EFFECT OF SOME STABILIZERS ON THE QUALITY AND STORAGE STABILITY OF YOGHURT

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Effect of Some Stabilizers on the Quality and Storage Stability of Yoghurt

A dissertation submitted to the Department of Food Technology, Central Campus of Technology, Tribhuvan University, in partial fulfillment of the requirements for the degree of B. Tech. in Food Technology

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Approval Letter

This ***dissertation*** entitled ***Effect of some stabilizers on the quality and storage stability of yoghurt*** presented by **Nabin Bista** has been accepted as the partial fulfillment of the requirement for the **B. Tech. degree** in **Food Technology**

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(Nabin Bista)

Abstract

The main aim of this dissertation was to prepare yoghurt with 2% starter culture of *Lactobacillus acidophilus* and *Streptococcus thermophilus* and evaluate its sensory and physiochemical properties. Stabilizers (pectin, guar gum and carboxymethyl cellulose) were used in this study at the rate of 0.1%, 0.2% and 0.3% each. These samples were optimized based on sensory attributes i.e., appearance/color, flavor, texture/mouthfeel, and overall acceptability. Sensory data were analyzed by two-way ANOVA (no blocking) using GenStat and means were compared using LSD at 5% level of significance. Again, yoghurt with optimized stabilizers were compared based on sensory attributes i.e., appearance/color, flavor, texture/mouthfeel, and overall acceptability. Sensory data were analyzed by two-way ANOVA (no blocking) using GenStat and means were compared using LSD at 5% level of significance.

From sensory evaluation, Sample coded as G (0.1% CMC) was found to be significantly ($p<0.05$) superior in sensory quality. The moisture, protein, fat, ash, acidity, pH, and lactose of this formulation were found to be 83%, 3.13%, 2.57%, 0.84%, 0.72%, 4.5 and 3.77% respectively. The shelf life of this product was estimated in terms of pH, syneresis and total plate count, and the shelf life for the best sample was found to be 11 days at refrigerated temperature (5°C).

Contents

List of Tables

List of Figures

List of Abbreviations

Abbreviation	Full form
SMP	Skim milk powder
NFDM	Non-fat dry milk
CMC	Carboxymethyl cellulose
LBG	Locust bean gum
MSNF	Milk solid not fat
GG	Guar gum
LAB	Lactic acid bacteria
NADH	Nicotinamide adenine dinucleotide
ATP	Adenosine triphosphate
EPS	Exopolysaccharide
UHT	Ultra-high temperature
HTST	High temperature short time
ANOVA	Analysis of variance
LSD	Least significant difference

Part I

Introduction

1.1 General Introduction

Fermented milk products, such as yoghurt, have been consumed for thousands of years, and the concept that they are beneficial to health is likely just as old. However, scientific backing for these views has only just begun to emerge. Fermented milk products are high in protein, vitamins, and minerals, just like the milk from which they are formed. However, in addition to these primarily nutritional features, there is growing support for a variety of other health benefits, as well as helping to preserve milk with a longer shelf life (Buttriss, 1997). Microorganisms used as starters in the manufacturing of cultured dairy foods are classified into two categories based on their optimal temperature ranges. Lactic acid bacteria kept at temperatures over 35°C are known as thermophilic bacteria, whereas those incubated at temperatures between 20 and 30°C are known as mesophilic starters, and they work in symbiosis with one another (Chandan *et al.*, 2008).

Yoghurt is an acidified coagulated dairy product produced by the controlled fermentation of milk by thermophilic lactic acid bacteria such as *Lactobacillus bulgaricus* and *Streptococcus thermophilus*. These organisms are employed as yoghurt cultures to generate a distinctive mild clean lactic flavor and typical aroma (Alakali *et al.*, 2008). It has a creamy, friable custard-like consistency with a clear and noticeable acid flavor and often comprises 12-14% total milk solids. Yoghurt is often made by pasteurizing the mixture and modifying the milk proteins to create the right viscosity and gelation with the least amount of syneresis in the product (Morr, 1985). Milk, starter culture, cream, SMP, sugar, stabilizers, and other components are commonly used in the making of yoghurt. Milk is standardized to produce the appropriate sort of yoghurt, such as whole milk for full fat yoghurt, low fat milk for low fat yoghurt, and skim milk for skimmed yoghurt. A good strain of starting culture not only impacts the flavor and aroma, but it can also speed up the process, lowering yoghurt production expenses. The starter culture's role is to ferment lactose (milk sugar) to produce lactic acid. Lactic acid increases pH, causing milk to coagulate or form the soft gel that is characteristic of yoghurt. Cream is used to increase or decrease the fat content. The solids content is adjusted using skimmed milk powder (SMP). Stabilizers can also improve the body and texture of yoghurt by enhancing firmness, avoiding whey separation (syneresis),

and assisting in keeping the fruit evenly distributed in the yoghurt. Alginates (carrageenan), gelatins, gums (locust bean, guar), pectin, and starch are some of the stabilizers used in yoghurt (Bhattarai *et al.*, 2015).

The use of stabilizers can improve the quality of yoghurt. Stabilizers can be obtained from a variety of sources. Some are created artificially (synthetically), such as carboxyl methyl cellulose; many are derived from plants, the cheapest and most extensively used being maize starch; and a few, such as gelatin, are derived from animals. Sodium carboxyl methyl cellulose, sometimes known as CMC or cellulose gum, is a synthetic water-soluble cellulose ether (Alakali *et al.*, 2008). Guar gum, xanthan gum, and locust bean gum (LBG) are all utilized as thickeners in the food business. They improve the texture by raising the viscosity of the continuous phase and decreasing syneresis. Carrageenan comes in a variety of forms and is utilized as a gelling agent. In the presence of calcium, κ -carrageenan forms a stiff and brittle gel, whereas ι -carrageenan forms a soft gel, and λ -carrageenan does not form a gel but functions as a thickening (Emine and Ihsan, 2017). CMC stabilizes protein dispersions, particularly at their isoelectric point of pH value. As a result, milk and dairy products are more resistant to casein precipitation. HM pectin is employed as protein stabilizers to prevent agglomeration and sedimentation, as well as to reduce or prevent whey separation at low pH (for example, in stirred yoghurts and fruit milk drinks). LM pectin are typically employed as gelling agents to produce texture and prevent syneresis (for example, in set and stirred yoghurt) (Swelam, 2012).

1.2 Statement of the problem

Milk is a major component of the traditional diet in many regions of Asia. In these communities, most of the milk produced is consumed in the home and is rarely sold. However, high temperatures and lack of refrigeration facilities have led to the inability to process and store fresh milk. Hence, conversion of any surplus liquid milk to relatively shelf stable products such as yoghurt, cheese, acidified milk, butter, and ghee has traditionally been done (Temesgen *et al.*, 2015).

According to Guarner *et al.* (2005) yoghurt bacteria are considered as “probiotics”. Live lactic acid bacteria in yoghurt have health-promoting properties such as protection against gastrointestinal upsets, improved lactose digestion by maldigests, a lower risk of cancer, lower blood cholesterol, an improved immune response, and the ability to help the body

assimilate protein, calcium, and iron (Perdigon *et al.*, 1998; Van de Water and Naiyanetr, 2003).

The homogenization process, heat treatment, and yoghurt processing conditions all influence the viscosity of the yoghurt, whereas syneresis is usually caused by several factors such as high incubation temperature, low solid contents in the milk, an excessive whey protein to casein ratio, and physical mishandling of the product during processing, storage, and transportation. The two major issues with yoghurt are changes in viscosity and whey protein leakage (syneresis), both of which have a negative impact on yoghurt quality. To overcome these flaws and improve product functionality, the most typical technique is to utilize different stabilizers, which are substances added to food items to smoothen and provide a consistent structure. Additionally, stabilizers aid in keeping flavoring ingredients distributed, resulting in the preservation of yoghurt viscosity. Stabilizers also form tight networks with casein molecules, which reduces syneresis and improves yoghurt texture (Rafiq *et al.*, 2018).

1.3 Objectives

1.3.1 General Objectives

The general objective of the study was to study the effect of some stabilizers on the quality and storage stability of yoghurt.

1.3.2 Specific Objectives

The specific objectives of the study were to:

1. To analyze the proximate composition of milk (% fat, SNF, acidity, protein, pH).
2. To optimize the concentration of stabilizer required for yoghurt.
3. To find a stabilizer suitable for lowering syneresis in yoghurt.
4. To study the shelf-life of yoghurt through chemical analysis.
5. To evaluate the cost of yoghurt.

1.4 Significance of the study

Yoghurt is a lactic-acid fermented product with an acidic, pungent flavor. Yoghurt was first made to preserve milk. Yoghurt has a longer shelf life than milk. Drinking yoghurt, dietetic yoghurt, shrikhand, and other yoghurt-based products are available on the global market. Yoghurt consumption is increasing as the world's population grows; hence yoghurt production should expand as well. Based on the hygiene requirements maintained during the manufacturing of yoghurt, as well as the microbiological quality of the components and packaging materials, the shelf life of the yoghurt is approximately three weeks when refrigerated (Tamime and Robinson, 1999). Yoghurt also contains a considerable number of high-quality proteins, traces of mono- and disaccharides, and significant amounts of minerals such as sodium, potassium, calcium, and magnesium. Yoghurt also has various medicinal effects, such as improving digestion and immunological function and lowering serum cholesterol levels (Crittenden *et al.*, 2003).

Normal yoghurt is a perishable food product with a short shelf life even when refrigerated. The main impediment to large-scale yoghurt production is its ability to maintain quality at both room and chilled temperatures. The bacteria in yoghurt not only enhance the acidity during storage, but they also increase the acidity at cooling temperatures. The only technology used by most dairies to enhance shelf-life is chilling. If low temperature is the only preservation barrier, the frequent lack of electricity in places such as Nepal may restrict the shelf-life due to the growth of culture bacteria and other contaminating microorganisms. The product may deteriorate during distribution for a variety of reasons (Bhattarai *et al.*, 2015).

Global demand for various types of yoghurts has increased due to greater concern about product quality and consumer satisfaction. The mouthfeel, flavor, and texture of yoghurt are all key characteristics of its quality. Stabilizers increase viscosity, alter texture, creaminess, and mouth feel, and aid in the prevention of whey separation from yoghurt (Alakali *et al.*, 2008). Stabilizers not only extend the shelf-life of yoghurt in Nepal, but also keep the food from deteriorating in the event of an electrical outage.

1.5 Limitations of the study

1. Only three stabilizers (Pectin, guar gum and CMC) were used for the study.
2. Only the product with the best concentration of stabilizer was used for the analysis of its composition and shelf-life.
3. Blending of different stabilizers was not carried out.

Part II

Literature review

2.1 Historical Background

Milk fermentation is one of the earliest methods used by humans to preserve milk with a long shelf life. The actual origin of milk fermentation is unknown; nonetheless, it appears to date back to the birth of civilization. Early civilizations such as the Samarians, Babylonians, Pharaohs, and Indians were said to have advanced agricultural and animal husbandry skills (Tamime and Robinson, 1999).This can be supported by the findings of Copley *et al.* (2003) , who discovered dairy fat remnants in ceramic shards from Neolithic Bronze-age and Iron-age towns, implying that dairying existed in Britain roughly 6500 years ago. However, it seems doubtful that milk fermenting was done during this period. As a result, the origin of fermented milk products such as yoghurt remains unknown. It has been reported that Anatolian goatherds preserve their milk by thickening it before drying it in the sun and transporting it in animal bellies (Adhikari, 2018).

Humans have traditionally employed fermentation to preserve milk. It is believed to have originated in the Middle East before the Phoenician era. Traditional fermented milks such as laban rayeb and laban khad have been consumed in Egypt from around 7000BC. Dadhi (modern-day yoghurt) is mentioned in the Vedas (Indo-Aryan treatises) dating back to 5000 years BC. Dadhi or dahi is still an important part of the South Asian diet. It is manufactured in most Indian families and consumed on a regular basis. The Turks are credited with coining the term yoghurt in the eighth century, which appeared as yoghurut. As a result, it is assumed that Turkish nomads in Asia made yoghurt. According to another tradition, the Balkans were the first to prepare or invent yoghurt. Prokish, or sour milk, was made from sheep's milk by Thrace's peasantry. South Asian (India, Pakistan, Nepal, and Bangladesh) and Southwest Asian (Iran, Iraq, Balkans, Turkey, and Syria) regions are among the greatest producers and consumers of fermented milk products (including yoghurt). The invasion of Mongols, Tartars, and other Asian rulers into Russia and Europe is thought to have helped to the spread of yoghurt and fermented milk to other regions of the world (Chandan *et al.*, 2017).

Yoghurt has been consumed from the beginning of time. It is unknown how yoghurt was discovered, however it is speculated that it was by chance, possibly by Mesopotamians

around 5000 BC (Kosikowski and Mistry, 1997). During this time, herders would milk goats and sheep and transport the milk in pouches formed from the stomachs of the animals. These stomachs contained chymosin, a natural enzyme that when mixed with milk formed a gel or coagulum. Given the warm climate in this region of the world, the storage conditions at the time, and the natural starting culture in the milk, either yoghurt or cheese was produced. Fermentation most likely started within a few hours. These folks most likely noticed that this soured milk product kept longer, and they began to prefer the flavor of yoghurt over that of fresh milk. These people ultimately understood the health benefits of eating yoghurt, and many years later, some observers wrote of enjoying a longer and healthier life as a direct result of frequent intake of the fermented products (Andrews, 2000).

Yoghurt has its origins in Russia's Caucasus Mountain region. The residents of this difficult terrain were mostly nomadic, surviving on the milk and meat of cows, sheep, goats, and yaks. Kefir, a fermented milk product native to this region, is a liquid cultured product whose name translates to "good feeling." It was also known as a healing drink and was regarded as a "gift of the gods." Kefir was widely consumed by all families, and the bacteria culture used to ferment it was highly treasured and well-guarded. Kefir's widespread appeal in Russia dates to the early 1900s.The society wanted to publicize this substance because of its known health and anti-aging properties (Tribby, 2009).

