & Sharma., 1961) by titrating 9gm of the diluted Yogurt samples against the standard N/10 NaOH solution until the substance reached a faint pink color corresponding to the end point of the phenolphthalein which was used 2-3 drops during titration as indicator. Then it was expressed in terms of % lactic acid.

i. 9gm of the diluted curd sample was taken in a porcelain beaker.
ii. 2-3 drops of phenolphthalein was added as an indicator.
iii. Titrated it against 0.1N NaOH.
iv. End point was indicated by appearance of a faint pink color which persisted for few seconds.

**Calculation:**

$$\%\text{Titratable acidity} = \frac{\text{ml of alkali used} \times (\text{N})\text{of NaOH} \times 0.09}{\text{Total volume of the sample}} \times 100$$

#### 3.3.2 Determination of pH

The pH of the preparations was measured using a digital microprocessor pH meter (p HepÒ3, Hanna Instruments, USA). The pH meter was standardized using reference pH 4.0 and 7.0 buffer solutions.

#### 3.3.3 Determination of Moisture, Protein and Ash

The Yogurt samples were tested for proximate analysis having dry matter (DM%), moisture%, Crude Protein (CP%), and ash% using standard laboratory procedures (AOAC, 2005). Dry matter estimation was done by oven dry method.

**Determination of moisture**

Moisture percentage was determined after determination of DM (dry matter). The enamel disc or crucible was dried in an oven regulated at 105°C which was cooled in a desiccator and weighted. 5gm of sample was weighted into the enamel disc and kept into the oven (105°C) for 24 hours. The enamel disc was removed from the oven with metal tong. After that it was cooled in desiccator and the final weight was taken after getting constant weight (AOAC, 2005).

$$\%DM = \frac{\text{Weight of Crucible with Dry Sample} - \text{Weight of Empty Crucible}}{\text{Weight of sample}} \times 100$$

% Moisture = 100 - % DM

Crude protein estimation was accomplished by Kjeldahl Method. Ash was measured by igniting the pre-ashing sample on a Muffle furnace at a temperature of 600°C for four to six hours.

**Determination of Ash**

The crucible was cleaned & dried in hot air oven. Then it was cooled in desiccator and weighted. 5 grams of sample was placed there and the sample was burned up to no smoke in heater. The crucible with sample was cooled and transferred to the muffle furnace. Then the sample was ignited at 550-600°C for 6-8 hours until white ash. The furnace was cooled at 150°C & the sample was transferred to desiccators and weighted (AOAC, 2005).

$$\%Ash = \frac{\text{Weight of Crucible and Ash} - \text{Weight of Crucible}}{\text{Weight of sample}} \times 100$$

**Determination of protein content**

About 0.5 gram sample was weighted and one spoonful catalyzer mixture (KOH, NaOH, and Se) was added there. 10ml Conc. $H_2SO_4$ was added and the digestion flask was placed in Kjeldahl Digestion Set. After that heat was increased gradually & continued up to clear residue (45 min-1hr). The Flask was then removed & cooled. 10ml 2% Boric Acid solution and 2 drops mixed indicator was taken in a conical flask. The conical flask was fitted in the collection arm of distillation set. 50ml distilled $H_2O$ was added in the digestion tube and fitted in the distillation flask. 40ml of 40% NaOH was added there & the distillation was continued up to 100ml of distillate. The Distillate was titrated against 0.1N HCl. Titration was continued until the color changed into pink. Then the titration volume was calculated (AOAC, 2005).

$$\text{Protein\%} = \frac{\text{Titration value} \times \text{Normality of HCl } (0.1) \times 0.014 \times 6.25}{\text{Sample weight}(0.5\text{g})} \times 100$$

### 3.4 Sensory evaluation of prepared yogurt

Sensory characters such as color and appearance, aroma, taste, body and texture, and overall acceptability of the prepared yogurt samples were evaluated by a panel judge consisting of 5 members of the Dairy Science laboratory of CVASU, using hedonic scale developed for the purpose. The sensory characters of the prepared samples were measured by the panel expert following the method as described by Shekhar et al. (2012), the test was aimed at quality appraisal of the products. The sensory evaluation of prepared yogurt was performed by the panel of judges based on "9-point hedonic scale" (1, 2, 3, 4, 5, 6, 7, 8 and 9 represent dislike extremely, dislike very much, dislike moderately, dislike slightly, neither like nor dislike, like slightly, like moderately, like very much and like extremely, respectively).

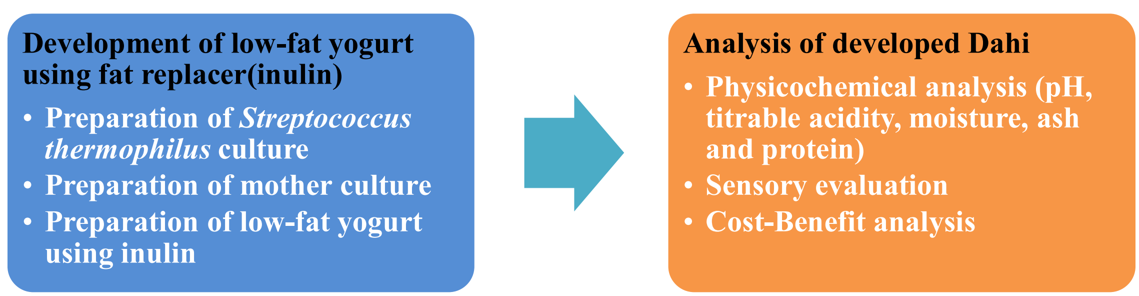



**Figure 6. Experimental work flow chart**

### 3.5 Cost-Benefit analysis:

Cost of development of low-fat yogurt was calculated considering the expense of skim milk, fat replacer (inulin) and skim milk powder etc. Skim milk cost was considered from sale price of raw fresh whole milk marketed through seller. Inulin and skim milk cost was calculated from the purchasing cost. Cost-benefit analysis is shown in **Table 10.**

