PREPARATION AND SHELF LIFE STUDY OF CINNAMON OLEORESIN INCORPORATED YOGHURT

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Preparation and Shelf life study of Cinnamon Oleoresin Incorporated Yoghurt

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

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Approval Letter

This *dissertation* entitled *Preparation and Shelf life study of Cinnamon Oleoresin Incorporated Yoghurt* presented by Gyanu Khadka has been accepted as the partial fulfillment of the requirements for the B. Tech. in Food Technology.

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Abstract

The yogurt was prepared using cow milk with different concentration of cinnamon with 2% starter culture of *Lactobacillus acidophilus* and *Streptococcus thermophilus*. Design expert® version 10 one factorial design was employed for the formulating the recipe of yoghurt. The obtained five formulations coded A (0 ml), B (0.1 ml), C (0.2 ml), D (0.3 ml) & E (0.4 ml) of cinnamon oleoresin incorporated yoghurt were prepared in laboratory. The samples were subjected to sensory evaluation by 9 points hedonic scoring method. Based on these quality parameter and sensory analysis score, the data were analyzed by two ways ANOVA (no blocking) using Genstat and means were compared using LSD at 5% level of significance.

From sensory evaluation, formulation of 0.1 ml cinnamon incorporated yoghurt was found to be significantly ($p<0.05$) best using LSD at 5% level of significance. The total solid, fat, acidity, protein, total ash, lactose content pH and polyphenol content were found 16.02%, 4.43%, 0.6%, 4.64%, 0.6%, 3.52%, 4.71 and 103.36 mg/g sample respectively. Shelf life of the best product was estimated in terms of pH, acidity, syneresis and total microbial count. The product having microbial count greater than 10.5×10^4 cfu\ml respectively was unacceptable. From this statement we can conclude that the shelf life was found to be 5 days at room temperature and 10 days at refrigeration.

Keywords: yoghurt, cinnamon, sensory analysis, syneresis, shelf life

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List of Abbreviations

Abbreviations	Full form
ANOVA	Analysis of variance
CFU	Colony Forming Unit
DM	Dry Matter
EPS	Exopolysaccharide
FAO	Food and Agriculture Organization
IDF	International Dairy Federation
LSD	Least Significant Difference
MSNF	Milk Solid Not Fat
PAL	Phenylalanin ammonia lyase
SNF	Solid Not Fat
TPC	Total Plate Count
UHT	Ultra High Temperature
WHO	World Health Organization

Part I

Introduction

1.1 General introduction

Milk is regarded as being nature's most complete food. It earns this reputation by providing many of the nutrients which are essential for the growth of the human body. Being an excellent source of protein and having an abundance of vitamins and minerals, particularly calcium, milk can make a positive contribution to the health of a nation. The realization of its nutritional attributes is clearly illustrated by the implementation of numerous 'school milk programmes' worldwide (Fellows and Hampton, 2000).

Fermented-milk products such as yoghurt and soured milk contain bacteria from the Lactobacilli group. These bacteria occur naturally in the digestive tract and have a cleansing and healing effect. Therefore the introduction of fermented products into the diet can help prevent certain yeasts and bacteria which may cause illness (Fellows and Hampton, 2000). Fermented milk products have been reported to have a positive effect on the human digestive system and are also implicated in the control of serum cholesterol. Both milk protein and lactose in fermented milk are more easily digestible than those in the original milk. Proteins are partly degraded by the action of the bacterial proteolytic system. The lactose content is lower than in the parent milk, as part of it is converted to lactic acid and/or alcohol. Lactic acid gives rise to the characteristic sour taste associated with fermented products. Yoghurt and fermented milks may contain more folate than the original milk because some strains of lactic acid bacteria also synthesize folate. Fermentation not only makes milk more digestible, but is also a means of increasing the shelf-life and microbiological safety of the products (Muehlhoff *et al.*, 2013).

Yogurt is one of the popular fermented milk products having different names and forms (Tamime and Robinson, 2007). It is a mixture of milk (whole, low-fat, or nonfat) and even cream fermented by a culture of lactic acid-producing bacteria, *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophilus*. Other bacteria may be added to the culture. The popularity of yogurt is due to various health claims and therapeutic values. Along with these, the flavor of yogurt has played an important role in increasing its consumer demand. Sweeteners (for example, sugar, honey, and aspartame), flavorings (for example, vanilla and coffee), and other ingredients (for example, fruits, spices, preserves, and stabilizers such as gelatin to improve the textural property) are added that modify the flavor of yogurt. The aroma, body, and taste of yogurt and other cultured dairy products can vary depending on the type of culture and milk, amount of milk fat and nonfat milk solids, fermentation process, and temperature used (Routray and Mishra, 2011).

1.2 Statement of problems

With the development of processing technologies and the growing competition in the food market, the urge to provide nutritious food with appealing flavor properties has increased. Yogurt is a basic dairy product that has been consumed for centuries as a part of the diet, even when its beneficial effects were neither fully known nor scientifically proven (Routray and Mishra, 2011). In recent years, there has been a significant increase in the popularity of yogurt as a functional food. Yogurt is a conventional food known for its therapeutic, nutritional and sensory properties and is most popular and preferred vehicle for probiotic culture. The quality of yogurt products and acceptance by consumers are largely determined by the rheological, physical properties and sensorial properties of yogurt (Patel, 2011). Different types of fruit flavors like strawberry, apple etc. are being used during yoghurt manufacturing (Routray and Mishra, 2011).

Spices like cinnamon in the form of oleoresins have the full flavor, aroma and pungency of fresh or dried spices and oleoresins are used at very low concentrations because they are highly concentrated. They give more uniform flavor with less variability than their ground spice counterparts without changing the colour or texture of the final products. Singh *et al.* (2011) reported that addition of anise oleoresins to plain yogurt do not have any adverse effect on the physicochemical properties. They were also reported to possess good antioxidant properties (Singh *et al.*, 2011). But oleoresins are sensitive to environmental factors such as oxygen, light and moisture. So, the yoghurt incorporated with oleoresins of cinnamon, their quality and storage stability were studied in this study to know about the effect on quality of cinnamon incorporated yoghurt during storage. Similarly, the effect of cinnamon on pH, acidity, TPC were also studied in our study.

1.3 Objectives

1.3.1 General objectives

The general objective of the study was to prepare yoghurt incorporated with cinnamon and to study its shelf life.

1.3.2 Specific objectives

The specific objectives were:

1. To optimize the cinnamon proportion for the preparation yoghurt.
2. To study the physical and chemical properties of thus prepared yoghurt.
3. To study the microbial quality of thus prepared yoghurt.
4. To study the shelf life period of thus prepared yoghurt.

1.4 Significance of study

The search for unique food ingredients and flavours with enhanced health properties is at present one of the key global market trends (Netzel *et al.*, 2007). Spices are a novel source of functional flavoring agents. There is now mounting scientific evidence of health benefits of spices including antioxidant, antimicrobial, anti-inflammatory and anticarcinogenic properties (Srinivasan, 2004). The popularity of yogurt as a food component depends mainly on its sensory characteristics, of which aroma and taste are most important (Routray and Mishra, 2011). Since, spices contains antioxidant properties, flavoring compounds and other health benefits this type of research may be proven beneficial to consumers by improving the flavor of yoghurt as well as other health benefits of yoghurt. The introduction of flavored yoghurt in market would meet the consumer choice and increase the consumption of yoghurt.

1.5 Limitations

i. Texture of yoghurt was determined only by sensory due to the absence of equipment.
ii. Yeast, mold count and coliform was not studied.
iii. Variation of sugar and skim milk could not be carried out.

Part II

Literature review

2.1 Milk

Milk is a lacteal secretion of mammary gland of milch animals. It is composed of lipids, carbohydrates, proteins and other many organic compounds and inorganic salts dissolved or dispersed in water. Lipid is composed primarily of fat although there are small amount of phospholipids, sterols, fat soluble vitamins A and D, carotene and xanthophylls. Protein content of milk is classified as a) casein, b) lactalbumin and c) lactoglobulin. Lactose is the carbohydrate in the milk (Meyer, 1960).

Different salt and minerals are found in the milk. Plentiful vitamins are present but vitamin C is limiting. Milk contains a number of enzymes; some of them apparently secreted in the milk and other are formed by microorganism (Meyer, 1960).