Yoghurt is derived from Turkish word “Jugurt “reserved for any fermented food with acidic test. Yoghurt in different forms with appropriate local names is made throughout the world, in Botswana it is called Madila, Lesotho Amasi, Namibia Omashikwa, Omaze Uozongombe, Zambia Mabisi Sawa and in Ethiopia Ergo. Currently yoghurt of many types including kefir, Greek style yoghurt, Swiss and fruit yoghurts can be found(Temesgen *et al.*, 2015). Yoghurt comes in a variety of flavors, including kefir, Greek style yoghurt, Swiss, and fruit yoghurts. Yoghurt is a popular fermented dairy product that is consumed all over the world. Lactic acid fermentation of milk is achieved through the action of a starting culture containing Streptococcus thermophilus and Lactobacillus delbrueckii ssp. Bulgaricus (Fadela *et al.*, 2009). Yoghurt comes in a variety of forms, including stirred, set, and frozen liquid yoghurt. Because the nutritious value of milk proteins is highly conserved during the fermentation process, proteins in yoghurt and milk are of outstanding biological quality. As a result, yoghurt is advised for the sick and elderly (Ebringer *et al.*, 2008).

Yoghurt is a popular fermented milk product that has acquired considerable market acceptability as a healthy snack. It contains a high concentration of nutrients in comparison to its energy and fat content, making it a nutrient-dense diet. Yoghurt, for instance, can supply the body with considerable amounts of calcium in a bioavailable form. Furthermore, yoghurt has various health benefits in addition to providing basic nourishment, such as enhanced lactose tolerance, a probable involvement in body weight and fat loss, and a variety of health qualities connected with probiotic microorganisms (Mckinley, 2005).

2.2 Development of dairy industry in Nepal

Traditionally, dairy farming in Nepal was done on a small scale. Most milk and milk products are consumed at home. Surplus milk is turned into ghee and sold in cities. This is still practiced in most parts of the country where there is a market for fresh produce. There is no such thing as milk. This is not the case in the surrounding metropolitan areas, where farmers choose to sell fresh milk rather than produce ghee because it diminishes their profit margin (Dahal, 2009).

Nepal has a relatively short history of dairy development. It all started in Tushal (Kabhre) in 2009 B.S. With the financial aid of the New Zealand government, a sophisticated milk processing factory with a capacity of 500 liters was erected in 1956 at Lainchaur, Kathmandu. DDC (Dairy Development Corporation) was established in 1969 under the Agriculture Development Act of 2001 to undertake an effective dairy development program. Similarly, in 1974, a modern milk processing factory with a capacity of 2000 liters was created in Biratnagar, Nepal's eastern area. Hetauda's 3000-liter capacity was established in 1974. In 1977, another factory with a capacity of 5000 liters was developed in Balaju, Kathmandu. Additionally, Pokhara Milk Supply Scheme, Lumbini Milk Supply Scheme, and Kohalpur and Surkhet Milk Supply Scheme were established (Dahal, 2009).Kathmandu dairy development program is also known as the central dairy since milk is supplied to the dairy from all of Nepal's dairies. In addition, National Dairy Growth Board (NDGB) was established as a further step in the growth of dairy in Nepal. This body oversees developing policy, planning, and developing the dairy profession as a liaison between the private and public sectors (Bhattarai *et al.*, 2015).

2.3 Milk

Milk is a lacteal secretion of mammary gland of milch animals. It is made up of lipids, carbohydrates, proteins, and a variety of organic and inorganic salts that have been dissolved or dispersed in water. Lipid is mostly made up of fat, but it also contains phospholipids, sterols, fat-soluble vitamins A and D, carotene, and xanthophylls. Milk's protein content is divided into three categories: a) casein, b) lactalbumin, and c) lactoglobulin. Lactose is a carbohydrate found in milk. Milk contains a variety of salts and minerals. There are plenty of vitamins, but vitamin C is scarce. Milk contains a variety of enzymes, some of which appear to be released by the milk and others which are created by microorganisms (Wolin, 1960).

Fresh milk has a pH of 6.5 to 6.7 and an initial acidity of 0.14 to 0.16%. pH and acidity measurements are frequently used as acceptance tests and to determine the quality of milk. These tests are used to monitor activities such as cheese production and yoghurt production (S. Rafiq *et al.*, 2016). Approximately 80% of milk proteins are caseins, which include α-, β-, κ-, and ϒ caseins. Casein micelles and fat globules give milk most of its physical properties, as well as taste and flavor to dairy products. Milk processing, by definition, entails the imposition of a changing colloidal system. This is because the colloidal particles in milk change their nature and behavior. Changing the pH, for example, causes disintegration and rearrangement of the micelles, and if the pH is low enough, new particles of isoelectric casein are created. Furthermore, heating to high enough temperatures causes serum protein binding to the micelle to break down (Lucey, 2002).

2.4 Milk Fermentation and biochemical changes

The International Dairy Federation defines a fermented milk product as one made from skimmed milk or not with organisms. The microflora is kept alive until it is sold to consumers and may or may not contain pathogenic microorganisms (Gandhi, 2000). Milk fermentation is any change in the chemical or physical properties of milk or dairy products caused by the activity of microbes or their enzymes. It happens when bacteria break down milk sugars and other milk components to produce lactic acid, alcohols, carbon dioxide, and other byproducts. Milk's fermentable constituents include lactose, fat, and citric acid. Lactose, a disaccharide, is the primary carbon supply, while fat and citric acid provides hydrogen and oxygen, respectively (Davies and Law, 1984). Fermentation in milk can either add to

desirable flavor and texture in products like cheese and yoghurt, or it can result in spoilt and deteriorated products. Microbial cultures with known qualities are added to milk or dairy product substrate to assure the growth of appropriate fermentation (Yuliana and Rangga, 2010).

Lactic acid bacteria (LAB) species are classified into several genera under the Lactobacillaceae family. They are prospective microorganisms that have been frequently used in food fermentation around the world because of their well-known status as generally regarded as safe (GRAS) microorganisms. They are also recognized for their fermentative activity, which improves food safety, organoleptic qualities, nutrient enrichment, and health benefits (Widyastuti and Febrisiantosa, 2014). The milk fermentation process has relied on the activity of LAB, which plays a critical role in converting milk as a raw material to fermented milk products. As starting cultures in the milk fermentation business, numerous industrial strains of LAB are used. LAB starter cultures were isolated, selected, and confirmed through a series of activities. Several behaviors as the features of each selected strain of LAB have been established and exploited in the industrial manufacture of fermented milk products. The most essential qualities of LAB are their ability to acidify milk (Mäyrä-Mäkinen and Bigret, 1993) and provide flavor and texture by transforming milk protein due to their proteolytic activity (Griffiths and Tellez, 2013; Kongo, 2013).

Lactic acid bacteria (LAB) use lactose as their primary carbon source for growth and energy. Lactase first hydrolyzes it into galactose and glucose (Greenberg and Mahoney, 1982) , which is then converted to D- or L-lactic acid via the glycolytic process, Embden-Meyerhof-Parnas pathway (Hemme *et al.*, 1980). Lactic acid fermentation is divided into two major pathways: homolactic fermentation, which generates lactic acid, and heterolactic fermentation, which generates an equimolar amount of lactic acid, carbon dioxide, and ethanol (Vakil and Shahani, 1970). Proteolysis breaks down protein, increasing the peptide and free amino acid content in fermented milk products (Alm, 1982). LAB lipases hydrolyze lipids sparingly, preferring lower molecular weight triglycerides but not higher molecular weight triglycerides (Collins *et al.*, 2003). Despite the presence of lipases in *S. thermophilus* and *L. delbrueckii subsp. bulgaricus*, they have no effect on the free fatty acid level of fermented milk products (Fernandes *et al.*, 1991). Minerals and vitamins are required for LAB growth (as mineral catalysis and mediators in the enzymatic process respectively), but their requirements are minimal and would not appreciably alter the total amount of fermented

milk products. Some minerals' bioavailability may be altered as a result of pH changes generated by fermentation (Hayek *et al.*, 2019).

2.5 Merits of milk fermentation

The most important merits of milk fermentation are:

- Preventing milk from becoming spoiled by undesirable bacteria, which happens because of the fermentation's buildup of lactic acid and other antimicrobial metabolites.
- Variety in foods is achieved by changes in body, texture, and flavor.
- The digestibility of fermented products, particularly protein, is improved, which may be beneficial in those with digestive issues.
- In some cases, the fermentation process can lower the bulk and the initial material, which increases the goods' storage life. Examples include classic dried, transportable fermented milk-cereal mixtures that can be consumed anywhere (Vedamuthu, 1982).
- Antibiotics produced by microorganisms employed as a culture in fermented milk products have a detrimental effect on the harmful microbes present in the intestine and inhibit their growth.
- For the nutritional treatment of certain disorders like dysentery, gastritis, anemia, kidney stones, etc., several fermented milk products are helpful.
- Fermented milk, such as yoghurt, has the ability to build weight more effectively than milk feeding (Hargrove and Alford, 1978).

2.6 Fundamental microbiology of yoghurt

The two species benefit each other; *S. thermophilus* removes oxygen and produces mild acid conditions that favor *L. bulgaricus* and the lactobacillus by hydrolyzing lactose and casein. *S. thermophilus* grows best at pH 6.5, stopping at pH 4.2-4.4, whereas *L. bulgaricus* grows best at pH 5.5, stopping at pH 3.5-3.8 (Rasic and Kurmann, 1978).

L. bulgaricus produces 1.7-1.8% of the lactic acid, while *S. thermophilus* produces 0.6-0.8%. Lactic acid is highly significant in yoghurt or fermented milks (Tamime and Robinson, 1999). Lactic acid contributed to the soluble calcium phosphate fraction by interacting with the colloidal calcium phosphate complex contained in the casein micelle. This causes progressive calcium loss from the micelles and, as a result, casein coagulation at pH 4.6-4.7.

The lactic generated by thermophilic bacteria provides yoghurt with its harsh and acidic taste, improving the product's flavor. According to many researchers, *S. thermophilus* mostly creates L (+) lactic acid, whereas *L. bulgaricus* primarily produces D (-) lactic acid. As a result, most yoghurt contains 45-60% L (+) lactic acid and 40-55% D (-) lactic acid (Garvie, 1978).

Yoghurt starter cultures are mildly proteolytic, and the peptides and amino acids produced serve as precursors for the enzymatic and chemical reactions that result in taste components. Protein degradation is primarily connected with *L. bulgaricus*, however *S. thermophilus* and *L. bulgaricus* also produce peptidase enzymes (Heller, 2001).

2.7 Starter Culture

A starter culture is a product that contains a high concentration of lactic acid bacteria, which can cause milk to acidify (Gandhi, 2006). For many years, yoghurt production has benefited from the symbiotic interaction between *Streptococcus thermophilus* and *Lactobacillus bulgaricus*, the thermophilic lactic acid bacteria. *L. bulgaricus*, which is more proteolytic, hydrolyzes milk casein to create specific peptides and amino acids that stimulate *S. thermophilus*. Formic acid and carbon dioxide produced by *S. thermophilus* promote the growth of *L. bulgaricus* (Radke-Mitchell and Sandine, 1986).

The starting culture used to ferment milk to make yoghurt influences the yoghurt's characteristics. Exopolysaccharide-producing *L. bulgaricus* and *S. thermophilus* strains are occasionally utilized in yoghurt manufacturing. The polysaccharides are secreted by these ropy cultures as a capsule or slime. Exopolysaccharides, which are mostly glucose and galactose but vary depending on the culture, crosslink with the bacterial cell surface and the protein in the yoghurt matrix. Although these bonds do not inherently affect the gel's firmness, they do contribute to an increase in viscosity and other rheological qualities, which affect the overall mouthfeel of yoghurt (Schonbrun, 2002).

Modern industrial processes use "starters" in the production of modern dairy products. A starter is a safe microbe that, when cultured in milk, imparts desirable and predictable flavor and texture qualities. A single strain culture comprises a single strain of bacterial species, whereas a mixed/multi strain culture has a mixture of more than one strain or species. Starter

cultures are transferred from a central laboratory to an operating plant in many forms, such as liquid culture, frozen concentrate, lyophilized culture, and so on (Chandan, 1982).

2.8 Types of starter culture

2.8.1 Pure and mixed culture

A further classification is created into pure cultures and mixed cultures. A pure culture is made up of only one kind of lactic acid bacteria, whereas a mixed culture is made up of various types of lactic acid bacteria. Pure cultures can be made up of one or several strains from the same species. Acidification with a mixed culture is the most prevalent kind, and in rare instances on its own. Individual dairies used to cultivate DL cultures as "dairy cultures", frequently the same culture for decades (Kunz *et al.*, 1983)

2.8.2 Mesophilic and thermophilic culture

Mesophilic cultures, which include *Lactococcus* and *Leuconostoc,* thrive best at temperatures ranging from 20 to 30°C. These mesophilic lactic cultures are employed in the manufacturing of numerous cheese kinds that have the following significant characteristics:

1. Acid producing activity.
2. Gas production, and
3. Enzymatic activity for cheese ripening, such as proteases and peptidases enzymes.

Thermophilic cultures thrive best at temperatures ranging from 37 to 45°C. Thermophilic cultures are commonly used in the manufacture of yoghurt, acidophilus milk, and Swiss cheese. Streptococcus and Lactobacillus species are common in thermophilic cultures. These cultures combine with milk to generate the common yoghurt starting culture. This growth is regarded as symbiotic since the rate of acid formation is faster when two bacteria are grown together than when single strains are produced (Dave and Shah, 1997).

2.8.3 Liquid culture

Liquid cultures are no longer widely available in commercial practice. For producing a liquid culture, organisms are propagated in a suitable media, such as milk or whey, and kept active through periodic transfers. In general, a liquid culture includes roughly 10^9 organisms per milliliter of starter (Neilson and Ullum, 1989).

2.8.4 Powdered culture

Powdered cultures are produced by freeze-drying a liquid culture that has been cultured to a high bacterial concentration. Drying under vacuum is referred to as freeze drying. This is a mild approach that reduces the bacterial count throughout manufacturing. Before using ordinary freeze-dried cultures, they must be re-inoculated into a mother culture (Neilson and Ullum, 1989).

2.8.5 Frozen culture

Deep frozen cultures are produced by deep freezing a concentrated liquid culture at the phase of bacteria growth where activity is at its peak. Lyophilization is used to preserve them in tiny vials. Super-concentrated, deep-frozen cultures are obtained by adding growth factors to a milk substrate, continually neutralizing the lactic acid produced with ammonium hydroxide, and then concentrating the culture in a desludging centrifuge/bactofuge. Palletization occurs when the concentrate is frozen as individual drops in liquid nitrogen. The culture is kept at -196°C until it is shipped to the dairies in foamed plastic boxes with dry ice (Neilson and Ullum, 1989).