**Benefit-Cost Ratio = ∑ Present Value of all the expected Benefits / ∑ Present Value of all the associate Costs.**

### 3.6 Statistical analysis

All data (proximate composition and sensory evaluation) were determined and stored in Microsoft Excel 2010 spread sheet to evaluate statistical analysis. All the collected data were subjected to statistical analyses by using STATA 13 (StataCrop IP Lakeway Drive, USA). One way ANOVA was performed to find out the significance of difference between yogurt samples ($p \leq 0.05$).


**Figure 7. Development of low-fat yogurt using fat replacer (inulin)**

(A) Culture in broth, (B) Measuring the inulin powder, (C) Inulin addition in milk, (D) Kept in Incubator, E) Developed yogurt, F) & G) Inulin Powder

A

B

C

D

**Figure 8. Analysis of yogurt samples**
(A) Determination of pH, (B) Determination of Acidity,
(C, D) Sensory evaluation by judge panel.

# Chapter-IV
# Results

The results of developed low-fat yogurt using fat replacer, obtained through the investigation are presented below through tables and graphs.

**4.1 Development of low-fat yogurt using fat replacer**

In this study, low-fat yogurt was developed using *Streptococcus thermophilus*. No artificial flavoring, coloring, thickening agents nor any chemical preservatives and stabilizer were added in any of the formulated yogurt. The development of yogurt with these cultures was successful as the preparation yielded a complete process.

The developed low-fat yogurt can be a novel or potential dietary food item or popular low fat dairy food products for obese as well as healthy people, as it has a better nutritive value with low-fat and with increased shelf life and greater acceptability, which could be introduced commercially for large scale low-fat dairy food production to meet the huge demand of the low-fat food across the globe.

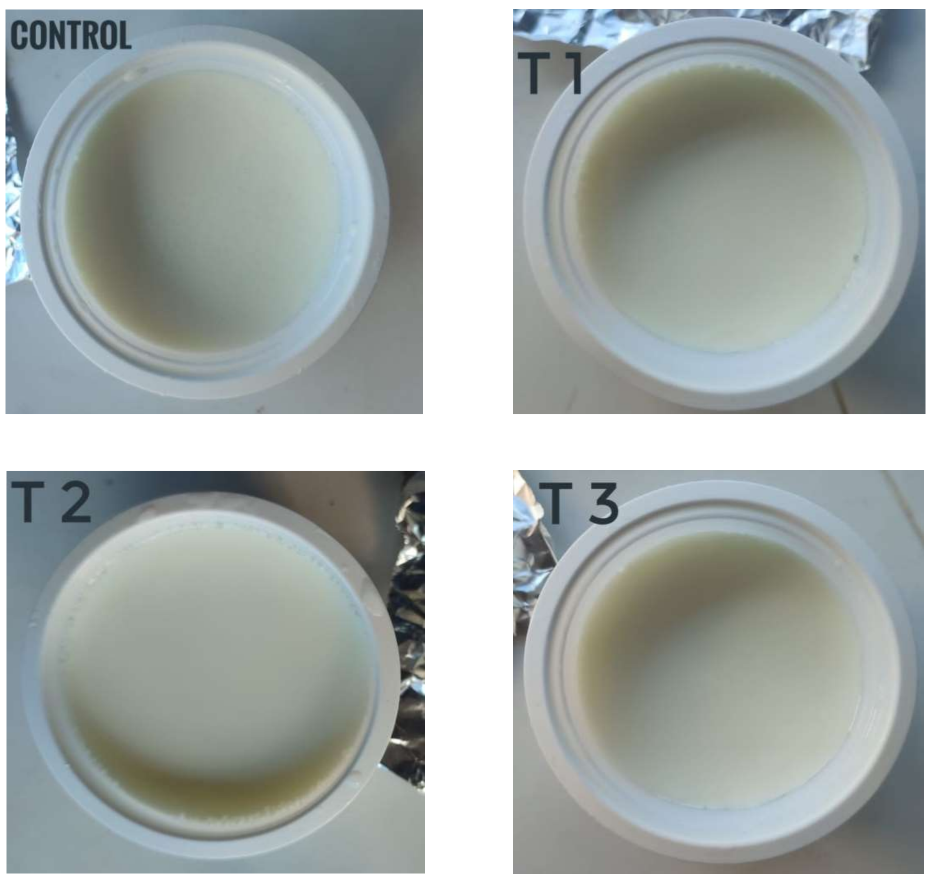



**Figure 9. Developed low-fat yogurt using fat replacer (Inulin)**

### 4.2 Physicochemical analysis

The developed yogurt samples were analyzed for titratable acidity, pH, moisture, ash and protein. All the determinants were done in triplicate and the results were expressed as the average. The pH and titratable acidity were done on day 1 and 7.