2.2 Types of fermented milk

2.2.1 Traditional fermented milk

Fermented milks were prepared in India in 800 B.C. and in Vedic period. Fermented milks have been prepared since ancient times in Mongolia, Tibet and in the Middle East. In the sub-arctic regions, the Laplanders also prepared small quantities of fermented milks. The Yakuts (Russia) also prepared fermented milks called *koumys* (Tamrakar, 2017).

2.2.2 Non-traditional fermented milk

The non-traditional fermented milk appeared after 1900. They were new products under the names: biograde, bioghurt, bifighurt, biokys, progurt, yakult, acidophilus milk, bifidum milk cultured butter milk etc. A certain numbers of products with artificial or enzyme acidification can be found outside the usual standards of fermented dairy products. A more important development is the use of human intestinal germs for the preparations of fermented milk which includes bacteria like *Lactobacillus acidophilus* and *Bifidobacterium bifidum* (Kurmann, 1983)

2.3 Advantages of milk fermentation

The most important advantages of fermented foods are:

- Keeping milk from spoiling by undesirable bacteria which is due to the accumulation of lactic acid and other antibacterial metabolites during fermentation.
- Production of variety in foods that is accomplished by change in body, texture and flavor.
- Digestibility of fermented product, especially protein, is improved and this may be important in people with digestive disorders.
- In some instances, the process of fermentation may reduce the bulk, the starting material and these results in the increased storage life of products. Examples are traditional fermented milk-cereal mixture which are dried and can be easily transported from one area to another (Vedamuthu, 1982).
- Fermented milk products contain antibiotics produced by microorganisms used as culture, which cause adverse effect on the harmful microorganisms present in the intestine and controls their growth.
- Some fermented milk products are useful for the nutritional treatment of some diseases like dysentery, gastritis, anemia, kidney stones etc.
- Fermented milk such as yoghurt has ability to increase weight than milk feeding. (Hargrove and Alford, 1978)

2.4 Yoghurt

2.4.1 Historical background

The history of yogurt goes back over six thousand years. It is believed that the word “yogurt” evolved from the Turkish word *jugurt* (Rasic and Kurmann, 1978). Today, yogurt is known by different names in different regions in the world. In Iraq, it is known as roba and Finland it is calledfiili. It is assumed that limited availability of milk due to dry desert surroundings in Middle East led to development of a yogurt like product. In Turkey, it was thought to be consumed as a preserved milk product (Tamime and Robinson, 1985). Traditionally, Greek yogurt is prepared from ewe’s milk, yet cow milk is used commercially. In South Asia the yogurt is called dahi, and it exhibits soft coagulum, lumpy texture and mild acidic flavor. In India, raita is made from dahi with addition of grated cucumber or grated bottle gourd, black pepper, cumin seeds and coriander. Bulgarian yogurt has a unique flavor and taste due to different microbial strains in the yogurt preparation. In Indonesia different varieties of yogurt called dadiah are prepared by fermenting milk in a bamboo container surrounded with banana leaves. Taratur is a variety of yogurt made in Albania and Republic of Macedonia by mixing yogurt with vegetables, walnuts, garlic, oil, and water. *Rahmjoghurt*, yogurt with higher milk fat content (10%), is produced in Germany and other European countries. Matsoni is another variety of yogurt product made by using *Lactococcus lactis* which gives it a distinctive viscous texture. In Middle Eastern countries, such as Jordan and Palestine, yogurt named Jameed is combined with salt and dried for preservation (Anon., 2009).

2.4.2 Raw materials used for yoghurt preparation

In general, yogurt is made with a variety of ingredients including milk, sugars, stabilizers, fruits and flavors, and a bacterial culture *(Lactobacillus bulgaricus)*. During fermentation, these organisms interact with the milk and convert it into a curd. They also change the flavor of the milk giving it the characteristic yogurt flavor of which acetaldehyde is one of the important contributors. The primary byproduct of the fermentation process is lactic acid. The acid level is used to determine when the yogurt fermentation is completed which is usually three to four hours. The suppliers of these yogurt cultures offer various combinations of the two bacterial types to produce yogurts with different flavors and textures (Anon., 2018).

To modify certain properties of the yogurt, various ingredients may be added. To make yogurt sweeter, sucrose (sugar) may be added at approximately 7%. For reduced calorie yogurts, artificial sweeteners such as aspartame or saccharin are used. Cream may be added to provide a smoother texture. The consistency and shelf stability of the yogurt can be improved by the inclusion of stabilizers such as food starch, gelatin, locust-bean gum, guar gum and pectin. These materials are used because they do not have a significant impact on the final flavor. The use of stabilizers is not required however, and some marketers choose not to use them in order to retain a more natural image for their yogurt (Anon., 2018).

To improve taste and provide a variety of flavors, many kinds of fruits are added to yogurt. Popular fruits include strawberries, blueberries, bananas, and peaches, but almost any fruit can be added. Beyond fruits, other flavorings are also added. These can include such things as vanilla, chocolate, coffee, and even mint. Recently, manufacturers have become quite creative in the types of yogurt they produce using natural and artificial flavorings (Anon., 2018).

The composition of milk used for yoghurt preparation is presented in Table 2.1.

Table 2.1 Composition of raw milk

Constituents	Percentage (%)
Lactose	4.5
Solid Not Fat	8
Protein	3.3
Fat	3.5-4
Minerals	0.7

Source: IDF (1991a)

2.4.3 Different types of yoghurts

Yoghurt is typically classified into the following groups:

a) Set yoghurt

This type of yoghurt is incubated and cooled in the final package and is characterized by a firm jelly like texture (Aswal *et al.*, 2012).

b) Stirred yoghurt

This type of yoghurt is incubated in a tank and the final coagulum is "broken" by stirring before cooling and packing. The texture of stirred yoghurt will be less firm than a set yoghurt somewhat like a very thick cream. A little reformation of coagulum will occur after packaging (Aswal *et al.*, 2012).

c) Drinking yoghurt

It also has the coagulum “broken” prior to cooling. In drinking yoghurt the agitation used to “break” the coagulum is severe. Very little reformation of coagulum may occur (Aswal *et al.*, 2012).

d) Frozen yoghurt

Frozen yoghurt is inoculated and incubated in the same manner as stirred yoghurt. However cooling is achieved by pumping through a Whipper / chiller / freezer in a fashion similar to ice-cream. The texture of the finished product is mainly influenced by the whipper/ freezer and the size and distribution of the ice crystals produced (Aswal *et al.*, 2012).

e) Concentrated yoghurt

This type of yoghurt is inoculated and fermented in the same manner as stirred yoghurt. Following the “breaking” of the coagulum the yoghurt is concentrated by boiling off some of the water, this is often done under vacuum to reduce the temperature required. Heating of low pH yoghurt can often lead to protein being totally denatured and producing rough and gritty textures. This is often called strained yoghurt due to the fact that the liquid that is released from the coagulum upon heating used to be “strained” off in a manner similar to making soft cheese (Aswal *et al.*, 2012).

f) Flavoured yoghurt

The flavours are usually added at or just prior to filling into pots. Common additives are fruit or berries, usually as a puree or as whole fruit in syrup. These additives often have as much as 50% sugar in them, however with the trend towards healthy eating gaining momentum, many manufacturers offer a low sugar and low fat version of their products. Low or no sugar yoghurts are often sweetened with saccharin or more commonly aspartame (Aswal *et al.*, 2012).

2.4.4 Classification of yoghurt

There are different types of yoghurt produced worldwide. However a Particular yoghurt may be subdivided into different groups based on the aspects presented in Table 2.2.

Table 2.2 Classification of yoghurt

S.N	Basis of classification	Different groups of yoghurt
1.	Chemical standards(Fat)	Full, skimmed/medium or skimmed/low fat
2.	Physical nature of product	Set, stirred or fluid/drinking; the latter is considered Stirred yoghurt of low viscosity
3.	Flavors	Plain/natural, fruit or flavored;
4.	Post fermentation processing	Enzyme hydrolysis, vitamin fortification, vegetable oils, heat treatment

Source: Aswal *et al.* (2012)

Set yoghurt is fermented in a retail container, filled after milk inoculation and is incubated in an incubation room at a suitable temperature normally 40- 43°C for approximately 2.5 to 4 h (Desai *et al.*, 1994). Stirred yoghurt is a non-Newtonian fluid, obtained by promoting the growth of *Lactobacillus delbrueckii* subsp. *Bulgaricus* and *Streptococcus salivarius* subsp. *thermophilus* at a mild temperature (between 40°C and 43°C) until a desired acidity level is reached. In stirred yoghurt, milk is inoculated and incubated in a fermentation tank, the yoghurt gel being broken up during the stirring, cooling and packaging stages. Due to several factors there may be variations in the rheological properties of stirred yoghurt. These can be of a physical nature such as those related with total solid content, milk composition and type of starter culture or processing conditions-related such as homogenization, thermal pre-treatment of the milk and post-incubation stages (including: stirring, pumping, cooling and packaging) (Tamime and Deeth, 1980).