2.9 Preparation of starter culture

Culturing the two organisms together results in a symbiotic relationship since each organism's growth rate and acid production are larger than in a single culture. The optimal temperature for rod and coccus growth is 45°C and 40°C respectively. A 1:1 ratio is widely considered excellent. A 2% inoculum with 2.5-hour incubation at 44°C yields good yoghurt. *S. thermophilus* has an acidity range of 0.85-0.95%, but *L. bulgaricus* has an acidity range of 1.20-1.50% (Neilson and Ullum, 1989).

2.10 Metabolism characteristics of LAB in yoghurt

2.10.1 Carbohydrate metabolism and acid production

Microbial cells get their energy from a variety of sources, including the cytochrome system, which uses electrons from reduced nicotinamide adenine dinucleotide (NADH), enzymes that run the anaplerotic pathways, the tricarboxylic acid cycle, and fermentation. Lactic acid bacteria (*lactococci*, *leuconostoc, lactobacilli*, *streptococci,* and *bifidobacteria*) lack all the previous three systems and must rely solely on carbohydrate fermentation for energy (Lawrence *et al.*, 1976). Most of the energy is obtained by substrate-level phosphorylation

and cytoplasmic membrane adenosine triphosphate enzymes (ATPases). In general, dairy starter cultures metabolize carbohydrate (lactose is the major sugar found in milk) by either homo- or hetero-fermentative metabolic pathways. Lactose is fermented homofermentatively by *S. thermophilus*, *L. delbruecki subsp. bulgaricus*, and *Lactobacillus acidophilus*, but heterofermentatively by *Bifidobacterium spp* (Tamime and Robinson, 1999).

Lactose catabolism by *S. thermophilus*, *L. delbruecki subsp. Bulgaricus*, *L. acidophilus*, and *bifidobacteria* produces lactic acid primarily, or lactic and acetic acids when *bifidobacteria* are used in the starter culture. Lactic acid is necessary in the production of yoghurt for the following reasons. First, lactic acid aids in the destabilization of casein micelles by gradually converting the colloidal calcium/phosphate complex (in the micelles) to the soluble calcium phosphate fraction, which diffuses into the milk's aqueous phase. As a result, the calcium in the micelles gradually depletes, resulting in casein coagulation at pH 4.6- 4.7 and the development of the yoghurt gel. Second, lactic acid provides yoghurt with its flavor (i.e., sharp, and acidic). It can also enhance or contribute to the product's nutty and/or aromatic flavor (Tamime and Robinson, 1999).

2.10.2 Production of flavor components

Starter cultures are principally responsible for producing flavor components that add to yoghurt's aroma. These compounds can be classified into:

- Acids that are not volatile (lactic, pyruvic, oxalic, or succinic).
- Acids that are volatile (formic, acetic, propionic, or butyric).
- Compounds containing carbonyls (acetaldehyde, acetone, acetoin, or diacetyl).

Both organisms convert nearly all the sugar to lactic acid with minimal byproducts. These are crucial for the distinctive yoghurt flavor, with *S. thermophilus* producing diacetyl and *L. bulgaricus* producing acetaldehyde (Schulz and Hingst, 1954).

2.10.3 Protein metabolism

Proteolytic activity of bacterial strains employed in the production of fermented milks may be of minor importance, but it is an important consideration when choosing bacterial strains for starter cultures when manufacturing cheese. Although yoghurt and other starter cultures

are thought to only be weakly proteolytic, *S. thermophilus* and *L. delbruecki subsp. bulgaricus* may induce a large amount of proteolysis during the fermentation. The hydrolytic breakage of the peptide bonds that make up the backbone of protein molecules is catalyzed by proteolytic enzymes (Tamime and Robinson, 1999).

S. thermophilus grows predominantly on glutamic acid, histidine, and methionine, as well as cystine, valine, leucine, isoleucine, tryptophan, arginine, and tyrosine. The absorption of branched chain amino acids has been examined. It is an active transporter that requires an exogenous energy source, is temperature and pH dependent, and is inhibited by L-cysteine (Zourari *et al.*, 1992). When there are more cocci than rods, whey protein hydrolysis is reduced. Free fatty acids can inhibit proteolytic activity and improve coagulum texture. During the production of lactose hydrolyzed yoghurt, high proteolytic activity is observed. The hydrolysis of peptides to free amino acids and subsequent use of these amino acids is a critical metabolic activity in LAB, and proteolysis has been identified as the major factor affecting the rate of flavor and texture development in yoghurt (Bintsis, 2018).

2.10.4 Lipid Metabolism

Acyl glycerol accounts for 96-98% of total milk lipids/fats, with the remainder being made up of phospholipids, sterols, fat-soluble vitamins (A, D, E, and K), fatty acids, waxes, and squalene. Lipids can be found in the following phases of milk: fat globules, fat globule membranes, and milk serum. The proportions of these fractions can vary depending on factors such as mammal species, breed, stage of lactation, and feed type (Walstra and Jenness, 1984). The acyl glycerol found in milk are produced by esterifying the glycerol's alcohol radicals with one, two, or three fatty acid residues, yielding mono-, di-, or triacylglycerols (triglycerides). In general, enzymatic hydrolysis of milk lipids occurs at the ester bonds, finally producing free fatty acids and glycerol (Tamime and Robinson, 1999).

Triacylglycerol lipase enzymes in yoghurt may be derived from the starter culture or from microbial contamination that survived milk heat treatment. Lipases, which exist naturally in milk, are inactivated at standard pasteurization temperatures (Deeth and Fitz-Gerald, 2006). As a result, any decrease in fat percentage, rise in fatty acid levels (free or esterified), or increase in volatile fatty acid content in yoghurt can be linked to lipid metabolism by bacteria such as *S. thermophilus* and *L. delbruecki subsp. Bulgaricus* (Tamime and Robinson, 1999).

2.10.5 Vitamin metabolism

Milk contains both fat-soluble and water-soluble vitamins. Milk can lose significant amounts of vitamins when exposed to excess dissolved oxygen and/or moderate heat. The most susceptible vitamins are C, B_6, B_{12}, and folic acid; probiotic yoghurts made with *S. thermophilus*, *L. acidophilus*, and *L. casei* GG (this organism has been reclassified as *L. rhamnosus* GG) reduced the bioavailability of vitamins B_1, B_2, and B_6 for healthy adult humans (Elmadfa *et al.*, 2001).

During the fermentation process, the yoghurt starter bacteria use some of the vitamins in milk to fuel their growth. This component, in some measure, causes the product's nutritional value to decrease. However, consumption amounts depend on the rate of inoculation, the type of yoghurt starter, and the fermentation conditions (Shahani and Chandan, 1979). EPS-producing yoghurt starter organisms reduced thiamin and biotin levels in the product, whereas non-EPS cultures boosted biotin, folic acid, and riboflavin levels. A long yoghurt incubation (14-16 hours at 30°C) reduced folic acid synthesis while increasing thiamin and nicotinic acid concentration in the product (Kneifel *et al.*, 1989).

2.10.6 Production of exopolysaccharides

Some bacteria strains use carbohydrates in the growing medium to create EPS materials, such as *Streptococcus mutans*, *Streptococcus bovis*, and *Leuconostoc mesenteroides subsp. mesenteroides*, all of which can produce extracellular dextran (Caulfield *et al.*, 1979). Neutral exopolysaccharides are produced by some strains of *S thermophilus* and *L bulgaricus*. Slime generated by *L bulgaricus* and *S thermophilus* strains includes galactose, glucose, mannose, and trace quantities of rhamnose, xylose, and arabinose. Polysaccharide production enhances viscosity and texture, increases mechanical handling resistance, and reduces sensitivity to syneresis (Cerning, 1990).

2.10.7 Production of antimicrobial compounds

Lactic acid bacteria produce metabolites such as hydrogen peroxide and organic acids, which have an inhibitory and antagonistic impact and are a key target for pathogens (Gram-positives and Gram-negatives) and food spoilage microbes (Papadimitriou *et al.*, 2015). Yoghurt has a high concentration of bioactive peptides with antioxidant action, which are produced during fermentation (Nguyen and Hwang, 2016). Pathogens are susceptible to the

broad-spectrum antibacterial effects of thiocyanate and hydrogen peroxide (Seif *et al.*, 2005). Several processes, including the formation of organic acids, hydrogen peroxide, inhibitory peptides, and bacteriocins, as well as competition for colonization sites with pathogenic bacteria, show that LAB has inhibitory effect against pathogenic bacteria, particularly Gram-negative pathogens (Davoodabadi *et al.*, 2015).

2.11 Coagulum formation in yoghurt

The foundation for a huge variety of cultured dairy products is the acid coagulation of milk. By lowering their charge, dissolving part of the insoluble calcium phosphate crosslinks, and altering internal protein bonds, acidification has a direct influence on the stability of casein micelles. At some crucial point, when electrostatic repulsion is diminished and is unable to repel attractive forces like hydrophobic contacts, aggregates and eventually gels begin to develop. Acid-induced milk gels become more rigid over time because of continuing casein particle-to-casein link formation inside the network. For a brief time after gelation, the loss of insoluble calcium phosphate crosslinks inside the casein particles that are already forming the gel matrix causes an increase in the loss tangent parameter to be seen in gels formed from warm milk. The rate of acidification, temperature, degree of whey protein denaturation, protein concentration, and presence of a polysaccharide stabilizer are all factors that affect the texture and physical characteristics of acid-induced gels (Lucey, 2016).

The biological and physical reactions of milk lead to the production of yoghurt gel. Yoghurt's starter uses lactose as fuel, producing lactic acid as well as other important components that are inescapable. The calcium caseinate phosphate complex is made unstable by the gradual formation of lactic acid. As the pH approaches the isoelectric point (pH 4.6–4.7), aggregates of casein micelles and/or the individual micelles clump together and partially coalesce. The interaction of α-lactalbumin and β-lactoglobulin with κ-casein (connected by -SH and -SS bridges) is most likely what partially shields the micelles from total instability or disruption. As a result, the gel network or matrix has a regular structure and captures all the basic mix's outer parts, including the water phase (Tamime and Robinson, 1999).

2.12 Methods for improving the body (viscosity) of yoghurt

Traditional yoghurt was produced by heating milk in open pans and concentrating it to two-thirds volume this manner. The higher the solids concentration, the thicker or more viscous

the yoghurt. If sheep milk is used, the yoghurt will be thicker since it contains roughly 50% more solids than regular cow milk (Tamime and Robinson, 1985).

When yoghurt is produced from non-concentrated or unfortified cow milk, it produces a beautiful gel, but it is delicate and readily destroyed by vibration. To circumvent this challenge, SMP at a 4-5% level is used. The simplest and least expensive method is to add carbohydrate gum such as carrageenan, alginate, agar, etc. at a concentration of around 0.3%. This is safe but has little nutritional benefit because milk fat is routinely homogenized (Tamime and Robinson, 1985).

Ropy strains of S. thermophilus and L. bulgaricus may both be used at the same temperature (43ºC) as regular yoghurt. However, the higher the viscosity, the lower the temperature and the longer the incubation period. As a result, a temperature of 30-32ºC and an incubation duration of 12-15 hours may be employed (Carr *et al.*, 1975).

2.13 Types of yoghurt

2.13.1 Set type yoghurt

Before being packaged in the filling machine, processed milk is put straight into the intermediate tank and inoculated with starter culture and/or flavor. The yoghurt cups are filled and placed in the 42°C incubation chamber. After 3 hours, the cups are cooled to 15-20°C using cold air from the chamber or cooling tunnel (Pant, 1992).

2.13.2 Stirred type yoghurt

It is soured in a tank before being stirred, chilled, and packaged. Stirred yoghurt has a particular consistency, being thick and smooth, and should be eaten rather than drunk. A stabilizer of 0.5 to 0.7% is used to impart gel structure, provide smooth body and texture, and avoid wheying off or syneresis during packing. This variety of yoghurt is more popular since it can be plain, fruity, or flavored (Tamime and Robinson, 1999).

2.13.3 Drinking type yoghurt

The product is stored and handled similarly to stirred yoghurt, except fruit syrup is utilized and the coagulum is homogenized during fermentation. Firstly, coagulum is set, heated, and the product has a shelf-life of three weeks at 10°C; secondly, pasteurization of yoghurt at 75°C for a few seconds, followed by cooling and packing gives the product a few weeks

shelf-life at 10°C; and thirdly, UHT drinking yoghurt is heated at 110°C for 4 seconds, cooled, and filled into sterilized container under aseptic conditions. The latter has a shelf life of several months at room temperature (Pant, 1992).

2.13.4 Frozen yoghurt

The yoghurt base is produced in the traditional manner. The milk should be UHT treated before fermentation with starting culture and producing natural stirred yoghurt, which is then blended with 65-80% yoghurt foundation, 20-35% fruit syrup base, and 0.85% stabilizer and emulsifier. After that, the product is frozen in a standard ice cream freezer (outlet temperature -6°C). Finally, the yoghurt is packaged and sent at temperatures ranging from 0 to -6°C (Tamime and Robinson, 1985).

2.13.5 Dried yoghurt

Yoghurt powder is produced by fermenting nonfat milk with regular yoghurt cultures until the desired pH is reached, followed by a drying stage, most likely by freeze-drying. Furthermore, blended yoghurt powder is created by combining cultured nonfat milk, cultured whey, cultured whey protein concentrate, cultured dairy solids, nonfat dry milk, and lactic acid, all of which have comparable flavor and functionality to regular yoghurt powder (Childs and Drake, 2008).

The primary goal of producing yoghurt powder is to keep the product stable and ready for use. It can be used to replace fresh yoghurt in beverages and dips, as well as in the confectionary industry as a coating material for coating dried fruit, nuts, pretzels, cereal, and other snack items (Krasaekoopt and Bhatia, 2012).

2.13.6 Therapeutic yoghurt

The fact that most strains of *S. thermophilus* and *L. bulgaricus* do not survive in the digestive system may be a limiting issue if yoghurt is used for antibiotic treatment or any other medicinal reason. Yet, using *Lactobacillus acidophilus* and *Bifidobacterium bifidum* as yoghurt starting cultures may violate certain existing definitions of yoghurt; yet the resulting milk product is said to have significant medicinal potential. Lactose-hydrolyzed yoghurt, for example, is advantageous to lactose-intolerant patients (Tamime and Robinson, 1999).