#### 4.2.1 Titratable acidity

**Figure 10** shows the mean values of acidity of the different samples on day 1 and day 7. The acidity values for Control, $T_1$, $T_2$, and $T_3$ on day 1 were 0.85, 0.85, 0.91 and 0.92 respectively, whereas the values on day 7 were 1.03, 1.07, 1.13 and 1.11, respectively. There was an increase in acidity with the progress of the storage time.

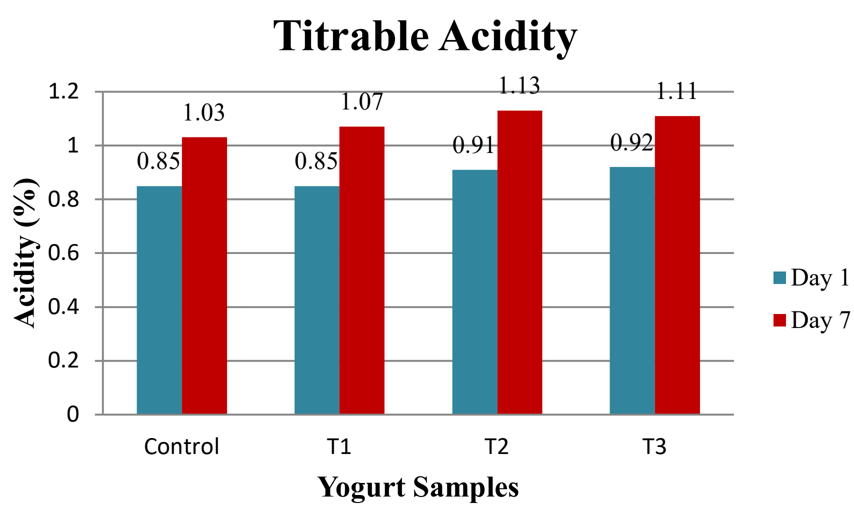



**Figure 10. Acidity (%) of the yogurt samples on day 1 and day 7**

#### 4.2.2 pH

The pH values of the samples are presented in **Figure 11**. The figure indicates that mean values of pH level on day 1 were 4.61, 4.59, 4.5 and 4.53, respectively, whereas 4.42, 4.42, 4.42 and 4.38, respectively, were observed on day 7.

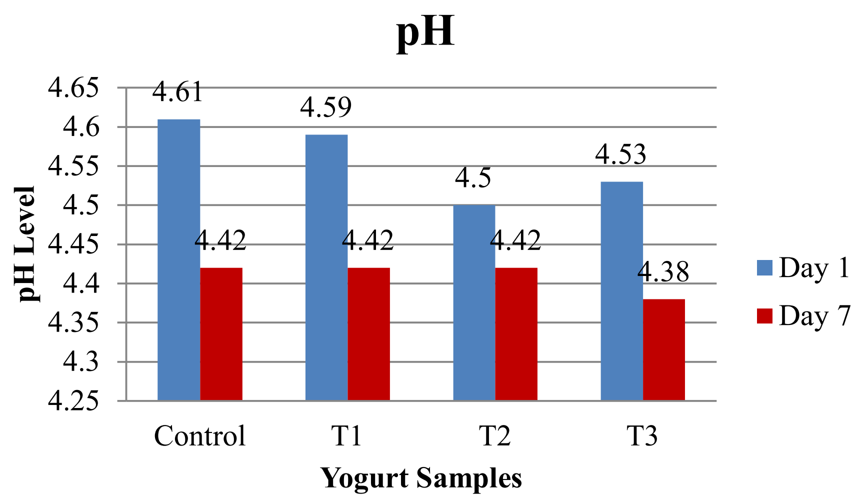



**Figure 11. pH of the Yogurt samples on day 1 and day 7**

### 4.2.3 Chemical composition

The developed yogurt samples were analyzed to determine moisture, ash and protein content. The chemical composition was shown in **Table 8**.

**Table 7. Chemical composition (Moisture, Protein and Ash) of developed low-fat yogurt**

| Yogurt Samples | Moisture | Ash% (Fresh Basis) | Protein% (Fresh Basis) |
|---|---|---|---|
| **Control** | 86.63 | 0.83 | 3.42 |
| **$T_1$** | 86.63 | 1.02 | 4.49 |
| **$T_2$** | 86.74 | 0.95 | 3.94 |
| **$T_3$** | 86.58 | 0.87 | 3.95 |
| ***p*** | 0.002 | <0.001 | <0.001 |

### 4.3 Sensory evaluation of developed yogurt

The sensory evaluation scores of developed yogurt samples are presented in **Table 9**. The maximum average overall acceptability score (8.1) was observed in control samples followed by $T_3$ (7.5), $T_1$ (7.3) while minimum score of (7.1) was obtained by treatments $T_2$. The highest sensory scores were recorded in the control samples.