2.4.5 Starter cultures

There is a symbiotic relationship between the two species of bacteria i.e. *Lactobacillus bulgaricus* and *Streptococcus thermophilus* that's why there is more rapid acid development than in the single strain culture (Tamime and Deeth, 1980).

Streptococcus thermophilus produces lactic acid and small quantities of formic acid, which promotes outgrowth of *Lactobacillus delbrueckii* subsp. *bulgaricus*. On the other hand *Lactobacillus delbrueckii* subsp. *bulgaricusspecies* produce aminoacids to stimulate the growth of *Streptococcus thermophilus* (Tamime and Deeth, 1980).

Chemical composition of the milk base (total solids and fat content) have significant effects on the activity of starter cultures. Ozer *et al*. (1998) have studied the behavior of starter cultures in concentrated yoghurt produced by different methods. They discovered that production method and milk total solids content influenced the growth and activity of starter cultures (Ozer *et al.,* 1998).

2.4.5.1 *Lactobacillus delbrueckii* subsp. *bulgaricus*

It is a gram-positive rod shaped, filamentous, non motile, non spore forming bacteria. It requires a low pH (around 5.4-4.6) to grow effectively so the bacterium is regarded as aciduric or acidophilic. The bacterium has complex nutritional requirements including the inability to ferment any sugar except lactose, from which it produces lactic acid that helps to preserve yoghurt. It is often helpful to sufferers of lactose intolerance whose digestive systems lack the enzymes to break down lactose to simpler sugars. While fermenting milk, it produces acetaldehyde, which is one of the main yoghurt aroma components (Fox, 1993).

2.4.5.2 *Streptococcus thermophilus*

The genus *Streptococcus* comprises several harmful pathogenic species such as *Streptococcus pyogenesor*, *Streptococcus pneumoniae*, together with a single, Generally recognized as safe species. *S. thermophilus* since this bacterium is widely used for the manufacture of dairy products (Cvetan, 1998).

2.4.5.3 *Lactobacillus acidophilus*

Lactobacillus acidophilus shows a range of health benefits which include: providing immune support for infections and cancer, reducing occurrence of diarrhea in human, aiding in lowering cholesterol, improving the symptoms of lactose intolerance. Studies have shown that dietary supplementation with *L. acidophilus* decrease the number of colon cancer cells in a dose dependent manner (Rao *et al.*, 1999). The better survival of *L. acidophilus* when added along with the starter was presumably due to the transient tolerance of the culture to hydrogen peroxide (Speck, 1976).

2.4.5.4 *Bifidobacterium bifidium*

Bifidobacteria are one of the most important group of intestinal organisms with regard to human health. *Bifidobacterium spp*. Belongs to the dominant anaerobic flora of the colon. The main species present in human colon are *Bifidobacterium adolescentis, B. bifidum, B. longum* subsp.*infantis, B. breveand, B. longum*. *B. bifidum* was found to be tolerant to the acidity of a model gastrointestinal tract system, with only a 20 % decrease in numbers as the pH decreased from 5.0 to 1.8 over an 80 min period (Holzapfel *et al.*, 2001). Important property of probiotic bifidobacteria is acid tolerance, enabling the cells to survive gastric acidity and volatile fatty acids produced during fermentation in the intestine (Charteris *et al.*, 1998). In the presence of yoghurt starter organisms the growth of bifidobacteria seems to be suppressed. Bifidobacteria have a higher resistance to acid and bile present in the gastrointestinal tract than yoghurt bacteria. The ingestion of bifidus fermented milk led to an increase in total bifidobacteria which was related to the colonic transit of the exogenous bifidobacteria (Bouhnik *et al.*, 1996). In yoghurt like product, addition of probiotic cultures to the normal starters generally results in slower growth of the probiotic strains than if they were added alone in milk (Roy *et al.*, 1996).

These starters produce environments that inhibit the growth of not only pathogens and spoilage microorganisms but also of probiotic. The phenomenon could partially be related to the production of bacteriocins or other inhibitors such as lactic and other organic acids and hydrogen peroxide produced by the starter cultures (Vinderola *et al.*, 2002). In addition, starter cultures grow faster, acidification occurs rapidly, and fermentation times are much shorter in their presence which resulted in reduced availability of nutrients (Shah, 2000); thus probiotic cultures do not have time to grow extensively. Many authors also reported the capacity of bifidobacteria to synthesize galacto oligosaccharides (Hung and Lee, 2002).

2.4.6 Biochemistry of yoghurt

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

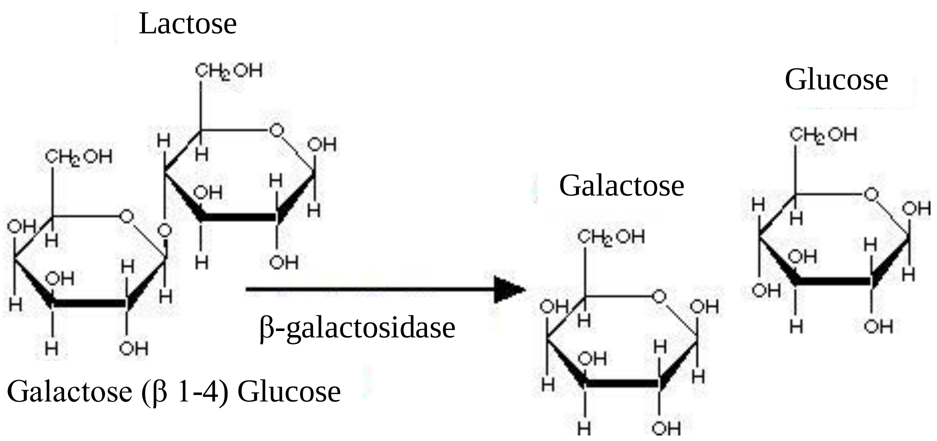

Fig. 2.1 Catabolism of lactose into glucose and galactose

Source: Anon. (2017)

Yogurt production begins with the breakdown of lactose into glucose and galactose (Fig. 2.1), a process catalyzed by β-galactosidase. The glucose produced from this catabolic step then enters glycolysis, producing pyruvate. It has been proposed that yogurt bacteria utilize the Embden-Meyerhof-Parnas pathway of glycolysis. Pyruvate then enters lactate fermentation, also known as homolactic fermentation, as it produces only lactic acid molecules. In other types of fermentation, such as ethanolic or heterolactic fermentation, the production of ethanol leads to other fermented foods and beverages such as sauerkraut, kimchi, and wine (Zourari *et al.*, 1992).

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

Yogurt production can occur both on a large scale as well as at home for individual consumption. As outlined by Cornell University's Milk Quality Improvement Program, yogurt production begins by heating the milk to 85-90°C to kill any unwanted bacteria, such as those that can spoil milk or are pathogenic, as well as to denature the milk proteins so that they form more of a gel-like texture by holding in the moisture. This pasteurization step is important both for the consumer and the active cultures that will be added, since it eliminates potential competitors in the environment. Once the milk is cooled to around 42°C, the starter culture is added. These starter cultures most often include *Lactobacillus delbrueckii subspecies bulgaricus* (known simply as *Lactobacillus bulgaricus* until 1984 and referred as such from here on) and *Streptococcus salivarius* subsp. *thermophilus* (or more commonly *Streptococcus thermophilus*. In fact, these two species are the only cultures required under the Code of Federal Regulations to be present in what can be called "yogurt", though there can be a wide variation in the strain that is used. Along with the starter culture, probiotics may be added, common ones being *Lactobacillus acidophilus, Lactobacillus casei*, and *Bifidobacteria* (Anon., 2017).