2.14 Factors affecting yoghurt quality

2.14.1 Casein and fat content

Yoghurt firmness is roughly equal to the cube of casein concentration. Natural variation in casein content can thus have a significant impact. Firmness is increased by evaporating the milk, adding skim milk powder, or using partial ultrafiltration. Because fat globules disrupt the network, the higher the fat concentration, the weaker the gel (Walstra *et al.*, 2005).

2.14.2 Homogenization

Homogenization of milk results in significantly increased firmness because the fat globules contain pieces of casein micelles in their surface coat, allowing them to participate on the network during acidification. As a result, the volume proportion of casein is effectively raised. However, skim milk homogenization makes no difference (Walstra *et al.*, 2005).

2.14.3 Heat treatment

Heat treatment improves milk hardness significantly. Denatured serum proteins tend to clump, creating massive, insoluble complexes that can increase milk viscosity. Generally, milk is heated for 5 to 10 minutes at 85°C to 90°C (Walstra *et al.*, 2005).

2.14.4 Acidity and pH

At a lower pH, the yoghurt is often firmer. The ideal pH range is between 4.1 and 4.6 (Walstra *et al.*, 2005).

2.14.5 Incubation temperature

The lower it is, the longer it takes to attain a given pH and hence hardness, but the finished product is considerably firmer (Walstra *et al.*, 2005).

2.15 Shelf life of yoghurt

The shelf-life of a product is the number of days after production that it may be consumed while remaining safe, preserving its quality appeal, and matching customer expectations. In other words, it should be microbiologically safe and organoleptically acceptable for the duration of its specified shelf-life (Ahmed, 2011).

Most yoghurts with a limited shelf life nevertheless include live (or "lives") culture organisms. There is some activity even though their metabolic rate is rather modest at 7°C.

This may be assessed during the shelf-life by measuring the pH, determining the titrable acidity, and tasting the product (Akpan *et al.*, 2007).

Yoghurt has a shelf life of around 3 weeks when kept refrigerated, depending on the hygienic standards followed during production, the microbiological quality of the ingredients, and the packaging materials (De *et al.*, 2014). Yoghurt has a shelf life of around 10 days at a chilled temperature of about 5°C, after which the bacterial growth, however restrained, will raise the level of acidity to such a degree that it will damage the flavor and finally make it unpleasant to most people. At some point, the bacteria are eliminated, and the yoghurt separates into curds and whey. Yoghurt is particularly vulnerable to yeast and mold attacks; therefore, it is important to take great care to ensure that the starter is clear of these organisms and that they do not enter during packing (Tamime and Deeth, 1980).

2.16 Techniques of shelf-life extension

Shelf life can be prolonged by various methods:

- Stopping the incubation process when the pH reaches 4.6-4.8, rapid cooling, and storage at 5°C.
- Aseptic operation on an enclosed manufacturing line, including aseptic addition of sterile additives and aseptic packing.
- Pasteurization in a continuous flow cooler of stirred cultured milk products with or without additives, aseptic chilling, filling, and sealing.
- Continuous flow heating, hot filling, package closure, and chilling after a suitably long pasteurization period.
- Cold filling, package closure, pasteurization within the packaging by heating, then cooling (Kessler, 2002).

2.17 Stabilizers and their classification

Some dairy products contain stabilizers and/or emulsifiers, however only stabilizers are added to the milk base for manufacturing yoghurt. In most nations, their application is controlled by governmental regulation. The categorization of these food-grade stabilizers/emulsifiers has long been a challenge, and several alternative methods have been proposed, including:

- All compounds are to be referred to as polysaccharide materials.
- The name includes botanical origin.
- Their general origin, i.e., plant, animal or synthetic.
- Chemical grouping (Tamime and Robinson, 1985).

Glicksman (1969), on the other hand, has modified the latter method, and his suggested categorization includes a reference to the processing technique, for example:

- Natural gums (those found in nature)
- Modified natural or semi-synthetic gums (i.e., chemical modifications of natural gums or gum-like materials)
- Synthetic gums (those prepared by chemical synthesis).

The main objective of adding stabilizers to the milk base is to improve and preserve ideal yoghurt properties such as body and texture, viscosity/consistency, appearance, and mouthfeel. As a result, the yoghurt coagulum is frequently exposed to mechanical treatment during production:

i. Stirring the coagulum in the fermentation tank to facilitate in-tank cooling or after the conclusion of the incubation period,
ii. Pumping the coagulum to a cooler on a plate or tube,
iii. Mixing to incorporate the fruit/flavors into the coagulum, pumping to the filling/packaging machine, and then post-fermentation heat treating of the coagulum to produce pasteurized, UHT, or long-life yoghurt; as a result, the yoghurt may become less viscous or, in extreme cases, may show whey separation. Stabilizers can be added to eliminate these defects (Tamime and Robinson, 1999).

Stabilizers are also known as hydrocolloids, and their mode of action in yoghurt consists of two primary functions:

i. Binding of water, and
ii. Promotion of an increase in viscosity.

Table 2.1 Classification and functions of gums which could be used during the manufacture of yoghurt

Natural	Modified	Synthetic[a]
Plant	Cellulose derivatives (1)[b]	Polymers
Exudates	Carboxymethylcellulose	Polyvinyl derivatives
Arabic (1,3)[b]	Methylcellulose	Polyethylene derivatives
Tragacanth (1)[b]	Hydroxyethylcellulose	
Karaya[b]	Hydroxypropylcellulose	
Extracts	Hydroxypropylmethylcellulose	
Pectin (2,3)[b]	Microcrystallinecellulose	
Seed flour	Microbial fermentation	
Carob (1)[b]	Dextran	
Guar (1)[b]	Xanthan (1,3)[b]	
Seaweeds	Miscellaneous derivatives[b]	
Extracts	Low-methoxy pectin	
Agar (2,3)[b]	Propylene glycol alginate	
Alginates (1,2,3)[b]	Pregelatinized starches	
Carrageenan (2,3)[b]	Modified starches	
Furcelleran (1,2,3)[b]	Carboxymethyl starch	
Cereal starches (1,2,3)	Hydroxyethyl starch	
Wheat	Hydroxypropyl starch	
Corn		
Animal		
Gelatin[b]		
Casein		
Vegetable		
Soy protein		

Source: Tamime and Robinson (1999)

Note: [a] Limited in their application in yoghurt. [b] Stabilizers permitted by FAO/WHO (1990), and the permitted level (single or combination with others) is $5gkg^{-1}$, except for pectin, gelatin and/or starch derivatives where it is $10gkg^{-1}$.

Figures in parentheses show the hydrocolloid's function: (1) thickening, (2) gelling agent, and (3) stabilizer. The permissible level of these stabilizing chemicals is set by legislation, and they are not authorized in natural or unflavored fermented milks.

Because of the existence of a negatively charged group, such as a hydrogen or carboxyl radical, or the presence of a salt with the ability to sequester calcium ions, the molecules of a stabilizer can establish a network of links between the milk components and themselves. These negative groups are concentrated in the interfacial locations, and the stabilizer achieves water binding into the milk base as follows:

- It binds the water as water of hydration.
- It interacts with the milk components (mostly the proteins) to enhance their water hydration level.
- It holds the protein molecules together in the shape of a network, which slows the free passage of water.

As a result, hydrocolloids in yoghurt serve two functions: (a) gelling or thickening agents, and (b) stabilizing agents. Table 2 lists the many compounds that may be added to milk to make viscous yoghurt, and these stabilizers can be added as single compounds or as a combination. Since most commercial formulations comprise a blend of stabilizing chemicals (unless otherwise stated), the latter strategy is more generally employed. The objective of combining these compounds is to achieve a certain function or, in most cases, to overcome one of the limiting features of a given component (Tamime and Robinson, 1999).

2.17.1 Common stabilizer used in yoghurt and yoghurt drinks

There are several stabilizers and their combinations for use in yoghurt. The following factors should be considered while selecting a stabilizer:

- Type of yoghurt being produced: vat/cup set, Swiss/blended type or drink/smoothie, mousse/whipped type.
- Formulation: fat content, total solids.
- Desired firmness and consistency of the finished product as per marketing objectives
- Desired ingredient labeling (natural, organic, etc.)

- Processing equipment available: batch process (ease of incorporation), continuous heating system, in-line dosing and mixing, cooling, and pumping of coagulum.
- Possible masking effect on the flavoring system (Chandan and O'Rell, 2006).

2.17.1.1 Pectin

Pectin, a complex heteropolysaccharide family composed primarily of partly methoxylated galacturonic acid residues, is widely distributed in practically all fruits and vegetables as the structural unit of fresh cells and the cell junction. Its structure is based on 1, 4-linked -D-galacturonic acid, which is interrupted by L-rhamnose residues with neutral sugar side chains (mostly D-galactose and L-arabinose). Because of its capacity to produce aqueous gels, pectin is widely employed as a functional component in the food business, and has been used in jams and jellies, fruit preparations, fruit drink concentrates, fruit juice, desserts, and fermented dairy products (Arioui *et al.*, 2017).

Pectin's are often used to stabilize stirred and set yoghurt, either alone or in conjunction with other hydrocolloids. For (refrigerated) cup yoghurt, low methoxy (LM) pectin is preferable. A very little quantity (0.07-0.15%) changes the consistency of the yoghurt, stiffening it and preventing any syneresis that may occur during handling, transit, and distribution. The lactoserum is retained by LM pectin in a highly flexible network created in interaction with calcium ions present in the yoghurt. Higher quantities of pectin in yoghurt may result in a gritty or sandy texture and lower viscosity in stirred yoghurt (Tamime and Robinson, 1999). To guarantee stability and manage viscosity in acidified milk drinks, high methoxy (HM) pectin is preferable. HM pectin stabilizes milk proteins, resulting in products with no sedimentation or whey separation, as well as a smooth tongue feel without "sandiness." The stabilization is achieved by the absorption of pectin onto the surface of the protein particles using shear force. The absorbed pectin charges all particles, generating repulsion between particles and prevents agglomeration, which would result in sedimentation, separation, and a gritty texture. The optimum HM pectin level is determined by:

- Protein concentration
- Protein particle size
- Heat treatment
- Length of shelf life(Chandan and O'Rell, 2006)

2.17.1.2 Carboxymethyl cellulose

Carboxymethyl cellulose (CMC) is an anionic, water-soluble derivative of cellulose, an anhydro-glucose linear polysaccharide. β-1,4- glycosidic linkages connect the repeating units. The main difference between CMC and cellulose at the molecular level is merely some anionic carboxymethyl groups (i.e., -CH_2COOH) in the CMC structure that replace the hydrogen atoms from certain hydroxyl groups found in the pristine cellulose architecture. CMC was synthesized for the first time in 1918. However, practical manufacture of these vital polymeric materials was first shown in Germany in the early 1920s (Rahman *et al.*, 2021).

CMC dissolves well in hot or cold water and is effective at high processing temperatures. Its principal role in yoghurt would be to thicken and bind moisture. It binds water in frozen yoghurt, reducing the production of big ice crystals that can form during temperature variations during storage. As a result, the frozen yoghurt has a smoother texture and better melt down properties. The stabilizer system used in yoghurt mix preparations is often a blend of several vegetable stabilizers. To achieve desired results, their ratios as well as the final concentration (usually 0.5-2.0%) in the product are carefully managed. Fruits and flavors are also significant elements in yoghurt production (Chandan and O'Rell, 2006).

2.17.1.3 Guar gum

Guar gum is derived from the seeds of the drought-resistant plant *Cyamopsis tetragonoloba*, which belongs to the Leguminosae family. The scientific names for the bean, guar gum flour, and galactomannan fraction are Indian cluster bean, guar, and guaran, respectively (Whistler and Hymowitz, 1979). Guar gum is similar to locust bean gum in that it mostly consists of the complex carbohydrate polymer made up of galactose and mannose, but in differing ratios (Mudgil *et al.*, 2014).

Guar gum can be used as a stabilizer in frozen yoghurt systems. Guar gum dissolves well in cold water and is unaffected by the high temperatures used in yoghurt pasteurization. Guar gum is non-gelling and is primarily employed as a viscosity booster, stabilizer, and moisture-binding agent. Guar gum adds body, texture, chewiness, and heat resistance to frozen yoghurt (Chandan and O'Rell, 2006).

2.17.1.4 Gelatin

Gelatin is a polypeptide with a high molecular weight derived from collagen, the major protein component of animal connective tissues such as bone, skin, and tendons. Gelatin became popular about the year 1700 and is derived from the Latin '*gelatus*', which means hard or frozen. Although the name gelatin is sometimes given to various gel formers, it should only be applied to collagen-derived protein compounds (Poppe, 1992).

Gelatin has long been used as a stabilizer in many types of yoghurts. It is used in chilled yoghurt at a concentration of 0.1-0.5%, depending on the hardness needed. Gelatin is also an excellent stabilizer for frozen yoghurt. Bloom strength 225 or 250 gelatin is usually used. The gelatin amount should be adjusted to meet yoghurt consistency guidelines. When yoghurt with relatively high milk solids is stirred, the amount of gelatin above 0.35% produces a curdy and lumpy look. Gelatin degrades during ultrahigh temperature processing, and its activity is temperature dependent. When temps fall below 10°C, the yoghurt becomes pudding-like. A rise in temperature significantly weakens the yoghurt gel formed by gelatin. Gelatin is desired due to its sheen-like appearance and capacity to withstand a great deal of damage while still producing a nice result. However, if just gelatin is used, the product may have a jelly-like consistency that stirs out lumpy, which is undesirable in most markets. As a result, it is more usual to combine gelatin with other stabilizers to reduce the rigid jelly effect and generate a body that stirs out smooth and free of lumps. The most prevalent combinations are modified starch-gelatin and gelatin-pectin (Chandan and O'Rell, 2006).