**Table 8. Sensory evaluation scores of developed low-fat yogurt**

| Yogurt Samples | Color & appearance | Aroma | Taste | Body and Texture | Overall acceptability |
|---|---|---|---|---|---|
| **Control** | 8.2 | 8.4 | 7.6 | 8 | 8.1 |
| **$T_1$** | 7.8 | 7 | 7.2 | 7.2 | 7.3 |
| **$T_2$** | 7.8 | 7 | 7 | 6.6 | 7.1 |
| **$T_3$** | 7.8 | 7.2 | 7.6 | 7.4 | 7.5 |
| ***P*** | 0.4182 | 0.06 | 0.899 | 0.338 | 0.242 |

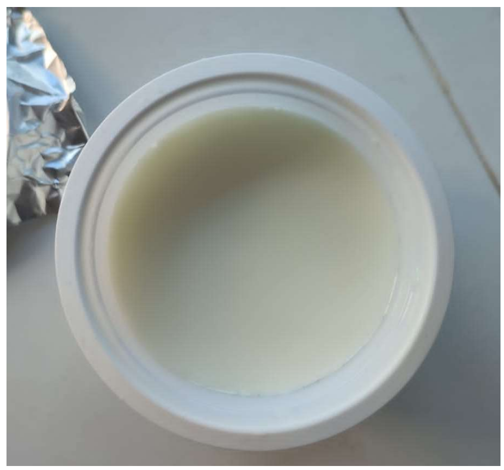


**Figure 12. Appearance of the $T_3$ sample containing 3% inulin**

### 4.4 Cost Benefit analysis

The cost benefit analysis is presented in **Table10.** Here $T_1$ Sample group shows highest benefit cost ratio (0.77) and $T_3$ sample group shows lowest benefit cost ratio (0.09).

**Table 9. Cost-Benefit Analysis of developed yogurt**

| **Cost-Benefit analysis (BDTK)** | **$T_{0=}$ 1litre whole milk + others** | **$T_{1=}$ 1litre skim milk + 1% Inulin + 4.2% skim milk powder + others** | **$T_{2=}$ 1litre skim milk + 2% Inulin + 3.2% skim milk powder + others** | **$T_{3=}$ 1litre skim milk + 3% Inulin + 2.2% skim milk powder + others** |
|---|---|---|---|---|
| Total cost per kg of Yogurt production | (80+10) = 90/= | (65+62+37.8+5) = 169.8/= | (65+124+28.8+5) = 222.8/= | (65+186+19.8+5) = 275.8/= |
| Price of Yogurt (Aarong Dairy)/ Low-fat Yogurt (Yoplait Original) in Market | 170/= (Aarong Dairy) | 300/= (Yoplait Original) | 300/= (Yoplait Original) | 300/= (Yoplait Original) |
| Benefit | 80 | 130.2/= | 77.2/= | 24.2/= |
| Benefit cost ratio(rounded) | 0.88 | 0.77 | 0.37 | 0.09 |

# Chapter-V
# Discussions

## 5.1 Development of low-fat yogurt using fat replacer (Inulin)

In this study, heat treated skim milk (0.2% fat) was used to make our yogurt, which contains inulin, skim milk powder and 2% culture. The most commonly used heat treatment in the yogurt industry include 85°C for 30 minutes or 90-95ºC for 5 minutes (Tamime and Robinson, 1999), whereas we used traditional boiling method for preparing milk to make yogurt. The sensory and morphological quality of yogurt can be affected by heat treatment (Shekhar et al., 2012). However, among the other heat treatment, boiling treatment of milk resulted in least syneresis of whey in the yogurt (Shekhar et al., 2012). In their study, yogurt prepared from boiled milk, showed the highest value in cases of firmness, consistency and index of viscosity. High heat treatment of milk increases gel firmness and reduces syneresis in the final product (Lucey et al., 1998; Vasbinder et al., 2004). The acid production rate in mixed culture is greater than the rate of acid production using single strain (Tamime and Deeth, 1980). In this study, sugar, artificial flavoring, coloring, thickening agents, chemical preservatives and stabilizer were not used to prepare our Yogurt.

## 5.2 Physicochemical analysis

### 5.2.1 Acidity

Replacement of milk fat with inulin significantly increased the titratable acidity (**Figure 10**). These results might be due to the prebiotic effect of inulin, which enhances the growth and ability of lactic acid bacteria to grow, to hydrolysis lactose (Gibson and Roberfroid, 1995; Kebary and Hussein, 1999; Kebary et al., 2004)

We found that the addition of 1%, 2% and 3% inulin to yogurt was slightly affected by its acidity during storage. These findings are not in agreement with Guven et al. (2005), who reported that the use of inulin did not significantly affect the acidity of yogurt.

In the present study, titratable acidity of all sample is in concurrence with the outcomes announced by Vijayendra and Gupta (2012). Also, Aryana and Olson (2017) reported that the most desirable yogurt resulted in a titratable acidity of 0.74 to 0.83%, when placing into cold storage and acidity of 0.91 to 0.93% during cold storage. We know that bacteria produce lactic acid from lactose during fermentation, which could result in enhancing the shelf life and microbial safety, improve texture and contribute to the pleasant sensory profile of the yogurt (Leroy and De Vuyst, 2004).

### 5.2.2 pH

I found that the addition of 1%, 2% and 3% inulin to yogurt was slightly affected by its pH during storage. These findings are not in agreement with those of Guven et al. (2005), who reported that the use of inulin did not significantly affect the pH of yogurt. It is clear from the data that the pH of Yogurt varied from one to another samples. The reason behind this might be the different time of determining pH, as the pH of the samples was determined in different period *i.e.,* day 1 and day 7, respectively. The increased pH might be due to higher alkalinity of the sample, and decreased pH indicates higher acidity. Samples become more acidic if the value of pH goes down than 7, whereas the more alkaline nature of the samples are considered when the value of pH goes above the level of 7 in any solution. The increased action of acid producing bacteria might contribute higher acidity, whereas low acidity could be due to lower acid production by the bacteria. The pH values of different yogurt samples were decreased with time.