The temperature of the milk is then maintained around 42°C until the pH reaches 4.5, a sign of sufficient lactic acid production. As the name *Streptococcus thermophilus* suggests, these bacteria are thermophilus that grow the best under elevated temperatures. Thus, the 42°C environment encourages the starter cultures to grow, while it inhibits the growth of non-thermophilus, such as pathogenic bacteria. Caution is needed, however, since *L. bulgaricus* and *S. thermophilus* may be thermophilus but are killed at temperatures higher than 55°C. Once a pH of 4.5 is reached, the yogurt is cooled to around 7°C to stop fermentation. It is worth noting however, that fermentation, though at a much slower rate, occurs even at a low temperature and therefore over-fermentation can occur and lead to excess lactic acid and dead bacteria, resulting in a sour, unpalatable yogurt. Thus, even though yogurt is already a product of fermentation, it can technically still spoil (Anon., 2017).

2.4.7 Process of yoghurt production

The general process of making yogurt includes modifying the composition of and pasteurizing the milk; fermenting at warm temperatures; cooling it; and adding fruit, sugar, and other materials.

2.4.7.1 Modifying milk composition

When the milk arrives at the plant, its composition is modified before it is used to make yogurt. This standardization process typically involves reducing the fat content and increasing the total solids. The fat content is reduced by using a standardizing clarifier and a separator (a device that relies upon centrifugation to separate fat from milk). From the clarifier, the milk is placed in a storage tank and tested for fat and solids content. For yogurt manufacture, the solids content of the milk is increased to 16% with 1-5% being fat and 11-14% being solids-not-fat (SNF). This is accomplished either by evaporating off some of the water, or adding concentrated milk or milk powder. Increasing the solids content improves the nutritional value of the yogurt, makes it easier to produce a firmer yogurt and improves the stability of the yogurt by reducing the tendency for it to separate on storage (Tamime and Robinson, 2007).

2.4.7.2 Pasteurization and homogenization

After the solids composition is adjusted, stabilizers are added and the milk is pasteurized. This step has many benefits. First, it will destroy all the microorganisms in the milk that may interfere with the controlled fermentation process. Second, it will denature the whey proteins in the milk which will give the final yogurt product better body and texture. Third, it will not greatly alter the flavor of the milk. Finally, it helps release the compounds in milk that will stimulate the growth of the starter culture. Pasteurization can be a continuous-or batch-process. Both of these processes involve heating the milk to a relatively high temperature and holding it there for a set amount of time. One specific method for batch process pasteurization is to heat a large, stainless steel vat of milk to 185° F (85° C) and hold it there for at least 30 min (Tamime and Robinson, 2007).

While the milk is being heat treated, it is also homogenized. Homogenization is a process in which the fat globules in milk are broken up into smaller, more consistently dispersed particles. This produces a much smoother and creamier end product. In commercial yogurt making, homogenization has the benefits of giving a uniform product, which will not separate. Homogenization is accomplished using a homogenizer or viscolizer. In this machine, the milk is forced through small openings at a high pressure and fat globules are broken up due to shearing forces (Tamime and Robinson, 2007).

2.4.7.3 Fermentation

When pasteurization and homogenization are complete, the milk is cooled to between 109.4-114.8° F (43-46°C) and the fermentation culture is added in a concentration of about 2%. It is held at this temperature for about three to four hours while the incubation process takes place. During this time, the bacteria metabolizes certain compounds in the milk producing the characteristic yogurt flavor. An important byproduct of this process is lactic acid (Tamime and Robinson, 2007).

Depending on the type of yogurt, the incubation process is done either in a large tank of several hundred gallons or in the final individual containers. Stirred yogurt is fermented in bulk and then poured into the final selling containers. Set yogurt, also known as French style, is allowed to ferment right in the container it is sold in. In both instances, the lactic acid level is used to determine when the yogurt is ready. The acid level is found by taking a sample of the product and titrating it with sodium hydroxide. A value of at least 0.9% acidity and a pH of about 4.4 are the current minimum standards for yogurt manufacture in the United States. When the yogurt reaches the desired acid level, it is cooled, modified as necessary and dispensed into containers (if applicable) (Tamime and Robinson, 2007).

2.4.7.4 Adding other ingredients

Fruits, flavors, and other additives can be added to the yogurt at various points in manufacturing process. This is typically dependent on the type of yogurt being produced. Flavor in non-fruit yogurts are added to the process milk before being dispensed into cartons. Fruits and flavors can also be added to the containers first, creating a bottom layer. The inoculated milk is then added on top and the carton is sealed and incubated. If the fruit is pasteurized, it can be added as a puree to the bulk yogurt, which is then dispensed into containers. Finally, the fruit can be put into a special package, which is mixed with plain yogurt upon consumption. The finished yogurt containers are placed in cardboard cases, stacked on pallets, and delivered to stores via refrigerated trucks (Anon., 2018).

2.4.8 Physicochemical composition of yoghurt

2.4.8.1 Lactose

The lactose content of yoghurt manufactured from cow milk is about 4.06%. This lactose content is relatively lower than that of raw milk (Bhagiel *et al.*, 2015). This decrease in lactose may be due to the breakdown of lactose into glucose and galactose by microorganisms during the fermentation period by the action of β-galactosidase (Zourari *et al.*, 1992).

2.4.8.2 Moisture content and total solid content

Ahmad (1994) reported that moisture content of yoghurt should range between 82% and 84%, as much water in yoghurt makes it less viscous and affects texture and mouth feel.

Hofi *et al.* (1994) stated that yoghurts should have total solids of between 15% and 16%. However Muhammed *et al.* (2005) reported higher total solids of 17.11% in yoghurt. According to Weaver (2015) low percentage of total solids in yoghurt can lead to malfunctions of the starter culture.

2.4.8.3 Protein

The protein content of set type yoghurt is about 3.89% which is slightly higher than that of milk (3%). The increase in protein content may be due to the protein synthesis during fermentation by the starter culture (Mohammad and El-Zubeir, 2011).

2.4.8.4 Fat

The percentage of fat in the final yoghurt has a significant effect on the mouthfeel", the normal range of fat content is from 0.5% to about 3.5%, however levels as low as 0% and as high as 10% are found in some speciality products (Hofi *et al.*, 1994).

2.4.8.5 pH and acidity

The initial pH of yoghurt is less than 5 and acidity is 0.6-1.3%. Acidity changes were found to be maximal during the first week of storage and minimal thereafter. Samples with low initial acidity (high pH) showed relatively the highest increase in acidity. After the first week of storage at 4'C, decreases in pH of 0.32 (4.59-4.27), 0.06 (4.18-4.12) and 0.01 (3.82-3.81) were obtained for samples with initial acidities of pH 4.59, 4.18, and 3.82, respectively (Salji and Ismail 1983).

Changes in titratable acidity shows an increase of 0.17% (0.79-0.96), 0.09% (1.01-1.10) and 0.10% (1.38-1.48) for samples stored at 4'C and with initial titratable acidities of 0.79%, l.0l%, and 1.38%, respectively. The change in titratable acidity at 7ºC storage was 0.21%

(0.79-l.l0), 0.22% (1.01-1.23) and 0.24% (1.38-l .62) for the same samples, respectively. Changes in titratable acidity does not seem to be much affected by the initial acidity of the samples as did the changes in pH. pH measurement, therefore, can be considered a more sensitive tool than titratable acidity in monitoring acidity changes during refrigerated storage of yoghurt (Salji and Ismail 1983).

2.4.9 Aroma compounds in yoghurt

The odor and taste of soured milk products are characterized by numerous volatile bacterial metabolites, some of which are by-products of lactic acid fermentation or are produced by other reaction mechanisms. Lactic acid itself is suggested to be one of the major compounds significantly contributing to yogurt flavor (Beshkova *et al.*, 1998). More than 90 flavor compounds have been identified so far (Lubbers *et al.*, 2004). It has been reported that the aroma and taste of yogurt are mainly because of the presence of nonvolatile or volatile acids and carbonyl compounds, and especially the group of carbonyl compounds is believed to have a significant influence on the final yogurt aroma due to their relatively higher concentrations (Kaminarides *et al.*, 2007).

The most important aromatic components are acetaldehyde, acetone, acetoin, and diacetyl in addition to acetic, formic, butanoic, and propanoic acids. The typical aroma of yogurt is characterized chiefly by acetaldehyde, so it is suggested as a major flavor compound. Pette and Lolkema (1950) were the first to suggest that acetaldehyde was the most important constituent of yogurt aroma. It was later suggested that high concentrations of acetaldehyde are necessary to produce a desirable flavor in yogurt. The higher concentration of acetaldehyde (in the range of 5 to 21 ppm) is reported to be due to the low utilization rate of this compound (Hamdan *et al.*, 1971).