Table 2.2 Common stabilizers for yoghurt and yoghurt drinks

Stabilizers	(%) Concentration in yoghurt mix
Whey protein concentrate (WPC) and/or Milk protein concentrate (MPC)	0.7-1.5
Modified starch (tapioca/corn)	0.8-2.0
Gelatin (225/250 Bloom)	0.1-0.5
Agar	0.25-0.7
Pectin (low methoxy for yoghurt)	0.08-0.2
Pectin (high methoxy for yoghurt beverages)	0.3-0.5
Locust bean gum (in combination)	0.3-0.5
Xanthan gum (in combination)	0.01-0.05
Carrageenan (in combination)	0.01-0.05
Natural corn starch	1.5-2.0
Carboxymethyl cellulose	0.1-0.2

Source: Chandan and O'Rell (2006)

According to Athar *et al.* (2000), Yoghurt was prepared by using seven various stabilizers like pectin, guar gum, carboxy methyl cellulose (CMC), carrageenan, sodium alginate, cornstarch and gelatin @ 0.4% in milk containing 3.5% milk fat and total solids 16.6%.At 0, 5, 10, and 15 days of storage, pH, acidity, lactose, and syneresis levels were all determined. During 15 days at 10°C, all samples showed a steady fall in pH and an increase in acidity. Lactose content decreases in all yoghurt samples after storage owing to lactic acid conversion. When compared to the control, cornstarch was shown to lower syneresis, followed by gelatin, pectin, guar gum, CMC, carrageenan, and sodium alginate.

2.17.2 Stabilizer effect on yoghurt quality

According to Athar *et al.* (2000), the quality of yoghurt samples is evaluated on the basis of changes in pH, acidity, lactose and whey separation (syneresis) during storage at 10°C±1 for 15 days. The results are described below:

- **pH**

The pH decreased less in samples treated with cornstarch, gelatin, and pectin during storage than in those treated with guar gum, CMC, carrageenan, and sodium alginate. As a result,

cornstarch, gelatin, and pectin had a greater influence on pH change than other stabilizers. After 15 days of storage, the largest drop in pH change in case of control was observed.

- **Acidity**

In all the treatments, along with control, a steady rise in acidity was seen during yoghurt sample preservation. In comparison to other treated samples, the control sample showed the greatest rise. Samples treated with cornstarch, gelatin, and pectin produced less acidity.

- **Lactose**

The results of seven stabilizers were examined, and it was found that the lactose content of the yoghurt samples stabilized with cornstarch decreased less than the yoghurt samples treated with the other six stabilizers. It may be because lactic acid-producing bacteria used the CH_2O present in cornstarch along with the lactose, which led to a lower decrease in lactose content in cornstarch compared to samples of yoghurt that had been treated with pectin, guar gum, CMC, carrageenan, gelatin, and sodium alginate. The results also showed that the lactose content drop in the control group was more pronounced. This drop may be the result of a microorganism converting lactose to lactic acid.

- **Syneresis/ Whey separation**

The stabilizer had a significant influence on syneresis. Cornstarch outperformed all other stabilizers in terms of performance. The syneresis increased with storage time in all cases, including control. The average value for all treated yoghurt samples increased with time as well.

Part III

Material and methods

3.1 Materials

The materials collected for the preparation of yoghurt with the use of stabilizers were as follows:

3.1.1 Milk

The standardized (3% fat and 8% SNF) and pasteurized milk was collected from the local market of Dharan.

3.1.2 Milk solid not fat / SMP

Skim milk powder was used as the source of MSNF, and it was collected from the Kamdhenu Dairy, Tarahara.

3.1.3 Sweetener

Sugar was used as a sweetener. It was bought from the local market of Dharan.

3.1.4 Starter Culture

Starter culture, a liquid culture containing *L. bulgaricus* and *S. thermophilus* in correct proportion (1:1) was collected from the Kamdhenu Dairy, Tarahara.

3.1.5 Stabilizers

Stabilizers (Pectin, Guar gum and CMC) were collected from the laboratory of Central Campus of Technology.

3.1.6 Containers

Plastic cups were bought from Baraha department store of Dharan. The size of cup was 100 ml and plain in design.

3.2 Methods

3.2.1 Proximate analysis of milk

3.2.1.1 Acidity

Acidity was determined by titrimetric method as per AOAC (2005).

3.2.1.2 Fat

Fat content in milk was determined by Gerber method as described by AOAC (2005).

3.2.1.3 Protein

Protein was determined Kjeldahl method as described in AOAC (2005).

3.2.1.4 Ash

Ash content was determined as described by Ranganna (1986).

3.2.1.5 pH

The pH value was determined by the direct reading with the digital pH meter as given by Kc and Rai (2007).

3.2.1.6 Total Soluble Solids

The total soluble solids of milk were determined by using Hand refractometer.

3.2.1.7 Lactose

Lactose content was determined by Lane and Eynon method as per Pearson (1976).

3.2.2 Preparation of yoghurt

Milk was preheated to 45ºC and skim milk powder was added at the rate of 2% with proper stirring. Then, milk was again heated to 65-70ºC and sugar was added at the rate of 4% and stirred well. Stabilizers such as pectin, guar gum and carboxymethyl cellulose were added at the rate of 0.1, 0.2 and 0.3% each. After that, pasteurization was done at 85- 90ºC for about 30 minutes. The pasteurized milk was cooled to 44ºC, and 2% starter culture was inoculated. It was then incubated at 44ºC for about 2-3 hours until the coagulum was formed. Set type

yoghurt thus obtained was cold stored at 4-8ºC. Control was prepared without addition of stabilizer by following similar steps shown in Fig 3.1.

3.2.3 Optimization of Pectin level

Three samples of yoghurt were prepared by using 4% sugar, 2% skim milk powder and varying amount of pectin (0.1%, 0.2% and 0.3%) and these samples were coded as Sample A, Sample B and Sample C. These samples were subjected to sensory evaluation in terms of appearance/ color, flavor, texture and overall acceptability and the scores so obtained were subjected to statistical analysis to get optimum level of pectin for preparation of yoghurt.

3.2.4 Optimization of Guar gum level

Three samples of yoghurt were prepared by using 4% sugar, 2% skim milk powder and varying amount of guar gum (0.1%, 0.2% and 0.3%) and these samples were coded as Sample D, Sample E and Sample F. These samples were subjected to sensory evaluation in terms of appearance/ color, flavor, texture and overall acceptability and the scores so obtained were subjected to statistical analysis to get optimum level of guar gum for preparation of yoghurt.

3.2.5 Optimization of Carboxymethyl cellulose (CMC)

Three samples of yoghurt were prepared by using 4% sugar, 2% skim milk powder and varying amount of carboxymethyl cellulose (0.1%, 0.2% and 0.3%) and these samples were coded as Sample G, Sample H and Sample I. These samples were subjected to sensory evaluation in terms of appearance/ color, flavor, texture and overall acceptability and the scores so obtained were subjected to statistical analysis to get optimum level of carboxymethyl cellulose for preparation of yoghurt.

3.2.6 Comparison between the yoghurt using different optimized stabilizers

Four samples of yoghurt were prepared by using 4% sugar, 2% skim milk powder and optimized amount of stabilizer such as pectin, guar gum, and carboxymethyl cellulose. These samples were coded as yoghurt P for yoghurt containing pectin, yoghurt GG for yoghurt containing guar gum, and yoghurt CMC for yoghurt containing carboxymethyl cellulose. These coded samples were subjected to sensory evaluation and the scores so obtained were

subjected to statistical analysis and best yoghurt in terms of sensory score in comparison to control was taken.

3.2.7 Comparison between the best yoghurt using stabilizer and control

Yoghurt was prepared by using 4% sugar, 2% skim milk powder and optimized amount of stabilizer and coded as yoghurt X and control was prepared without addition of stabilizer while other proportion remained constant and coded as yoghurt C. Both samples of yoghurt were stored in refrigerated temperature and subjected to physicochemical analysis in every two days interval till products were acceptable for sensory evaluation to compare the shelf life of yoghurt with or without stabilizer.

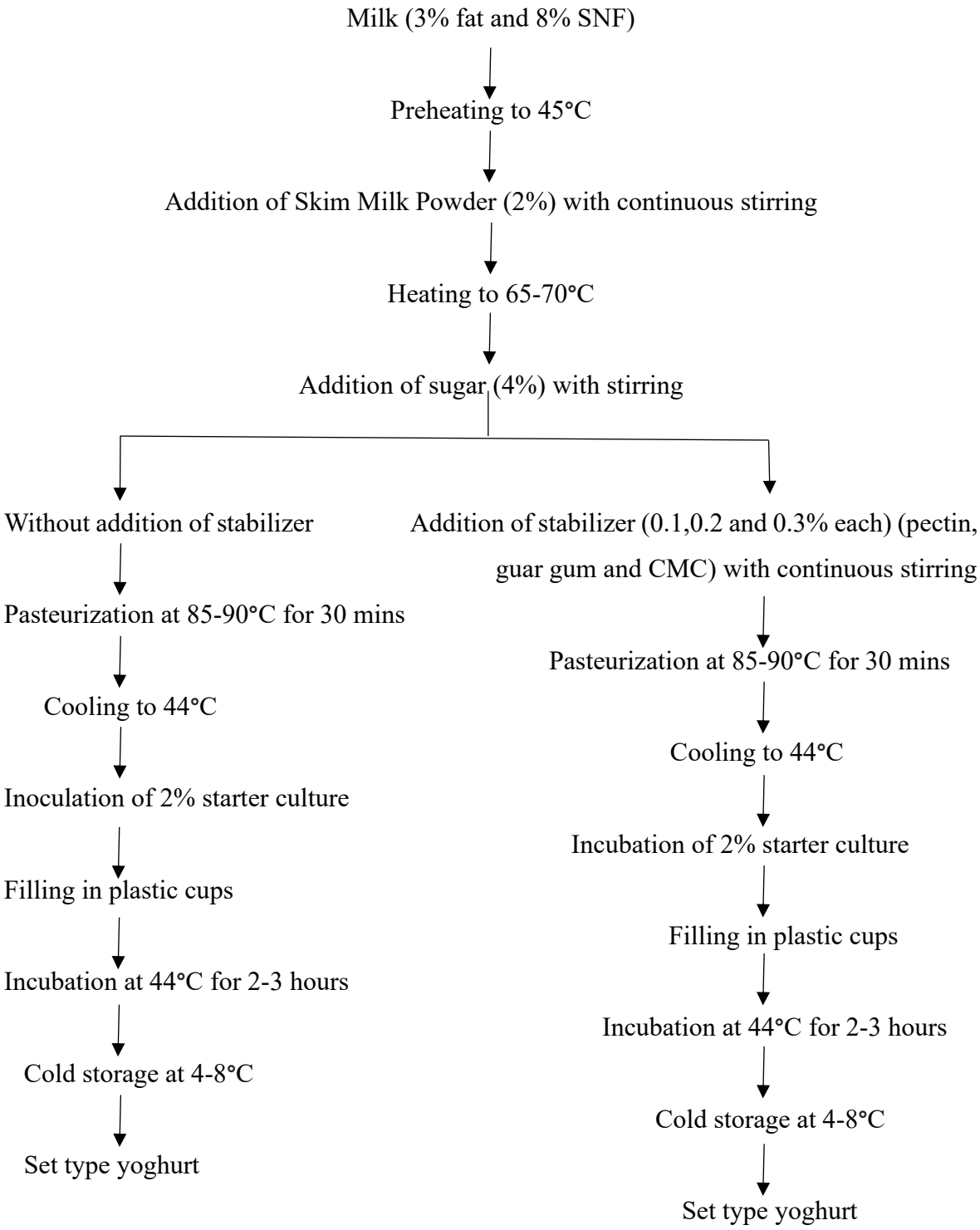

Fig 3.1 Flow chart of yoghurt preparation

Source : Bhattarai *et al.* (2015)

3.2.8 Analysis of yoghurt

3.2.8.1 Sensory Evaluation

Sensory evaluation was carried out using the 9-point hedonic scale described by Ranganna (1986). Sensory panelists were teachers and research students from Central Campus of Technology, Dharan. Sensory evaluation was carried out on the quality attributes viz., color and appearance, taste, body and texture, flavor, and overall acceptability. The specimen of the evaluation of card is shown in Appendix A.

3.2.8.2 Physical analysis

3.2.8.2.1 Syneresis

Degree of syneresis, expressed as proportion of free whey was measured by a method used by Lee and Lucey (2004).

A 20gm sample of yoghurt was placed on a filter paper resting on the top of a funnel. After 10 min of drainage in vacuum condition, the quantity of the remained yoghurt was weighed and syneresis was calculated as follows:

$$\% \text{ Free whey (g/20g)} = \frac{\text{Wt. of initial sample} - \text{wt. of sample after filtration} \times 100}{\text{Wt. of initial sample}}$$

3.2.8.3 Chemical analysis

3.2.8.3.1 Fat

Fat content was determined by the Gerber method as described in AOAC (2005).

3.2.8.3.2 Acidity

Acidity was determined by titrimetric method given by AOAC (2005).

3.2.8.3.3 Protein

Protein was determined Kjeldahl method as described in AOAC (2005).

3.2.8.3.4 Lactose

Lactose content was determined by the Lane and Eynon method as per Pearson (1976).

3.2.8.3.5 pH

The pH value was determined by the direct reading with the digital pH meter as given by Kc and Rai (2007).

3.2.8.3.6 Ash

Ash content was determined as described by AOAC (2005).

3.2.8.3.7 Moisture

Moisture content was determined as per the methods described in AOAC (2005).

3.2.8.4 Storage stability

The final product yoghurt X and control yoghurt C were stored at refrigerated temperature. pH and syneresis were studied at 1st, 3rd, 5th, 7th, 9^{th,} and 11th days of storage period.

3.2.8.5 Microbial examination

Total plate count (TPC) was carried out by using plate count agar as described by Burke *et al.* (2021).

3.2.8.6 Statistical analysis

The analyses were carried out in triplicate. Statistical calculations were performed in Microsoft office Excel 2016. Analysis of variance (ANOVA) was carried out for data from sensory evaluation. The significant differences between them were studied by using L.S.D. at 5% level of significance using GenStat release 12.1 software program developed by VSN International Ltd.

Part IV

Result and discussions

Yoghurt was prepared at CCT, Dharan, in a laboratory for the present study. Milk was preheated to 45ºC and skim milk powder was added at the rate of 2% with proper stirring. Then, Milk was again heated to 65-70ºC and sugar was added at the rate of 4% and stirred well. Stabilizers such as pectin, guar gum and carboxymethyl cellulose were added at the rate of 0.1, 0.2 and 0.3% each. After that, pasteurization was done at 85- 90ºC for about 30 minutes. The pasteurized milk was cooled to 44ºC, and 2% starter culture was inoculated. It was then incubated at 44ºC for about 2-3 hours until the coagulum was formed.