### 5.2.3 Chemical composition (Moisture, Protein and Ash) of developed yogurt

The chemical composition of the test samples is shown in **Table 7**. The chemical composition of different samples of our developed Yogurt varied significantly as is observed from the data found in this study. Significant variation was observed in the values of moisture, protein and ash contents of the different samples of developed Yogurt. Total protein and ash values increased with the addition of skim milk powder (SMP). It is obvious that the ash and protein% of $T_1$ sample were improved significantly compared to other samples. Low moisture content and ingredient composition might be reasons for enhanced nutrient contents of the $T_1$ Yogurt. These findings are in agreement with Akin et al. (2005), who reported that the use of inulin is affect the moisture, protein and ash content of yogurt.

### 5.3 Sensory evaluation of developed Yogurt

There was no significant difference in the sensory (color, aroma, taste, body & texture) scores of the yogurt samples during the storage and between treatments.

These findings are in agreement with those of Mazloomi et al. (2011), who also reported that the use of inulin did not significantly affect the sensory characteristics of yogurt.

In our experimental yogurt, various sensorial characters such as color and appearance, taste, aroma, body and texture, overall acceptability etc. were evaluated by the panel expert following the method as described by Shekhar et al. (2012). In the sensory evaluation, control group shows highest color and appearance, taste, aroma, body and texture and overall acceptability than the developed samples. Among the three developed sample group, $T_3$ samples shows better aroma, taste, body and texture and overall acceptability than the other two groups. Higher inulin content (3%) of $T_3$ might increase palatability, aroma or flavor nature of the yogurt, and which in turn, could enhance the consumer acceptability towards the products. Aryana and McGrew (2007) reported that yogurt with 1.5% inulin had better body and texture compared to a control yogurt.

The color & appearance of the different developed samples were not influenced by the treatments. The low-fat yogurt samples developed using 3% fat replacer (inulin) is more preferred by the panelists.

### 5.4 Cost Benefit analysis

We have calculated benefit-cost ratio for sample group and treatment group. In control group we have found 0.88 benefit-cost ratio which was satisfactory. In treatment group of the present study, 1% inulin containing yogurt showed more benefit cost ratio than other two sample groups. Yogurt containing 3% inulin showed less benefit cost ratio. It was because of price of small quantity inulin and skim milk powder is comparatively high than large scale procurement. It would be more cost effective if inulin and skim milk powder bought in large scale. On the other hand, if milk was bought in large amount and large amount of skim milk could process by cream separator then average price of skim milk would be cheaper.

# Chapter-VI
# Conclusion

It is clear from the results that the developed low-fat yogurt using *Streptococcus thermophilus* and fat replacer can be prepared successfully. Inulin was added to milk containing 0.2% of milk fat to give inulin levels of 1, 2 and 3%. The experimental yogurts were compared with control yogurt produced from whole milk. The total solids content of milk was standardized by adding skim milk powder to the experimental yogurt. The chemical composition, pH, titratable acidity contents were determined in the experimental yogurts after 1 and 7 days. In proximate analysis, 1% inulin added yogurt showed highest value of ash (1.02%) and protein (4.49%) in fresh basis than control and rest of the two samples. Sensory properties of the yogurts were evaluated during storage. pH and titratable acidity were not influenced by addition of inulin. With respect to the organoleptic quality of yogurt, inulin addition caused a slight decrease in organoleptic scores: the control yogurt had the highest score, and the lowest score was obtained in yogurt samples containing 1% of inulin comparatively. The yogurt containing 3% of inulin was more or less similar in quality characteristics to control yogurt made with whole milk. With respect to cost-benefit ratio yogurt containing 1% inulin showed more benefit cost ratio than yogurt containing 2% and 3% inulin sample groups. Overall, if we consider benefit-cost ratio and better ash% and protein% yogurt containing 1% inulin showed comparatively better than yogurt containing 2% and 3% inulin, on the other hand if we consider better organoleptic taste - yogurt containing 3% inulin showed better organoleptic scores than yogurt containing 1% and 2% inulin sample groups.

# Chapter-VII

# Limitations and Recommendations

**Limitations:**

1. Research fund was inadequate.
2. Lab facility should be more improved.
3. Specific minerals present in the ash content were not determined for insufficient fund and facility.
4. Due to pandemic research work could not start timely.

**Suggestions for future research work:**

From the present study, the following suggestions can be made for future work-

1. Storage study should be extended to evaluate the shelf life of the product for more days.
2. Determination of whey separation, consistency, acetaldehyde and volatile fatty acidity contents should be included for better analysis of low-fat yogurt.
3. Different levels of skim milk could be used for evaluate best level of fat% in low-fat yogurt.

## References


Aggarwala, A. C., & Sharma, R. M. (1961). A laboratory manual of milk inspection. *A laboratory manual of milk inspection.*, (4th edition).

Akın, N. (2006). *Modern yoğurt bilimi ve teknolojisi*. Selçuk Üniversitesi Ziraat Fakültesi Gıda Mühendisliği Bölümü, ISBN:975-00594-0-9. 456 , Damla Ofset, Konya.

Akin, S., Özer, D., & Özer, B. (2005). Effect of Inulin and Lactulose on Survival of" Lactobacillus Acidophilus" LA-5 and" Bifidobacterium Bifidum" BB-02 in Acidophilus-Bifidus Yoghurt. *Food science and technology international= Ciencia y tecnología de alimentos internacional*, *11*(1), 19-24.