According to Illupapalayam *et al.* (2014) the flovor of spices incorporated yoghurt was significantly different from the control (whole milk) yoghurt. The flavor of spiced incorporated yoghurt was preffered more than that of control sample. Similarly, Junaid *et al.* (2013) reported that there was significant difference in flavor of yoghurt incorporated with different fruit juice in small amount.

2.4.10 Microbiology of yoghurt

Duru and Ozgunes (1981) who reported total bacteria count of 8.17 log cfu/g for sweetened yoghurt, and an average total bacteria count of 8.30 log cfu/g in strawberry flavored yoghurt samples. The report recorded the lowest average of 8.14 cfu/g total bacteria corresponding to one day storage time while the highest count of 8.40 log cfu/g corresponded to 3 days storage. This means that among other factors, the total bacteria count of yoghurt could depend on ingredient formulation and storage time.

According to Mohammad and El-Zubeir (2011) total bacterial count, coliform count and yeast and mould counts, were affected significantly during the storage period. The total bacterial count and yeast and mould counts increased significantly from day 3 to 6 and then gradually with non-significant effect in day 9 of storage period. The coliform count was found to decrease significantly from day 3 to 6 and then without any significant change at day 9. The coliform count and yeast and mould counts followed the same trend in yoghurt produced from both recombined and fresh milk. Total bacterial count increased significantly from day 3 to 6 in both types of yoghurt and then gradually without any significance difference in day 9 of storage period in fresh milk yoghurt whereas in recombined milk yoghurt, the total bacterial count were slightly decreased at day 9 (Mohammad and El-Zubeir, 2011).

2.4.11 Syneresis

Syneresis or spontaneous whey separation on the surface of set yogurt is regarded as a defect. This problem can be reduced or eliminated by increasing the level of milk solids to ∼15% (Tamime and Deeth, 1980). Other alternatives include the use of stabilizers (i.e. starch, 18hermop and vegetable gum) or exopolysaccharide (EPS) producing starter cultures. Due to consumer awareness of natural products, the use of stabilizers is restricted in some countries (Amatayakul *et al.*, 2006). The syneresis was found to be higher if storage time is increased (Yilmaz-Ersan and Kurdal, 2014).

Dannenberg and Kessler (1988), when carrying out investigations on natural yoghurts of 4.6 pH value containing traditional, high-aromatic cultures, demonstrated their sensitivity to syneresis ranging from 38 to 58%, depending on the extent of the denaturation of β-lactoglobulin in the processed milk.

The formation or precipitation of protein-polyphenol complex was modeled by many researchers (Dönmez et al., 2017).

2.4.12 Factors affecting yoghurt quality

Numerous factors must be carefully controlled during the manufacturing process in order to produce a high-quality yoghurt with the required flavor, aroma, viscosity, consistency, appearance, freedom from whey separation and long shelf life:

- Choice of milk
- Milk standardization
- Milk additives
- Deaeration
- Homogenization
- Heat treatment
- Choice of culture

Pre-treatment of the milk thus includes a number of measures which are all very important to the quality of the end product. The mechanical treatment to which yoghurt is subjected during production also affects its quality (Bylund, 2015).

2.4.12.1 Choice of milk

In order to be able to produce a high quality yoghurt, the milk intended for yoghurt production must be of the highest bacteriological quality. It must have a low content of bacteria and substances which may impede the development of the yoghurt culture. The milk must not contain antibiotics, bacteriophages, residues of CIP solution or sterilizing agents. The dairy should therefore obtain the milk for yoghurt production from selected, approved producers. The milk must be very carefully analysed at the dairy (Bylund, 2015).

2.4.12.2 Milk standardization

The fat and dry solids contents of the milk are normally standardized according to the FAO/WHO code and principles described in the following sections.

2.4.12.2.1 Fat

Yoghurt may have a fat content of 0 to 10 %. A fat content of 0.5 – 3.5 % is, however, the most typical (Bylund, 2015). Yoghurt can be classified in the following groups according to the FAO/WHO code and principles are shown in Table 2.3.

Table 2.3 Type of yoghurt according to FAO

Type		Fat (%)
Yoghurt	Min. milk fat	3 %
Partially skimmed yoghurt	Max. milk fat	<3 %
	Min. milk fat	>0.5 %
Skimmed yoghurt	Max.milk fat	0.5 %

Source: Bylund (2015)

2.4.12.2.2 Dry matter (DM) content

According to the FAO/WHO code and principles the minimum MSNF is 8.2 %. An increase in the total DM content, particularly the proportion of casein and whey proteins, will result in a firmer yoghurt coagulum, and the tendency to whey separation will then be reduced (Bylund, 2015).

The most common ways to standardize the DM content are:

- Evaporation (10 – 20 % of the milk volume is normally evaporated)
- Addition of skim milk- or protein powder, usually 1 – 3 %
- Addition of milk concentrate
- Addition of UF or RO retentate from skim milk (Bylund, 2015).

2.4.12.3 Milk additives

Sugar or sweeteners and stabilizers may be used as additives in yoghurt production (Bylund, 2015).

2.4.12.3.1 Sugar or sweetener

The disaccharide sucrose, or a monosaccharide such as glucose, can be added alone, or in conjunction with fruit addition. To satisfy dieters, among whom diabetics are an important category, sweeteners should be used. A sweetener has no nutritive value, but tastes very sweet, even in very small doses (Bylund, 2015).

The fruit in question usually contains about 50 % sugar or a corresponding amount of sweetener, so the required sweetness can normally be supplied by adding 12 to 18 % fruit. It should be noted that adding too much sugar (more than ~8 %) to the milk before the inoculation/incubation period has an adverse effect on fermentation conditions, because it changes the osmotic pressure of the milk (Bylund, 2015).

2.4.12.3.2 Stabilizers

Hydrophilic colloids can bind water. They increase the viscosity and help to prevent whey separation in yoghurt. The type of stabilizer and the rate at which it should be added must be determined experimentally by each manufacturer. The product may acquire a rubbery, hard consistency if the wrong stabilizer, or an excess of stabilizer, is used (Bylund, 2015).

Correctly produced, natural yoghurt requires no addition of stabilizers, as a firm, fine gel with a high viscosity will occur naturally. Stabilizers can be used in fruit yoghurts and must be used in pasteurized and whipped yoghurt. Stabilizers (0.1 – 0.5 %) such as gelatin, pectin, starch and agar-agar are the most commonly used substances (Bylund, 2015).

2.4.12.4 Deaeration

The air content of the milk used to make fermented milk products should be as low as possible. However, some admixture of air is unavoidable if the MSNF content is increased by addition of milk powder. If this is done, the milk should be deaerated as part of the subsequent processing (Bylund, 2015).

When the MSNF content is increased by evaporation, deaeration is a part of that process (Bylund, 2015).

The advantages gained through deaeration are (Bylund, 2015)

- Improved stability and viscosity of the yoghurt

- Shortened fermentation time
- Improved working conditions for the homogenizer
- Less risk of fouling during heat treatment
- Removal of volatile off-flavours (deodorization)

2.4.12.5 Homogenization

The main motives for homogenizing milk intended for cultured milk production are to prevent creaming during the incubation period and to assure uniform distribution of the milk fat. Homogenization also improves the stability and consistency of fermented milks, even those with low fat contents. Homogenization with subsequent heating at high temperature, usually 90 – 95°C for about five min, has a very good influence on the viscosity of the final yoghurt (Bylund, 2015).

The viscosity is measured with a simple viscosimeter (SMR viscosimeter) at 20°C, and the result is given in seconds for 100 ml of product to pass a nozzle of a certain diameter. Figure 11.8 shows a viscosimeter provided with exchangeable nozzles, each of a diameter of 2–6 mm. The viscosity of full-stream homogenized milk runs parallel to the homogenization pressure, regardless of whether it has been subjected to ordinary heat treatment or not. The table also shows that high-temperature heat treatment makes the product more viscous (Bylund, 2015).

As a general recommendation, the milk should be homogenized at 20 – 25 Mpa and 65 – 70 °C to obtain optimum physical properties in the product. Homogenization is frequently utilized even in production of low-fat cultured milks. Some producers homogenize their yoghurt milk up to 40 Mpa (400 bar) and at temperatures up to 95 °C. For certain recipes these higher parameters have a positive influence on both viscosity and stability (Bylund, 2015).