4.1 Proximate composition of milk

Milk was collected from the local market of Dharan, and principal constituents of milk were analyzed which were shown in table 4.1.

Table 4.1 Proximate composition of milk

Parameters	*Values (% dry basis)
Protein (%)	3.3±0.09
Fat (%)	2.93±0.047
Lactose (%)	4.43±0.094
Ash (%)	0.69±0.05
Acidity (% as lactic acid)	0.13±0.005
pH	6.56±0.047
Total soluble solids	12.67±0.34

*Values in the table are arithmetic mean of triplicate samples. Figures in the parentheses indicate standard deviation.

Milk is a complex biological fluid. Milk of any single species varies with the individuality of the animal, the breed (in the case of commercial dairying species), health (mastitis and other disorders), nutritional state, stage of lactation, age, interval between milkings, and so on. Many of these characteristics are evened out in a bulked factory milk supply, but some

variability will continue and be fairly considerable in instances where milk production is seasonal (Fox *et al.*, 1998).

4.2 Optimization of pectin for preparation of yoghurt

Three yoghurt samples were prepared by adding pectin at 0.1%, 0.2%, and 0.3% concentrations while keeping the proportions of skim milk powder (2%) and sugar (4%) constant. Sensory evaluation of yoghurt samples was performed in terms of appearance/color, flavor, texture/mouthfeel, and overall acceptability. Figure 4.1 displays the yoghurt's mean sensory score.

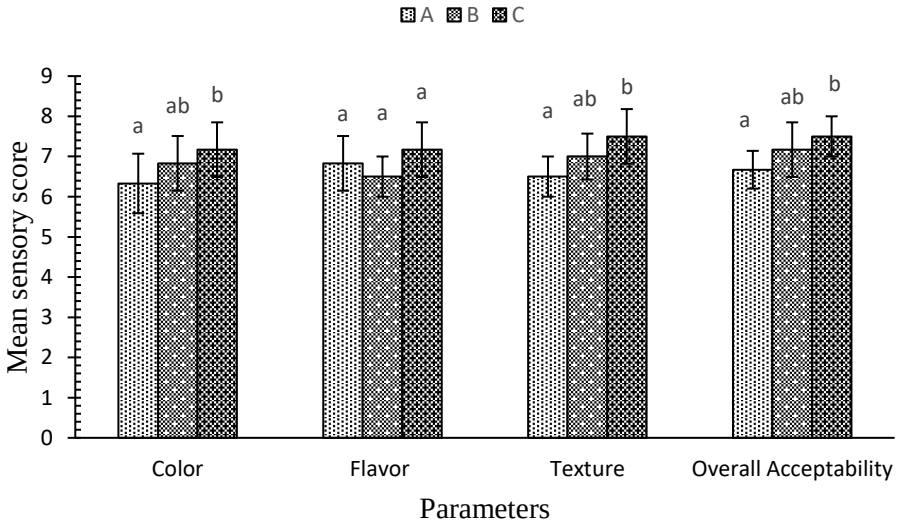

Fig 4.1 Optimization of pectin for preparation of yoghurt

Fig. 4.1 represents the mean sensory scores for color, flavor, texture, and overall acceptability for 0.1%, 0.2% and 0.3% pectin conc. in yoghurt. Values on the top of the bars bearing similar superscript are not significantly different at 5% level of significance. The vertical error bar represents ±standard deviation of scores given by panelist.

Where, Sample A was yoghurt containing 0.1% pectin, Sample B was yoghurt containing 0.2% pectin and Sample C was yoghurt containing 0.3% pectin.

In terms of superiority at 5% LSD of the formulations with respect to appearance/color, flavor, texture/mouthfeel, and overall acceptability, following conclusion can be drawn:

Appearance/color: Sample A≤ Sample B≤ Sample C

Flavor: Sample A= Sample B= Sample C

Texture/mouthfeel: Sample A≤ Sample B≤ Sample C

Overall acceptability: Sample A≤ Sample B≤ Sample C

Based on the frequency of occurrence as 'best' in each attribute type, Sample C appeared to be the best formulation. Hence, Sample C (0.3%) was taken for further study.

4.3 Optimization of guar gum for preparation of yoghurt

Three yoghurt samples were prepared by adding guar gum at 0.1%, 0.2%, and 0.3% concentrations while keeping the proportions of skim milk powder (2%) and sugar (4%) constant. Sensory evaluation of yoghurt samples was performed in terms of appearance/color, flavor, texture/mouthfeel, and overall acceptability. Fig 4.2 displays the yoghurt's mean sensory score.

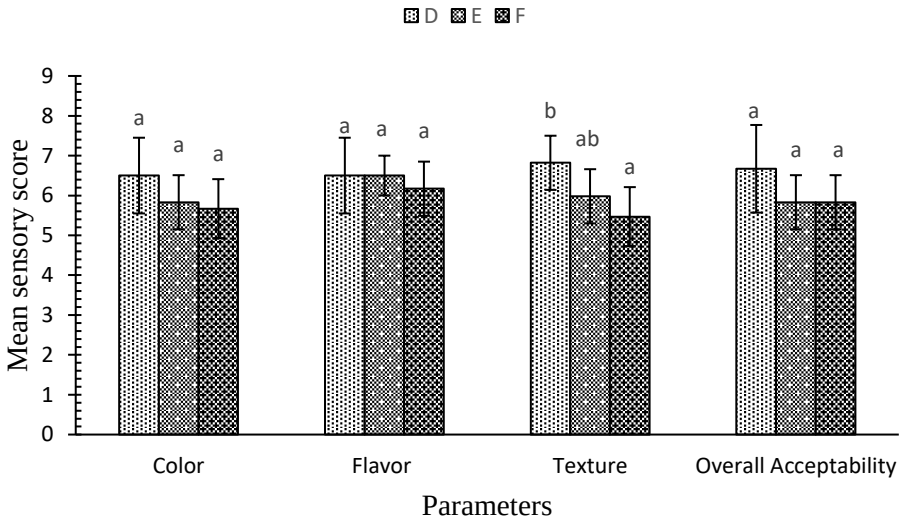

Fig 4.2 Optimization of guar gum for preparation of yoghurt

Fig. 4.2 represents the mean sensory scores for color, flavor, texture, and overall acceptability for 0.1%, 0.2% and 0.3% guar gum conc. in yoghurt. Values on the top of the bars bearing similar superscript are not significantly different at 5% level of significance. The vertical error bar represents ±standard deviation of scores given by panelist.

Where, Sample D was yoghurt containing 0.1% guar gum, Sample E was yoghurt containing 0.2% guar gum and Sample F was yoghurt containing 0.3% guar gum.

In terms of superiority at 5% LSD of the formulations with respect to appearance/color, flavor, texture/mouthfeel, and overall acceptability, following conclusion can be drawn:

Appearance/color: Sample D= Sample E= Sample F

Flavor: Sample D= Sample E= Sample F

Texture/mouthfeel: Sample D≥ Sample E≥ Sample F

Overall acceptability: Sample D= Sample E= Sample F

Based on the frequency of occurrence as 'best' in each attribute type, Sample D appeared to be the best formulation. Hence, Sample D (0.1%) was taken for further study.

4.4 Optimization of CMC for preparation of yoghurt

Three yoghurt samples were prepared by adding CMC at 0.1%, 0.2%, and 0.3% concentrations while keeping the proportions of skim milk powder (2%) and sugar (4%) constant. Sensory evaluation of yoghurt samples was performed in terms of appearance/color, flavor, texture/mouthfeel, and overall acceptability. Fig 4.3 displays the yoghurt's mean sensory score.

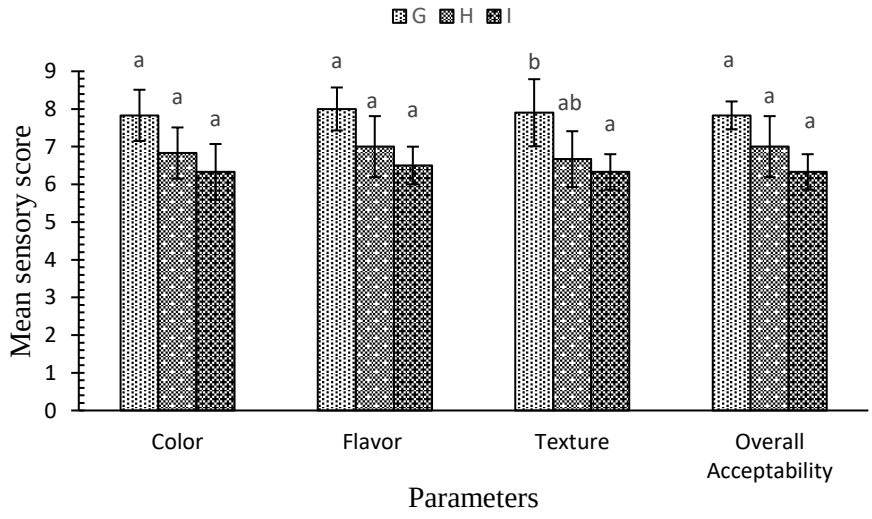

Fig 4.3 Optimization of CMC for preparation of yoghurt

Fig. 4.3 represents the mean sensory scores for color, flavor, texture, and overall acceptability for 0.1%, 0.2% and 0.3% CMC conc. in yoghurt. Values on the top of the bars bearing similar

superscript are not significantly different at 5% level of significance. The vertical error bar represents ±standard deviation of scores given by panelist.

Where, Sample G was yoghurt containing 0.1% CMC, Sample H was yoghurt containing 0.2% CMC and Sample I was yoghurt containing 0.3% CMC.

In terms of superiority at 5% LSD of the formulations with respect to appearance/color, flavor, texture/mouthfeel, and overall acceptability, following conclusion can be drawn:

Appearance/color: Sample G≥ Sample H≥ Sample I

Flavor: Sample G≥ Sample H≥ Sample I

Texture/mouthfeel: Sample G≥ Sample E≥ Sample I

Overall acceptability: Sample G≥ Sample H≥ Sample I

Based on the frequency of occurrence as 'best' in each attribute type, Sample G appeared to be the best formulation. Hence, Sample G (0.1%) was taken for further study.

4.5 Comparison of yoghurt prepared from pectin, guar gum and CMC

Sensory evaluation of all three formulations of the product which were carried out by a group of six semi-trained panelists evaluating color, flavor, texture, and overall acceptance of prepared yoghurt. The Analysis of Variance (ANOVA) was carried out using the least significant difference (LSD) at 5% level of significance.

4.5.1 Color

Regarding color of the prepared yoghurt, the analysis showed that the mean sensory scores for sample P, GG and CMC were found to be 7.5, 6.17 and 8.5 respectively. Statistical analysis showed that the effect of different stabilizers on color of the product was significant ($p<0.05$). LSD at 5% level of significance indicated that the sample CMC was significantly different from the rest of the sample and had the highest score.

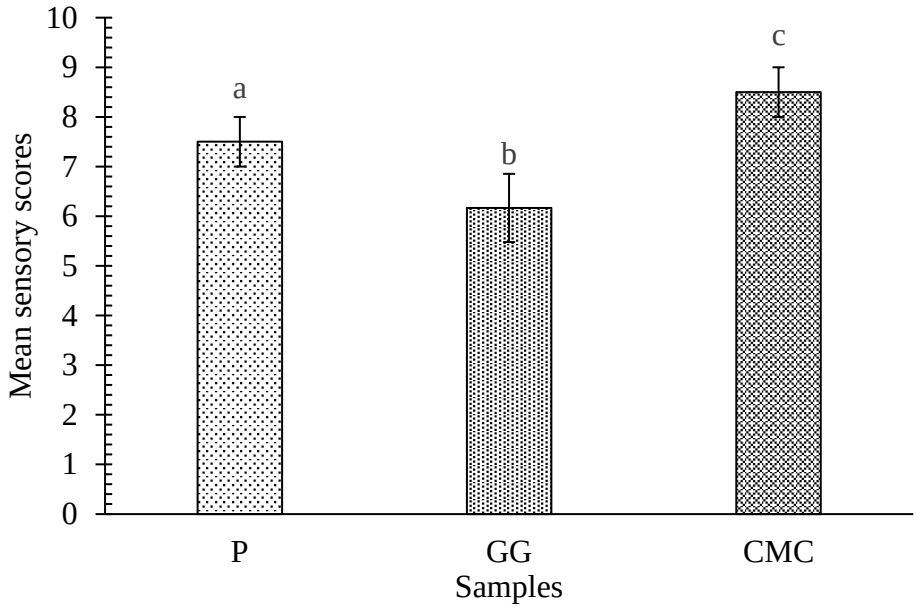

Fig 4.4 Effect of stabilizers on color of yoghurt

Fig. 4.4 represents the mean sensory scores for color of yoghurt. Values on the top of the bars bearing similar superscript are not significantly different at 5% level of significance. The vertical error bar represents ±standard deviation of scores given by panelist.

4.5.2 Flavor

Regarding flavor of the prepared yoghurt, the analysis showed that the mean sensory scores for sample P, GG and CMC were found to be 7.83, 5.83 and 8 respectively. Statistical analysis showed that the effect of different stabilizers on flavor of the product was significant ($p<0.05$). LSD at 5% level of significance indicated that the sample CMC was significantly different from the rest of the sample and had the highest score.

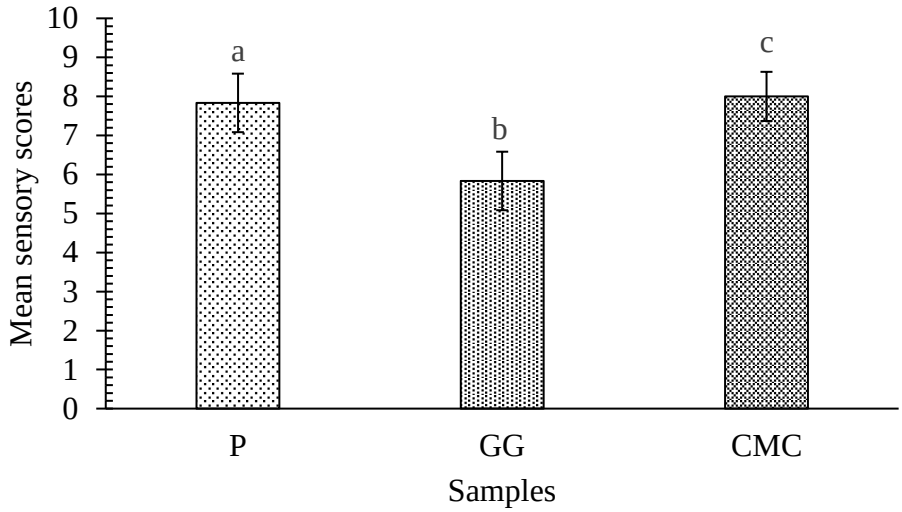

Fig 4.5 Effect of stabilizers on flavor of yoghurt

Fig. 4.5 represents the mean sensory scores for flavor of yoghurt. Values on the top of the bars bearing similar superscript are not significantly different at 5% level of significance. The vertical error bar represents ±standard deviation of scores given by panelist.