Akoh, C.C., (March 1998). Fat Replacers. Food technology, 52, (3), 47-53. Retrieved November 25, 2021.

Alhaj, O. A., Kanekanian, A. D., & Peters, A. C. (2007). Investigation on whey proteins profile of commercially available milk-based probiotics health drinks using fast protein liquid chromatography (FPLC). *British Food Journal*.

Amin, U. S., Kober, A. K. M. H., Akter, N., M. A. Hossain, M. A., & Rana, E. A. (2022). Isolation and identification of lactic acid bacteria from traditional fermented milk “Dahi” towards developing probiotic Dahi in Bangladesh. Journal of Modern Agriculture and Biotechnology (IN-PRESS).

Anukam, K. C., & Olise, N. A. (2012). Development of a Novel Probiotic Yogurt “PENTOYO” with a Fully Sequenced Lactobacillus pentosus KCA1 and its Survival during Storage at 4 oC. *Tropical Journal of Pharmaceutical Research*, *11*(6), 977-981.

AOAC. (2005). Official methods of Analysis. (17th ed.). Association of Official Analytical Chemists. Washington DC, USA.

Arunachalam, K. D. (1999). Role of bifidobacteria in nutrition, medicine and technology. *Nutrition research*, *19*(10), 1559-1597.

Aryana, K. J., & McGrew, P. (2007). Quality attributes of yogurt with Lactobacillus casei and various prebiotics. *LWT-Food Science and Technology*, *40*(10), 1808-1814.

Aryana, K. J., & Olson, D. W. (2017). A 100-Year Review: Yogurt and other cultured dairy products. *Journal of Dairy Science*, *100*(12), 9987-10013.

Aryana, K. J., Plauche, S., Rao, R. M., McGrew, P., & Shah, N. P. (2007). Fat-free plain yogurt manufactured with inulins of various chain lengths and Lactobacillus acidophilus. *Journal of Food Science*, *72*(3), M79-M84.

Coussement, P. A. (1999). Inulin and oligofructose: safe intakes and legal status. *The Journal of nutrition*, *129*(7), 1412S-1417S.

Duboc, P., & Mollet, B. (2001). Applications of exopolysaccharides in the dairy industry. *International Dairy Journal*, *11*(9), 759-768.

Foschino, R., Fiori, E., & Galli, A. (1996). Survival and residual activity of Lactobacillus Foschino acidophilus frozen cultures under different conditions. *Journal of Dairy Research*, *63*(2), 295-303.

Frøst, M. B., Dijksterhuis, G., & Martens, M. (2001). Sensory perception of fat in milk. *Food Quality and Preference*, *12*(5-7), 327-336.

Gibson, G. R., & Roberfroid, M. B. (1995). Dietary modulation of the human colonic microbiota: introducing the concept of prebiotics. *The Journal of nutrition*, *125*(6), 1401-1412.

Gilliland, S. E., & Lara, R. C. (1988). Influence of storage at freezing and subsequent refrigeration temperatures on β-galactosidase activity of Lactobacillus acidophilus. *Applied and environmental microbiology*, *54*(4), 898-902.

Guven, M., Yasar, K., Karaca, O. B., & Hayaloglu, A. A. (2005). The effect of inulin as a fat replacer on the quality of set-type low-fat yogurt manufacture. *International journal of dairy Technology*, *58*(3), 180-184.

Haque, Z. U., & Ji, T. (2003). Cheddar whey processing and source: II. Effect on non-fat ice cream and yoghurt 1. *International journal of food science & technology*, *38*(4), 463-473.

Huyghebaert A, Dewettinck K and de Greyt W (1996) Fat replacers. In Fat Replacers-Ripening and Quality of Cheese. IDF Bulletin 317: 1996, pp 10–15. Brussels: International Dairy Federation.

Katz, F. (2001). Active cultures add function to yogurt and other foods: Yogurt reinvents itself. *Food technology (Chicago)*, *55*(3), 46-49.

Kebary, K. M. K., & Hussein, A. S. (1999). Improving viability of bifidobacteria by microentrapment and their effect on some pathogenic bacteria in stirred yoghurt. *Acta alimentaria*, *28*(2), 110-132.

Kebary, K. M. K., Hussein, S. A., & Badawi, R. M. (2004). Impact of fortification of cow's milk with a modified starch on yoghurt quality. *Egyptian Journal of Dairy Science*, *32*, 111-124.

Kiessling, G., Schneider, J., & Jahreis, G. (2002). Long-term consumption of fermented dairy products over 6 months increases HDL cholesterol. *European journal of clinical nutrition*, *56*(9), 843-849.

Kim, Y., Faqih, M. N., & Wang, S. S. (2001). Factors affecting gel formation of inulin. *Carbohydrate polymers*, *46*(2), 135-145.

Kip, P., Meyer, D., & Jellema, R. H. (2006). Inulins improve sensoric and textural properties of low-fat yoghurts. *International Dairy Journal*, *16*(9), 1098-1103.

Kneifel, W., Jaros, D., & Erhard, F. (1993). Microflora and acidification properties of yogurt and yogurt-related products fermented with commercially available starter cultures. *International Journal of Food Microbiology*, *18*(3), 179-189.

Krasaekoopt, W., Bhandari, B., & Deeth, H. (2005). Comparison of gelation profile of yoghurts during fermentation measured by RVA and ultrasonic spectroscopy. *International Journal of Food Properties*, *8*(2), 193-198.