2.4.12.6 Heat treatment

The milk is heat treated before being inoculated with the starter in order to (Bylund, 2015)

- Improve the properties of the milk as a substrate for the bacteria culture
- Ensure that the coagulum of the finished yoghurt will be firm
- Reduce the risk of whey separation in the end product

Optimum results are achieved by heat treatment at 90 – 95°C and a holding time of about five min. That temperature/time combination denatures about 70-80% of the whey proteins (99 % of the β-lactoglobulin). In particular, the β-lactoglobulin, which is the principal whey protein, interacts with the κ-casein, thereby helping to give the yoghurt a stable body. UHT treatment and sterilization of milk intended for culturing do not, however, have the same favourable influence on viscosity, for reasons not yet fully understood (Bylund, 2015).

2.4.12.7 Choice of culture

Culture laboratories today produce a wide range of customized yoghurt cultures. Dairies can choose branded yoghurt cultures or mix cultures themselves to get their own requirements for the final yoghurt. Some cultures will give the final yoghurt different mouth thickness and gel firmness. Other cultures that influence fermentation time and post acidification. Cultures are also adapted to the type of yoghurt that should be produced (e.g. stirred type, set type, drink (Bylund, 2015).

2.4.13 Shelf life of yogurt

The shelf life of yogurt at a refrigerated temperature is around 34 weeks, depending on the standard hygiene observed during manufacture and the microbial quality of the ingredients and the packaging material. Various techniques have been used to extend yogurt's shelf life, including freezing and drying, gas flushing, adding preservatives, using aseptic equipment, applying multiple frequency microwave, and sterilizing with heat (Tamime and Robinson, 2007).

The shelf life of Greek yogurt and/or labneh is quite short. At refrigeration temperature, a shelf life of seven to 10 days is recommended. The relatively short shelf life of cloth bag labneh is largely responsible for the wide use of benzoates and sorbates to control growth of spoilage microorganisms due to aerial contamination during sun drying or pressing, although the latter can be minimized (Chandan and Kilara, 2010). The shelf life of yogurt cheese is similar to that of labneh anbaris, which is longer than that of labneh due to a higher total solids content and more concentrated lactic acid. At ambient temperature, the shelf life is about 12 to 18 months as long as the product is kept submerged under olive oil (Tamime and Robinson, 2007).

2.4.14 Fruit and flavoured yoghurts

Yoghurt with various fruits, flavouring and aroma additives is very popular, although the trend back towards natural yoghurt is clearly discernible in some markets. Common additives are fruit and berries in syrup, processed or as a purée. The proportion of fruit is usually about 15 %, of which about 50 % is sugar (Bylund, 2015).

The fruit is mixed with the yoghurt before or in conjunction with packing; it can also be placed in the bottom of the pack, before the latter is filled with yoghurt. Alternatively, the fruit can be separately packed in a twin cup integrated with the basic cup (Bylund, 2015).

Sometimes yoghurt is also flavoured with vanilla, honey, coffee essences, spices etc. Colouring and sugar in the form of sucrose, glucose or aspartame (a sugar-free diet sweetener) are often added together, with the flavouring. When necessary stabilizers may also be added to modify the consistency (Bylund, 2015).

The additives increase the DM (Dry Matter) content of the finished yoghurt; a typical composition for fruit flavored yoghurt is shown in Table 2.4.

Table 2.4 Composition of fruit flavored yoghurt

Parameters	Value (%)
Fat	0.5 – 3.0 %
Lactose	3.0 – 4.5 %
Milk solids non-fat (MSNF)	11.0 – 13.0 %
Stabilizer (if used)	0.3 – 0.5 %
Fruit	12.0 – 18.0 %

Source: Bylund (2015)

2.4.15 Benefits of yoghurt

Many of us like to enjoy yogurt-based foods such as smoothies or salad dressings on a regular basis. But only a few are aware of the tremendous benefits that come with yogurt. That's why eating yogurt can help develop some key aspects of our body, including bones, muscles, and skin. For these reasons and countless others yogurt has been an important part of our gastronomy for centuries (Anon., 2017a). For example, yogurt can reduce our risk of heart disease, and it can help us manage our weight. The health benefits of yogurt are:

1. Nutrient rich

Undoubtedly, the biggest benefit of consuming yogurt is that it is a well-rounded an overall nutritious food that should be included in diet. Whether a bowl of yogurt is preferred in the morning or an afternoon smoothie, yogurt has both the nutritional value and the taste to make it one of the best foods out there. It contains a range of healthy nutrients such as calcium and vitamin B, all of which are necessary for good body function. Moreover, yogurt contains some lesser known nutrients like phosphorus or potassium which are equally important (Anon., 2017a).

2. Good source of protein

For bodybuilders and indeed any other health conscious individual, yogurt can provide body with copious amounts of protein. In fact, just 200 g of yogurt contains 12 g of protein, which means that if consumed daily, yogurt can help the body to develop more muscle mass. Moreover, protein can increase metabolism, helping you to burn more calories throughout the day and keeping weight in control. Another great benefit of yogurt is that it creates a feeling of fullness, therefore deterring from eating other tempting snacks (Anon., 2017a).

3. Solve the problem of constipation

Nowadays, many people consume unhealthy diets that are loaded with highly processed ingredients, chemicals, as well as preservatives. That's why many of us find it difficult to go to the bathroom, and often experience irritating constipation. The lack of fiber in diets is the main culprit, but so is the lack of probiotics. Luckily, yogurt helps to balance out the bacteria in our stomach, helping us to digest food better and therefore making it easier to go the toilet (Anon., 2017a).

4. Immune system boost

If you consume yogurt frequently, then you may be able to considerably boost immune system as well as its ability to protect the body against harmful bacteria and viruses. Moreover, yogurt can be consumed as a healthy snack food, making this food one of the easiest and most effective ways to boost your immune system naturally. Try consuming yogurts that have high levels of probiotics in them; that way, can help control inflammation as well as reduce the risk of developing different illnesses and ailments. This is especially important for the flu season, which infects millions of people annually (Anon., 2017a).

5. Helps digestion

If one struggle with digestion, try eating some yogurt before or after a meal. Yogurt is widely believed to help the digestive process because it contains an abundant number of probiotics. Probiotics are a group of healthy and beneficial bacteria that can be found in the gut. The probiotics help to balance out the bacteria in the digestive system, therefore keeping everything smooth and in check. To get the most out of yogurt, consume yogurts that contain high amounts of cfus, which is often indicated on the label of the yogurt (Anon., 2017a).

2.5 Cinnamon

Cinnamon is a spice obtained from the inner bark of several tree species from the genus Cinnamomum. Cinnamon is used mainly as an aromatic condiment and flavoring additive in a wide variety of cuisines, sweet and savoury dishes, breakfast cereals, snack foods, and traditional foods. The aroma and flavor of cinnamon derive from its essential oil and principal component, cinnamaldehyde, as well as numerous other constituents, including eugenol (Bailey, 1911).

2.5.1 Cinnamaldehyde

Cinnamaldehyde is an organic compound with the formula $C_6H_5CH=CHCHO$. Occurring naturally as predominantly the trans (E) isomer, it gives cinnamon its flavor and odor (Service, 2018). It is a flavonoid that is naturally synthesized by the shikimate pathway (Gutzeit and Ludwig-Müller, 2014). This pale yellow, viscous liquid occurs in the bark of cinnamon trees and other species of the genus Cinnamomum. The essential oil of cinnamon bark is about 50% cinnamaldehyde (Singh *et al.*, 2007). The polyphenol content of cinnamon is 148 mg/g (Su *et al.*, 2005).

2.5.1.1 Biosynthesis

The biosynthesis of cinnamaldehyde begins with deamination of L-phenylalanine into cinnamic acid by the action of phenylalanine ammonia lyase (PAL) (Bang *et al.*, 2016). PAL catalyzes this reaction by a non-oxidative deamination. This deamination relies on the MIO prosthetic group of PAL. PAL gives rise to trans-cinnamic acid (Kong, 2015).