4.5.3 Texture

Regarding texture of the prepared yoghurt, the analysis showed that the mean sensory scores for sample P, GG and CMC were found to be 7, 5.5 and 7.83 respectively. Statistical analysis showed that the effect of different stabilizers on texture of the product was significant ($p<0.05$). LSD at 5% level of significance indicated that the sample CMC was significantly different from the rest of the sample and had the highest score.

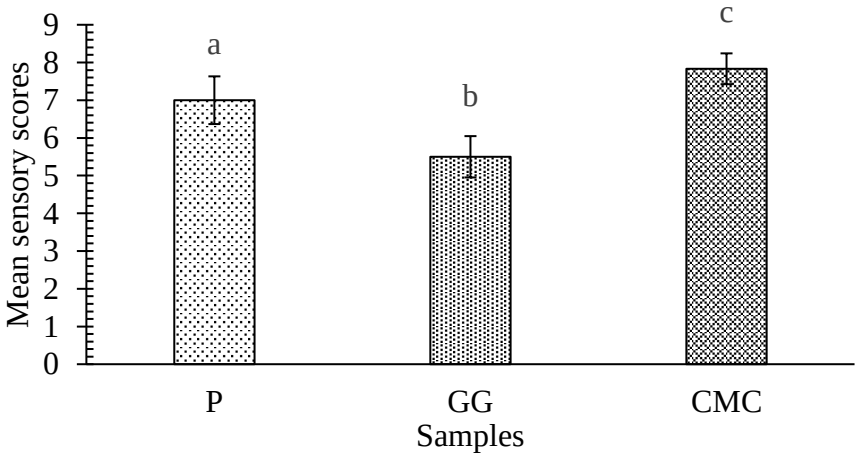

Fig 4.6 Effect of stabilizers on texture of yoghurt

Fig. 4.6 represents the mean sensory scores for texture of yoghurt. Values on the top of the bars bearing similar superscript are not significantly different at 5% level of significance. The vertical error bar represents ±standard deviation of scores given by panelist.

4.5.4 Overall acceptability

Regarding overall acceptability of the prepared yoghurt, the analysis showed that the mean sensory scores for sample P, GG and CMC were found to be 7.33, 5.83 and 8.17 respectively. Statistical analysis showed that the effect of different stabilizers on overall acceptability of the product was significant ($p<0.05$). LSD at 5% level of significance indicated that the sample CMC was significantly different from the rest of the sample and had the highest score.

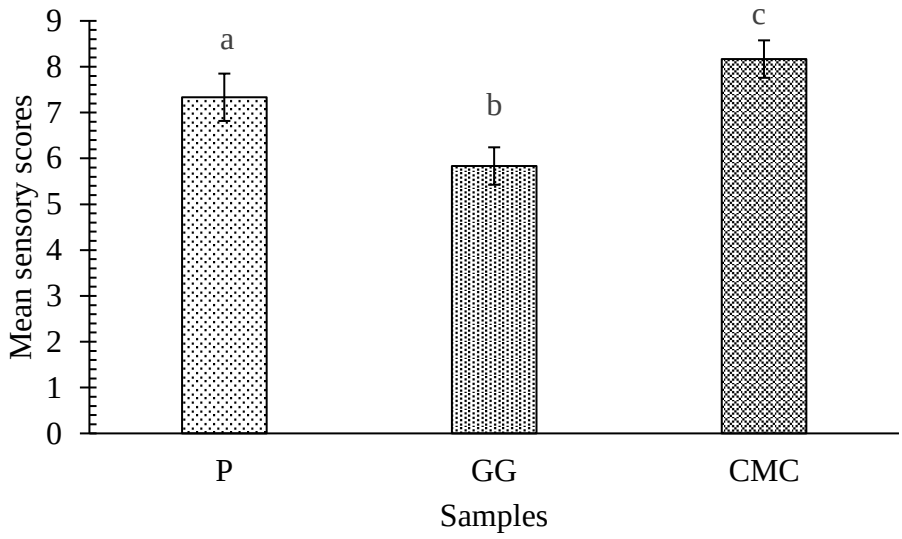

Fig 4.7 Effect of stabilizers on overall acceptability of yoghurt

Fig. 4.7 represents the mean sensory scores for overall acceptability of yoghurt. Values on the top of the bars bearing similar superscript are not significantly different at 5% level of significance. The vertical error bar represents ±standard deviation of scores given by panelist.

4.6 Chemical analysis of yoghurt

Two samples of yoghurt were prepared, one with 0.1% CMC added as a stabilizer and the other without; both samples contained 2% skim milk powder and 4% sugar. After 4 hours of incubation, both yoghurt samples were placed in refrigeration (5°C), and after one day, they were tested chemically for acidity, pH, protein, fat, ash, and lactose. The results were displayed in table 4.5.

Table 4.2 Chemical analysis of yoghurt

Parameters	Sample CMC (Best)	Sample C (Control)
Acidity (% as lactic acid)	0.72^{a}±0.021	0.84^{b}±0.017
Ash (%, db)	0.84^{a}±0.016	0.85^{a}±0.041
Fat (%, db)	2.57^{a}±0.047	2.77^{b}±0.047
Protein (%, db)	3.03^{a}±0.124	2.93^{a}±0.125
Moisture	83^{a}±0.816	84.33^{a}±1.247
pH	4.5^{b}±0.082	4.33^{a}±0.047
Lactose (%, db)	3.77^{b}±0.047	3.53^{a}±0.047

*Values in the table are arithmetic mean of triplicate samples. Figures in the parentheses indicate standard deviation. Values in the column having different superscripts are significantly different at 5% level of significance.

Sample C had a significantly higher acidity than Sample CMC. Similar result was observed in Alakali *et al.* (2008).

Sample CMC had a significantly higher pH than Sample C. Yoghurt's pH increases with the addition of CMC due to a decrease in total H+ ion concentration with the reduction of total acid. This is due to the inhibition of bacterium motility, which limits the culture activities of yoghurt. The result was similar to Sebayang (2019).

There was no significant change in the ash content between Sample C and Sample CMC. Similar result was observed in Alakali *et al.* (2008).

Sample C had significantly higher fat content than Sample CMC. The effects of CMC stabilizers of various concentrations result in a decrease in fat content as CMC concentration increases owing to the dilution effect. The dilution effect occurs due to the presence of stabilizer material, which diminishes nutritional content such as fats. The amount of stabilizer applied determines the dilution level. The result was similar to Sebayang (2019).

There was no significant change in the moisture content between Sample C and Sample CMC. Moisture of Sample C found in our study was comparable to the data reported by Matela *et al.* (2019).

Sample CMC had somewhat higher protein content compared to Sample C. This is in line with the assertion made by Fardiaz (1986), who claimed that CMC can improve viscosity and decrease protein precipitation at isoelectric points because of the interaction between carboxyl CMC and functional groups from proteins that carry a positive charge.

Sample CMC had higher lactose content than Sample C. The lactose content of yoghurt from Sample C was comparable to that reported by Gaglio *et al.* (2019).

4.7 Study of storage stability of best sample and control w.r.t pH and syneresis

Yoghurt samples containing 0.1% CMC were found best with respect to appearance/color, flavor, texture/mouthfeel, and overall acceptability which were used for preparation of yoghurt for further study. Hence yoghurt samples containing 0.1% CMC as Yoghurt CMC and without stabilizer as Yoghurt C (control) were subject for chemical analysis with respect to pH and syneresis under refrigerated condition.

4.7.1 Relation between pH of yoghurt sample under refrigeration

Yoghurt samples labelled as Sample C and Sample CMC were prepared and subjected for pH determination from day one to day 11 with two days interval under refrigerated condition and their relation were shown in fig 4.8

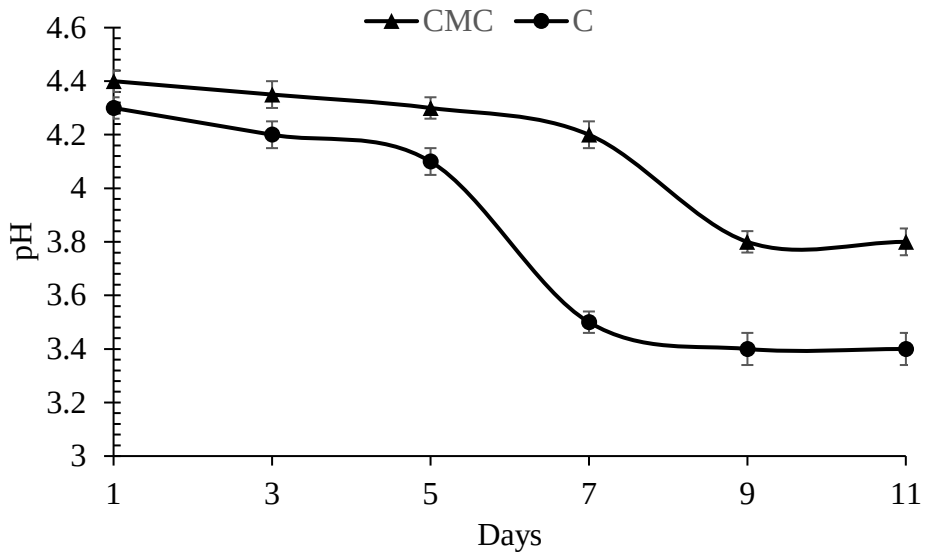

Fig 4.8 Relation between pH of yoghurt Sample C and Sample CMC with time

In fig 4.8, vertical bars represent the standard deviation.

The mean values of pH of yoghurt Sample C for day 1, 3, 5, 7, 9 and 11 were found to be 4.3, 4.2, 4.1, 3.5, 3.4 and 3.4 respectively whereas that of Sample CMC were found to be 4.4, 4.35, 4.3, 4.2, 3.8 and 3.8 respectively. It was observed that pH was significantly lower in Sample C than Sample CMC due to the formation of lactic acid with respect to storage time. Similar result was also observed by Andiç *et al.* (2013). Yoghurt samples were suitable for consumption for 11 days under refrigerated condition.

4.7.2 Relation between syneresis of yoghurt sample under refrigeration

Yoghurt samples labelled as Sample C, Sample P, Sample GG and Sample CMC were prepared and subjected for syneresis determination from day one to day 11 with two days interval under refrigerated condition and their relation were shown in fig 4.9

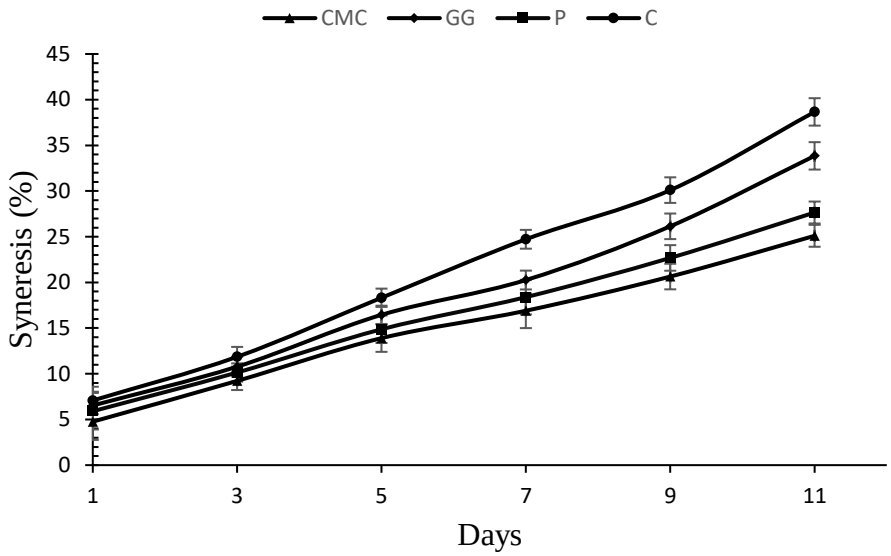

Fig 4.9 Relation between syneresis of yoghurt samples with time

In fig 4.9, vertical bars indicate the standard deviation.

The mean values of syneresis of yoghurt Sample C for day 1, 3, 5, 7, 9 and 11 were found to be 7.08, 11.86, 18.32, 24.72, 30.1 and 38.66 percentage respectively. Also, the mean values for Sample P were found to be 5.9, 10.14, 14.87, 18.39, 22.68 and 27.65 percentage respectively. Furthermore, the mean values for Sample GG were found to be 6.53, 10.78, 16.45, 20.26, 26.14 and 33.85 percentage respectively whereas that of Sample CMC were found to be 4.76, 9.22, 13.9, 16.9, 20.64 and 25.1 percentage respectively. It was observed that syneresis of Sample C was significantly higher than Sample CMC. With respect to time, syneresis was stable on Sample CMC than Sample C. Kumar and Mishra (2004) reported that the type and concentration of stabilizer (gelatin, sodium alginate, and pectin at concentrations of 0.20, 0.40, and 0.60%) had a significant impact on the level of syneresis, with the highest stabilizer concentration causing the lowest level of syneresis in mango soy fortified set type yoghurt. Yoghurt samples were suitable for consumption for 11 days under refrigerated condition.