Kruse, H. P., Kleessen, B., & Blaut, M. (1999). Effects of inulin on faecal bifidobacteria in human subjects. *British Journal of Nutrition*, *82*(5), 375-382.

Kucukoner, E., & Haque, Z. U. (2003). Liyofilize edilmis protein kaynakli yag ikame maddelerinin Edam peynirinin tekstur ve olgunlasmasina etkisi. *Gıda*, *28*, 227-233.

Leroy, F., & De Vuyst, L. (2004). Lactic acid bacteria as functional starter cultures for the food fermentation industry. *Trends in Food Science & Technology*, *15*(2), 67-78.

Lucey, J. A., Tamehana, M., Singh, H., & Munro, P. A. (1998). Effect of interactions between denatured whey proteins and casein micelles on the formation and rheological properties of acid skim milk gels. *Journal of Dairy Research*, *65*(4), 555-567.

Mazloomi, S. M., Shekarforoush, S. S., Ebrahimnejad, H., & Sajedianfard, J. (2011). Effect of adding inulin on microbial and physicochemical properties of low fat probiotic yogurt. *Iranian Journal of Veterinary Research*, *12*(2), 93-98.

Mckinley, M. C. (2005). The nutrition and health benefits of yoghurt. *International journal of dairy technology*, *58*(1), 1-12.

Murphy, O. (2001). Non-polyol low-digestible carbohydrates: food applications and functional benefits. *British Journal of Nutrition*, *85*(S1), S47-S53.

Niness, K. R. (1999). Inulin and oligofructose: what are they?. *The Journal of nutrition*, *129*(7), 1402S-1406S.

O'Brien, C. M., Mueller, A., Scannell, A. G. M., & Arendt, E. K. (2003). Evaluation of the effects of fat replacers on the quality of wheat bread. *Journal of Food Engineering*, *56*(2-3), 265-267.

Ohr L M (2004) Nutraceuticals and functional foods. Food Technology 58 71–75. Retrieved November 25, 2021, Web:https://www.ift.org/news-and-publications/food-technology-magazine/issues/2003/january/columns/products-and-technologies_nutraceuticals-and-functional-foods

Pereira, D. I., & Gibson, G. R. (2002). Effect of consumption of probiotics and prebiotics on serum lipid levels in humans. *Critical Reviews in Biochemistry and Molecular Biology*, *37*(4), 259-281.

Roberfroid, M. B. (2007). Inulin-type fructans: functional food ingredients. *The Journal of nutrition*, *137*(11), 2493S-2502S.

Romeih, E. A., Michaelidou, A., Biliaderis, C. G., & Zerfiridis, G. K. (2002). Low-fat white-brined cheese made from bovine milk and two commercial fat mimetics: chemical, physical and sensory attributes. *International Dairy Journal*, *12*(6), 525-540.

Rowland, I. R., Rumney, C. J., Coutts, J. T., & Lievense, L. C. (1998). Effect of Bifidobacterium longum and inulin on gut bacterial metabolism and carcinogen-induced aberrant crypt foci in rats. *Carcinogenesis*, *19*(2), 281-285.

Sandrou, D. K., & Arvanitoyannis, I. S. (2000). Low-fat/calorie foods: current state and perspectives. *Critical reviews in food science and nutrition*, *40*(5), 427-447.

Shah, N. (2003). Yogurt| the product and its manufacture.

Shah, N. P. (2000). Probiotic bacteria: selective enumeration and survival in dairy foods. *Journal of dairy science*, *83*(4), 894-907.

Shekhar, S., Joe, J., Kumar, R., Jyothis, J., Kumar, K., Priya, Y. A. R. M., & Pagote, C. N. (2012). Effect of heat treatment of milk on the sensory and rheological quality of dahi prepared from cow milk. *J Food Dairy Technol*, *1*(1), 9-15.

Siraj, N., Shabbir, M. A., Ahmad, T., Sajjad, A., Khan, M. R., Khan, M. I., & Butt, M. S. (2015). Organogelators as a saturated fat replacer for structuring edible oils. *International Journal of Food Properties*, *18*(9), 1973-1989.

Sodini, I., Montella, J., & Tong, P. S. (2005). Physical properties of yogurt fortified with various commercial whey protein concentrates. *Journal of the Science of Food and Agriculture*, *85*(5), 853-859.

Staffolo, M. D., Bertola, N., & Martino, M. (2004). Influence of dietary fiber addition on sensory and rheological properties of yogurt. *International Dairy Journal*, *14*(3), 263-268.

St-Onge, M. P., Farnworth, E. R., & Jones, P. J. (2000). Consumption of fermented and nonfermented dairy products: effects on cholesterol concentrations and metabolism. *The American journal of clinical nutrition*, *71*(3), 674-681.

Surono, I. S., & Hosono, A. (2011). Fermented milks, Types and standards of identity.

Tamime, A. Y., & Deeth, H. C. (1980). Yogurt: technology and biochemistry. *Journal of food protection*, *43*(12), 939-977.

Tamime, A. Y., & Robinson, R. K. (1999). Yoghurt Science and Technology, 2nd (ed) CRC Press. *Boca Raton FL New York*.

Tekinsen, O. C. (2000). Sut urunleri teknolojisi. *Selcuk Universitesi Basimevi. Konya, Turkey*, *1*, 86.

Varnam, A.H., & Sutherland, J.P. (1994). Milk and Milk Products: Technology Chemistry and Microbiology.