In the second step, 4-coumarate: CoA ligase (4CL) converts cinnamic acid to cinnamoyl-CoA by an acid-thiol ligation. 4CL uses ATP to catalyze the formation of cinnamoyl-CoA. 4CL effects this reaction in two steps. 4CL forms a hydroxycinnamate-AMP anhydride, followed by a nucleophile attack on the carbonyl of the acyl adenylate. Cinnamoyl-CoA is reduced by NADPH catalyzed by CCR (cinnamoyl-CoA reductase) to form cinnamaldehyde (Bang *et al.*, 2016).

2.5.1.2 Synthesis

Several methods of laboratory synthesis exist, but cinnamaldehyde is most economically obtained from the steam distillation of the oil of cinnamon bark. The compound can be prepared from related compounds such as cinnamyl alcohol, (the alcohol form of cinnamaldehyde), but the first synthesis from unrelated compounds was the aldol condensation of benzaldehyde and acetaldehyde (Kong, 2015).

2.5.2 Antimicrobial activity

Cinnamon *(Cinnamomun zeylanicum)* showed the highest antibacterial activity (12 mm inhibition zone) to the microorganisms tested as compared to other spice extracts. In a study it was found that *C.zeylanicum* was the most effective spice against all of the test strains. In another study it was found that the oils of cinnamon were the most inhibitory, each having a bacteriostatic concentration of 0.075% or less against all of five pathogens (*S. aureus, L. monocytogenes, Camphylobacter jejuni, Salmonella enteritidis, E. coli*) (Asimi *et al.*, 2013).

2.5.3 Chemical composition

Cinnamon is high in polyphenols, proanthocyanidins, antioxidant activity, and is a great source of manganese, fiber, iron, and calcium. It has been reported that a group of polyphenolic polymers found in cinnamon may function as antioxidants to potentiate insulin action, and therefore, may be beneficial in the control of glucose intolerance and diabetes. Two major types of polymeric procyanidins have been observed including A-type and B-type linkages. Among them, A-type has two interflavan linkages (IFL) while B-type has a single IFL, which results in a 2 amu unit difference (He *et al.*, 2007).

2.5.4 Health benefits

Cinnamon health benefits include a variety of health disorders, including diarrhea, arthritis, menstrual cramps, yeast infections, colds, flu, rheumatism and digestive problems. Cinnamon has been used for centuries and in many cultures. It has found a prominent position in traditional healing medicines, especially Ayurveda (the traditional Indian medicinal system) (Anon., 2015).

Today, the use of cinnamon has expanded to treating a variety of health disorders, including respiratory problems, skin infections, blood impurity, heart disorders, and diabetes. Cinnamon has also been used to treat diarrhea and other problems of the digestive system (Anon., 2015).

Part III

Materials and methods

3.1 Materials

The materials collected for the preparation of cinnamon incorporated yoghurt were as follows:

3.1.1 Milk

The standardized (3% fat and 8% SNF) and pasteurized milk was collected from local market of Dharan produced by Dairy Development Corporation (DDC).

3.1.2 Cinnamon oleoresin

Cinnamon oleoresin was bought from Druk Pvt. Ltd., Rijal-Tansi, Itahari.

3.1.3 Skim milk powder (SMP)

Skim milk powder was used as the source of MSNF and it was bought from the Bharaha department store of Dharan. Manufactured by Singhania Industries, Shreepur, Birgunj-16.

3.1.4 Sweetener

Sugar was used as a sweetener. It was bought from the Bharaha department store of Dharan.

3.1.5 Starter culture

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

3.1.6 Containers

Plastic cup as ice cream packaging materials were bought from Bharara department store of Dharan. The size of cup was 100 ml and plain in design.

3.1.7 Equipment and chemicals

The list of equment and chemicals used are given in Table 3.1 and Table 3.2 respectively.

Table 3.1 List of equipments used

S.N.	Physical apparatus	S.N.	Physical apparatus
1.	Heating arrangement	9.	Refrigerator
2.	Electric balance (Phoenix)	10.	Daily routine glassware
3.	Gerber centrifuge	11.	Muffle furnace (Accuma, India)
4.	Thermometer	12.	Hot air oven (Vitco, India)
5.	Refractrometer (Hand refractometer model WYT- 32, Zhongyou Optical Instruments).	13.	Stainless steel vessels
6.	Titration apparatus	14.	Incubator (Vitco, India)
7.	Desiccators	15.	Gerber butyrometer
8.	Kjeldahl digestion and distillation set		

Table 3.2 List of chemicals used

S.N.	Chemicals	Specifications
1.	40% Formaldehyde	Thermo fisher Scientific (40% by volume)
2.	Culture medium (plate count agar)	Titan Biotech Ltd.
3.	Starter culture	Kamadhenu Dairy Pvt. Ltd.
4.	Saturated potassium oxalate	Merck Life Pvt. Ltd. (India)
5.	0.0005% Fuchsin solution	Qualigens fine chemicals

3.2 Method

3.2.1 Recipe formulation

Design expert version 10, one factorial design is used to design the formulation of spices and other ingredients are kept constant as shown in Table 3.3.

Table 3.3 Sample formulation

Sample	Milk (ml)	Oleoresin (ml)	Sugar (%)	SMP (%)
A	100	0	3	4
B	100	0.1	3	4
C	100	0.2	3	4
D	100	0.3	3	4
E	100	0.4	3	4

3.2.2 Preparation of set type cinnamon oleoresin incorporated yoghurt

The standardized and pasteurized milk from Kamadhenu was taken for the preparation of yoghurt. The milk was mixed with 4% SMP and 3% sugar at 45°C. Heating of milk was further continued till the temperature reached to around 65-70°C for certain period. After that the heated milk was cooled to around 43-44°C. After cooling, the starter culture is added at the rate of 2% to each formulations. Then five formulations of the samples were made by adding 0, 0.1, 0.2, 0.3, and 0.4 ml of cinnamon per 100 ml yoghurt mix. The mix is placed in plastic cups. The yoghurt mix was then kept in an incubator which was maintained at a temperature of about 43°C and was kept for 3.5-4 h until the coagulum is formed. Now the prepared yoghurt was immediately cooled to 5-7°C and stored at that temperature in a refrigerator. The flow diagram for prearation of set type of yoghurt is shown in Fig. 3.1.