4.8 Microbial analysis

Yoghurt samples labelled as Sample CMC and Sample C were prepared and subjected for microbial count from day one to day 11 with two days interval under refrigerated condition and their relation were shown in fig 4.10

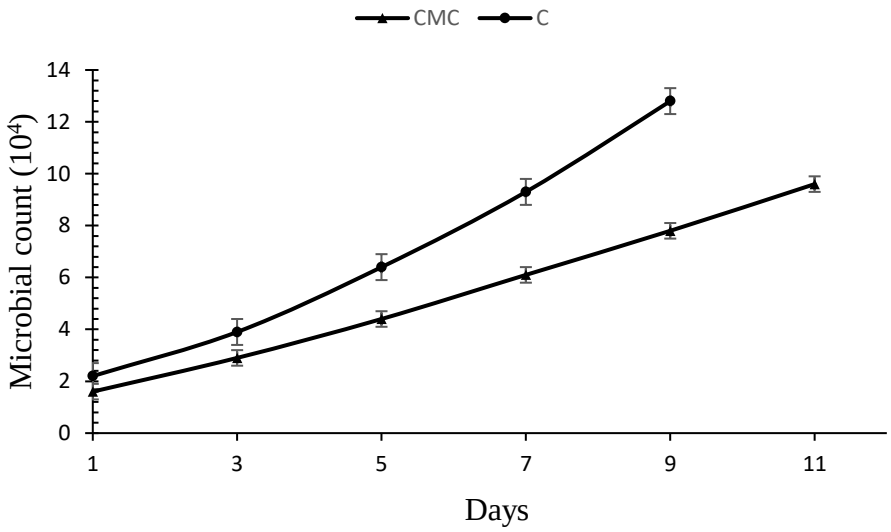

Fig 4.10 Microbial count under refrigeration of Sample CMC and Sample C with time

In fig 4.10, vertical bars indicate standard deviation.

Microbial analysis of the best yoghurt was carried out by observing the total plate count (TPC). TPC in yoghurt gradually increased from 1.6×10^4 to 9.6×10^4 CFU/ml after 11 days of refrigerated storage in Sample CMC whereas Sample C showed significant increment from 2.2×10^4 to 12.8×10^4 CFU/ml after 9 days. The increase in TPC of yoghurt is related to the formation of lactic acid even at low temperatures. The results are in agreement with Ahmed (2011). Similar results were obtained by De *et al.* (2014) for the consumable range of total bacterial count as in the range of (3.0×10^3-10.5×10^4 CFU\ml). Yoghurt samples were suitable for consumption up to 11 days.

4.9 Cost evaluation

The total cost of the best yoghurt was calculated. It is shown in appendix B. The price for 100 ml yoghurt was found to be NRs.14.3.

Part V

Conclusion and recommendations

5.1 Conclusion

Based on the research work conducted, the following conclusions can be concluded:

1. From the sensory evaluation of the product conducted on the attributes like appearance/color, flavor, texture/mouthfeel and overall acceptability, yoghurt samples treated each with 0.1% guar gum, CMC and 0.3% pectin were found to be better.
2. On comparison, 0.1% CMC was found to be better than 0.3% pectin and 0.1% guar gum among other attributes on sensory evaluation of the product.
3. The protein, fat, acidity, lactose, ash, moisture, and pH of best product were found 3.13%, 2.57%, 0.72%, 3.77%, 0.84%, 83% and 4.5 respectively.
4. Syneresis was significantly lower in yoghurt treated with CMC than without stabilizer whereas pH was significantly higher ($p<0.05$). Syneresis was increased with storage time, but pH was decreased significantly.
5. TPC was significantly lower in Sample CMC than Sample C ($p<0.05$). Yoghurt samples were suitable for consumption up to 11 days at refrigerated temperature.
6. The cost of 100ml yoghurt with 0.1% CMC as stabilizer was NRs.14.3.

5.2 Recommendations

From the above research work, following suggestions were recommended for future works:

1. Yoghurt can be prepared by using 0.1% CMC with respect to sensory attributes and syneresis.
2. Other more stabilizers can be used for the preparation of yoghurt.
3. Yoghurt can be prepared by blending different proportions of stabilizer.

Part VI

Summary

Yoghurt is a cultured dairy product produced by fermenting milk, with or without added non-fat dry milk (NFDM) with *Lactobacillus bulgaricus* and *Streptococcus thermophilus* bacteria. Milk was collected and analyzed for proximate composition. The mean value of total soluble solid, acidity, lactose, protein, fat, and ash content of the milk were found to be 12.67, 0.13, 4.43, 3.3, 2.93 and 0.69 percentages respectively while the pH was found to be 6.56.

Milk was preheated to 45ºC and skim milk powder was added at the rate of 2% with proper stirring. Then, milk was again heated to 65-70ºC and sugar was added at the rate of 4% and stirred well. Stabilizers such as pectin, guar gum and carboxymethyl cellulose were added at the rate of 0.1, 0.2 and 0.3% each. After that, pasteurization was done at 85- 90ºC for about 30 minutes. The pasteurized milk was cooled to 44ºC, and 2% starter culture was inoculated. It was then incubated at 44ºC for about 2-3 hours until the coagulum was formed. The set type yoghurt thus obtained was cold stored at 4-8ºC. Control was prepared without the addition of stabilizer.

Sensory evaluation based on appearance/color, flavor, texture/mouthfeel, and overall acceptability was carried out for the optimization of stabilizers. Yoghurt samples with 0.3% pectin, 0.1% guar gum and 0.1% CMC were found to be best with respect to sensory evaluation. After that, comparison of yoghurt with optimized stabilizers was carried out based on appearance/color, flavor, texture/mouthfeel, and overall acceptability where yoghurt prepared from 0.1% CMC was found to be best among them. Yoghurt containing 0.1% CMC and control (without stabilizer) were compared with respect to physiochemical analysis to determine the shelf life of yoghurt.

The shelf life of the best product was estimated with respect to pH, syneresis and TPC. pH decreased from 4.3 to 3.4 in case of Sample C and 4.4 to 3.8 in case of Sample CMC whereas syneresis was increased from 7.08 to 38.66% in case of Sample C and 4.76 to 25.1% in case of Sample CMC from day 1 to 11 storage period. TPC in Sample CMC was significantly less than Sample C. Therefore, yoghurt samples treated with stabilizer can be stored for about 11 days by keeping the sensorial attributes.

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Appendices

Appendix-A

Sensory evaluation card

Name: ……. Date: …….

Product: Yoghurt

Observe the product based on sensorial attributes. Use appropriate scale to show your attitude by checking at the point that best describes your feeling of the product. An honest expression of your personnel feeling will help you to choose the right product.

Quality description

1= Dislike extremely 4= Dislike slightly 7= Like moderately

2= Dislike very much 5= Neither like nor dislike 8= Like very much

3= Dislike moderately 6= Like slightly 9= Like extremely

Table A.1 Sensorial panelist is requested to give ranks on their individual choice.

Sample	**Color**	**Flavor**	**Texture**	**Overall acceptability**
A				
B				
C				
D				
E				

Comments (if any): ……...

Signature: …….

Appendix B

Table B.1 Cost evaluation of 100ml of yoghurt with 0.1% CMC

Particulars	**Quantity**	**Rate (NRs)**	**Amount (NRs)**
Milk	100 ml	85/ltr	8.5
Sugar	4 g	95/kg	0.38
SMP	2 g	980/kg	1.96
CMC	0.1g	2800/kg	0.28
Plastic cup	1 pc	80/100pcs	0.8
Sub total			11.92
Overhead cost	20%		2.38
Total			14.3

Appendix C

Table C.1 ANOVA output of sensory scores of appearance/color, flavor, texture/mouthfeel, and overall acceptability of yoghurt samples treated with 0.1%, 0.2%, and 0.3% pectin at 5% level of significance (two way no blocking).

Attributes	**0.1% pectin**	**0.2% pectin**	**0.3% pectin**	**LSD**	**F pr.**
Color	6.33	6.83	7.17	0.650	0.049
Flavor	6.83	6.50	7.17	0.939	0.328
Texture	6.50	7.0	7.5	0.730	0.157
Overall acceptability	6.67	7.17	7.5	0.559	0.023

Table C.2 ANOVA output of sensory scores of appearance/color, flavor, texture/mouthfeel, and overall acceptability of yoghurt samples treated with 0.1%, 0.2%, and 0.3% guar gum at 5% level of significance (two way no blocking).

Attributes	**0.1% guar gum**	**0.2% guar gum**	**0.3% guar gum**	**LSD**	**F pr.**
Color	6.50	5.83	5.67	1.076	0.237
Flavor	6.50	6.50	6.17	1.068	0.732
Texture	6.82	5.98	5.47	0.868	0.455
Overall acceptability	6.67	5.83	5.83	1.093	0.196

Table C.3 ANOVA output of sensory scores of appearance/color, flavor, texture/mouthfeel, and overall acceptability of yoghurt samples treated with 0.1%, 0.2%, and 0.3% CMC at 5% level of significance (two way no blocking).

Attributes	**0.1% CMC**	**0.2% CMC**	**0.3% CMC**	**LSD**	**F pr.**
Color	7.83	6.83	6.33	1.024	0.105
Flavor	8.0	7.0	6.5	1.076	0.103
Texture	7.9	6.67	6.33	1.093	0.065
Overall acceptability	7.83	7.0	6.33	0.802	0.022

Appendix D

Table D.1 ANOVA (no blocking) for appearance/color of yoghurt

Source of variation	**d.f.**	**s.s**	**m.s.**	**v.r.**	**F pr.**	**l.s.d**
Sample	2	16.44444	8.22222	92.50	<.001	0.3835
Panelist	5	4.94444	0.98889	11.13	<.001	0.5424
Residual	10	0.88889	0.08889			
Total	17	22.27778				

Table D.2 ANOVA (no blocking) for flavor of yoghurt

Source of variation	d.f.	s.s	m.s.	v.r.	F pr.	l.s.d
Sample	2	17.4444	8.7222	27.07	<.001	0.730
Panelist	5	4.4444	0.8889	2.76	0.081	1.033
Residual	10	3.2222	0.3222			
Total	17	25.1111				

Table D.3 ANOVA (no blocking) for texture/mouthfeel of yoghurt

Source of variation	d.f.	s.s	m.s.	v.r.	F pr.	l.s.d
Sample	2	16.7778	8.3889	44.41	<.001	0.559
Panelist	5	2.4444	0.4889	2.59	0.094	0.791
Residual	10	1.8889	0.1889			
Total	17	21.1111				

Table D.4 ANOVA (no blocking) for overall acceptability of yoghurt

Source of variation	**d.f.**	**s.s**	**m.s.**	**v.r.**	**F pr.**	**l.s.d**
Sample	2	16.7778	8.3889	68.64	<.001	0.4497
Panelist	5	1.7778	0.3556	2.91	0.071	0.6360
Residual	10	1.2222	0.1222			
Total	17	19.7778				

Appendix E

Table E.1 t-test (two sample assuming unequal variances) for acidity of best sample with control

	Sample C	**Sample CMC**
Mean	0.843333	0.723333
Variance	0.000433	0.000633
Observations	3	3
Hypothesized Mean Difference	0	
df	4	
t stat	6.363961	
P (T≤t) one-tail	0.001563	
t Critical one-tail	2.131847	
P (T≤t) two-tail	0.003126	
t Critical two-tail	2.776445	

Table E.2 t-test (two sample assuming unequal variances) for pH of best sample with control

	Sample C	**Sample CMC**
Mean	4.333333	4.5
Variance	0.003333	0.01
Observations	3	3
Hypothesized Mean Difference	0	
df	3	
t stat	-2.5	
P (T≤t) one-tail	0.043853	
t Critical one-tail	2.353363	
P (T≤t) two-tail	0.087707	
t Critical two-tail	3.182446	

Table E.3 t-test (two sample assuming unequal variances) for protein of best sample with control

	Sample C	**Sample CMC**
Mean	2.933333	3.033333
Variance	0.023333	0.023333
Observations	3	3
Hypothesized Mean Difference	0	
df	4	
t stat	-1.60357	
P (T≤t) one-tail	0.092037	
t Critical one-tail	2.131847	
P (T≤t) two-tail	0.184074	
t Critical two-tail	2.776445	

Table E.4 t-test (two sample assuming unequal variances) for fat of best sample with control

	Sample C	**Sample CMC**
Mean	2.766667	2.566667
Variance	0.003333	0.003333
Observations	3	3
Hypothesized Mean Difference	0	
df	4	
t stat	4.242641	
P (T≤t) one-tail	0.006618	
t Critical one-tail	2.131847	
P (T≤t) two-tail	0.013236	
t Critical two-tail	2.776445	

Table E.5 t-test (two sample assuming unequal variances) for ash of best sample with control

	Sample C	**Sample CMC**
Mean	0.853333	0.84
Variance	0.002533	0.0004
Observations	3	3
Hypothesized Mean Difference	0	
df	3	
t stat	0.426401	
P (T≤t) one-tail	0.349281	
t Critical one-tail	2.353363	
P (T≤t) two-tail	0.698562	
t Critical two-tail	3.182446	

Table E.6 t-test (two sample assuming unequal variances) for moisture of best sample with control

	Sample C	**Sample CMC**
Mean	84.33333	83
Variance	2.33333	1
Observations	3	3
Hypothesized Mean Difference	0	
df	3	
t stat	1.264911	
P (T≤t) one-tail	0.147614	
t Critical one-tail	2.353363	
P (T≤t) two-tail	0.295229	
t Critical two-tail	3.182446	

Table E.7 t-test (two sample assuming unequal variances) for lactose of best sample with control

	Sample C	**Sample CMC**
Mean	3.533333	3.766667
Variance	0.003333	0.003333
Observations	3	3
Hypothesized Mean Difference	0	
df	4	
t stat	-4.94975	
P (T≤t) one-tail	0.003881	
t Critical one-tail	2.131847	
P (T≤t) two-tail	0.007763	
t Critical two-tail	2.776445	

Appendix F

Table F.1 List of equipment used

Physical apparatus	Physical apparatus
Incubator	Thermometer
Hot air oven	Heating arrangement
Muffle furnace	Refrigerator
Gerber centrifuge	Stainless steel vessels
Gerber butyrometer	Titration apparatus
Desiccators	Kjeldahl digestion and distillation set
Refractometer	Electric balance
Daily routine glassware	

Table F.2 List of chemicals used

Chemicals	Chemicals
40% formaldehyde	pH buffer solution
Starter Culture	Distilled water
0.1N NaOH solution	Carrez-I
Saturated potassium oxalate	Carrez-II
0.0005% fuchsin solution	Phenolphthalein indicator
Conc. H_2SO_4 solution	Methylene blue indicator
Conc. HCl solution	Copper sulphate solution
Boric acid	Amyl alcohol

Color Plates

Fig: Protein Determination

Fig : Proximate Analysis

Fig : Incubation of yoghurt

Fig : Kjeldahl Distillation Set