Vasbinder, A. J., van de Velde, F., & de Kruif, C. G. (2004). Gelation of casein-whey protein mixtures. *Journal of Dairy Science*, *87*(5), 1167-1176.

Vijayendra, S. V. N., & Gupta, R. C. (2012). Assessment of probiotic and sensory properties of dahi and yoghurt prepared using bulk freeze-dried cultures in buffalo milk. *Annals of microbiology*, *62*(3), 939-947.

Xu, Z. M., Emmanouelidou, D. G., Raphaelides, S. N., & Antoniou, K. D. (2008). Effects of heating temperature and fat content on the structure development of set yogurt. *Journal of Food Engineering*, *85*(4), 590-597.

Zimeri, J. E., & Kokini, J. L. (2003). Rheological properties of inulin–waxy maize starch systems. *Carbohydrate polymers*, *52*(1), 67-85.

# Appendices

## Score card for sensory evaluation of Yogurt

Name of the evaluator: Date:

- Please evaluate the following samples using the 9-point hedonic scale.
- Write the preferred score in the columns as per evaluation.

| Sensory attributes | Sample number | | | |
|---|---|---|---|---|
| | A | B | C | D |
| Colour and appearance | | | | |
| Aroma | | | | |
| Taste | | | | |
| Body and texture | | | | |
| Overall acceptability | | | | |

| Hedonic rating | Score |
|---|---|
| Like extremely | 9 |
| Like very much | 8 |
| Like moderately | 7 |
| Like slightly | 6 |
| Neither like or dislike | 5 |
| Dislike slightly | 4 |
| Dislike moderately | 3 |
| Dislike very much | 2 |
| Dislike extremely | 1 |

**Suggestions and comments:** **Signature of the evaluator**

**Score card for sensory evaluation of Dahi**

Name of the evaluator: Prof. Goutam Kumar Debnath Date: 09/12/21

- Please evaluate the following samples using the 9-point hedonic scale.
- Write the preferred score in the columns as per evaluation.

| Sensory attributes | Sample number | | | | |
|---|---|---|---|---|---|
| | A | B | C | D | E |
| Color and appearance | 8 | 7 | 7 | 7 | |
| Aroma | 8 | 5 | 6 | 7 | |
| Taste | 8 | 4 | 4 | 6 | |
| Body and texture | 8 | 6 | 6 | 7 | |
| Overall acceptability | | | | | |

| Hedonic rating | Score |
|---|---|
| Like extremely | 9 |
| Like very much | 8 |
| Like moderately | 7 |
| Like slightly | 6 |
| Neither Like or dislike | 5 |
| Dislike slightly | 4 |
| Dislike moderately | 3 |
| Dislike very much | 2 |
| Dislike extremely | 1 |

**Suggestions and comments:**

Goutam

**Signature of the evaluator**

**Chemical composition (Moisture, Protein and Ash) of developed Yogurt**

| Treatment | DM (%) | Moisture (%) | Ash% (DM Basis) | Ash% (Fresh Basis) | Protein% (DM Basis) | Protein% (Fresh Basis) |
|---|---|---|---|---|---|---|
| $T_0$ | 13.38 | 86.62 | 6.22 | 0.832236 | 25.6 | 3.42528 |
| $T_0$ | 13.36 | 86.64 | 6.21 | 0.829656 | 25.65 | 3.42684 |
| $T_0$ | 13.37 | 86.63 | 6.24 | 0.834288 | 25.45 | 3.402665 |
| $T_1$ | 13.38 | 86.62 | 7.6 | 1.01688 | 33.77 | 4.518426 |
| $T_1$ | 13.35 | 86.65 | 7.65 | 1.021275 | 33.38 | 4.45623 |
| $T_1$ | 13.37 | 86.63 | 7.7 | 1.02949 | 33.65 | 4.499005 |
| $T_2$ | 13.2 | 86.8 | 7.12 | 0.93984 | 29.7 | 3.9204 |
| $T_2$ | 13.32 | 86.68 | 7.16 | 0.953712 | 29.8 | 3.96936 |
| $T_2$ | 13.26 | 86.74 | 7.14 | 0.946764 | 29.6 | 3.92496 |
| $T_3$ | 13.41 | 86.59 | 6.46 | 0.866286 | 29.4 | 3.94254 |
| $T_3$ | 13.42 | 86.58 | 6.45 | 0.86559 | 29.45 | 3.95219 |
| $T_3$ | 13.44 | 86.56 | 6.48 | 0.870912 | 29.49 | 3.963456 |

## Brief biography of the student

The author of this paper, Jewel Chandra Bhowmik was born in Noakhali, Chattogram, Bangladesh in 1993. He is the elder son of Jagadish Ch. Majumdar and Jayanti Rani Bhowmik. He passed the Secondary School Certificate Examination from Oterhat High School in 2009 with GPA 5.00 followed by Higher Secondary Certificate Examination from Govt. Science College in 2011 with GPA 5.00. He completed his graduation degree on Doctor of Veterinary Medicine (DVM) from Chattogram Veterinary and Animal Sciences University (CVASU), Bangladesh in 2017 with CGPA 3.41. During his graduation, he received clinical training from Madras Veterinary College and Veterinary College & Research Institute, Namakkal, Tamilnadu, India and. He has great interest to work in Dairy microbiology sector. Now, he is a candidate for the degree of MS in Dairy Science, Dept. of Dairy and Poultry Science, Faculty of Veterinary Medicine, CVASU.