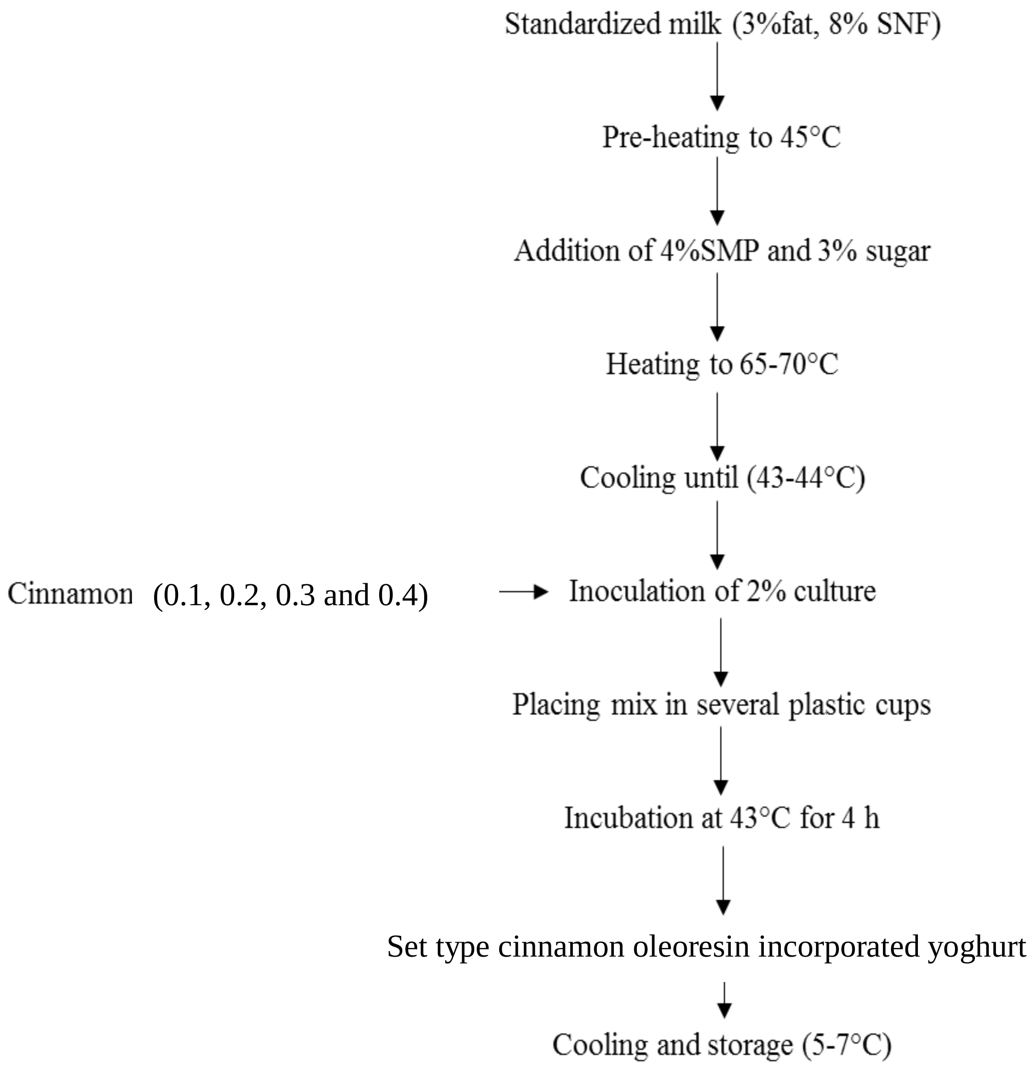

Fig. 3.1 Preparation of set type of yoghurt

Source: Aswal *et al.* (2012)

3.2.3 Analysis of yoghurt

3.2.3.1 Sensory evaluation

Sensory evaluation was carried out using 9-point hedonic scale described by Ranganna (2000). Sensory panelists were semi trained panelists from Central Campus of Technology, Dharan. Sensory evaluation was carried out on the quality attributes viz., colour and taste, and texture, flavour and overall acceptability. The specimen of the evaluation of card is shown in Appendix A.

3.2.3.2 Physical analysis

3.2.3.2.1 Syneresis

Degree of syneresis, expressed as proportion of free whey was measured by a method used by Lee and Lucey (2004). A 100 g 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:

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

3.2.3.3 Chemical analysis

3.2.3.3.1 Fat

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

3.2.3.3.2 Lactose

Lactose content was determined by the Lane and Eynon method as described in Ranganna (2000).

3.2.3.3.3 pH

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

3.2.3.3.4 Titrable acidity

Titrable acidity was determined by titrimetric method given in AOAC (2005).

3.2.3.3.5 Protein

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

3.2.3.3.6 Ash

Ash content was determined as described in Ranganna (2000).

3.2.3.3.7 Total carbohydrate

Total carbohydrate contents of samples were calculated by difference, that is the percentage of moisture, ash, protein and fat was subtracted from 100 % Pearson (1981).

3.2.3.3.8 Polyphenol content

Determination of total phenol in yoghurt was carried out with Folin-Ciocalteau reagent as mentioned by Sadasivam and Manickam (1997) with slight modification. Phenols react with phosphomoybdic acid in Folin-Ciocalteau reagent in alkaline medium producing a blue colored complex (molybdenum blue). 0.05 ml of the extract and 1ml of Folin- Ciocalteu reagent was mixed and incubated at room temperature for 15 min. Then 2.5 ml of saturated sodium carbonate was added and further incubated for 30 min at room temperature and absorbance measured at 760 nm. Also, the standard curve was prepared using 0-100 μg/ml solutions of gallic acid in ethanol. Total phenol values were calculated using the standard curve equation and expressed in terms of gallic acid equivalent (mg/ml) of dry mass.

3.2.3.4 Microbiological examination

Total plate count (TPC) was carried out by using plate count agar as described in IDF (1991b).

3.2.3.5 Data analysis

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. (VSNI) in the collaboration with practicing statisticians at Rothamsted Research and the organization in Australia, New Zealand, Switzerland and the UK. t-Test: Two-Sample Assuming Equal Variances was carried out using Microsoft Excel 10 to evaluate the significant difference between the synerisis of the two samples.

Part IV

Results and discussions

Yoghurt was prepared from the milk with 3% fat brought from Kamadhenu dairy. The culture of yoghurt was also brought from Kamadhenu dairy. The prepared yoghurt was taken from chemical and physical analysis. The results of analysis found as:

4.1 Physicochemical analysis of milk

The milk of Kamadhenu dairy was analysed. The value of physicochemical composition of milk was shown in Table 4.1.

Table 4.1 Physicochemical composition of milk

Parameters	Value
Fat (%)	3.1 (0.17)
TS (%)	13.2 (0.26)
Acidity (% as lactic acid)	0.13 (0.017)
pH	6.49 (0.026
Ash (%)	0.53 (0.12)
Lactose (%)	4.53 (0.12)
Protein (%)	4.68 (0.06)

Values are the mean of triplicates and values in brackets represent standard deviation.

The above physicochemical composition of milk was of the same quality as required for yoghurt production. The similar results of physiochemical properties of milk was reported by IDF (1991a).

4.2 Polyphenol content of cinnamon oleoresin

The polyphenol content of cinnamon was found to be 158.14 mg/g sample (4.56). It was similar as obtained by (Su *et al.*, 2005). The polyphenol content found by Kong (2015) was 149 mg/g sample. This was similar to our study.

4.3 Sensory evaluation of yoghurt

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

4.3.1 Color

Regarding color of cinnamon oleoresin incorporated yoghurt, the analysis shows that the mean sensory score for sample A, B, C, D, and E were found to be 7.3, 7.9, 7.4, 7.2 and 7.3 respectively. Statistical analysis shows that effect of cinnamon on color of the product was not significant ($p<0.05$). The mean sensory score for color for different samples is shown in Fig. 4.1.

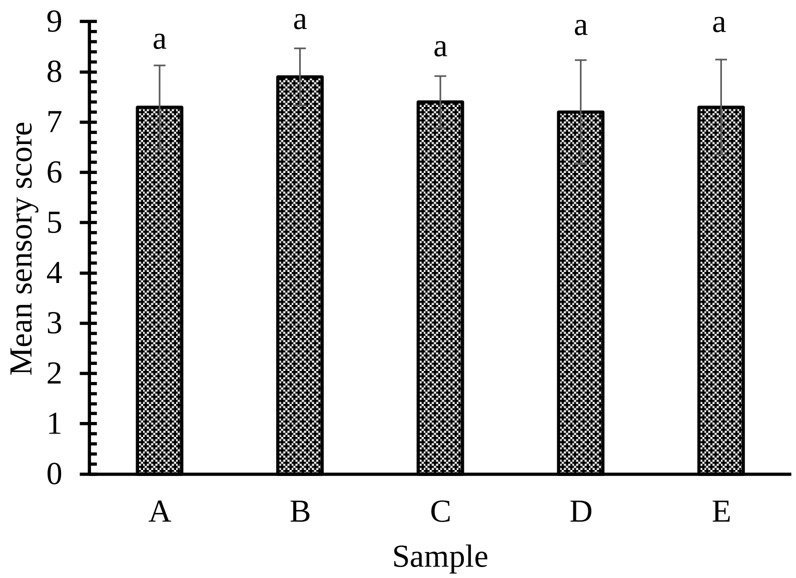

Fig. 4.1 Mean sensory score for color of cinnamon incorporated yoghurt

*Vertical error bars represent the value of standard deviation. Values of same subscript represents the samples were similar in terms of color.

Where A, B, C, D and E represents the yoghurt sample incorporated with 0. 0.1, 0.2, 0.3 and 0.4 ml cinnamon respectively.

According to Illupapalayam *et al.* (2014) the appearance or color of yoghurt incorporated with spices: cardamom, cinnamon and nutmeg were not significantly different from the control (whole milk) yoghurt. This is the similar results as obtained in our study. Similarly, Junaid *et al.* (2013) reported that there was no significant difference in color of yoghurt incorporated with different fruit juice in small amount but as the storage time increases the color was less preffered by panelist.

4.3.2 Flavor

Regarding flavor of cinnamon oleoresin incorporated yoghurt, the analysis shows that the mean sensory score for sample A, B, C, D, and E were found to be 6.6, 7.6, 7.4, 6.4 and 6.3 respectively. Statistical analysis shows that effect of different cinnamon portion on flavor of the product was significant ($p<0.05$). B had scored higher sensory mean value for flavor than other and were significantly different from other samples. While, A, C and D were not significantly different from each other. According to sensory evaluation following results were obtained. The mean sensory score for Flavor for different samples is shown in Fig. 4.2.

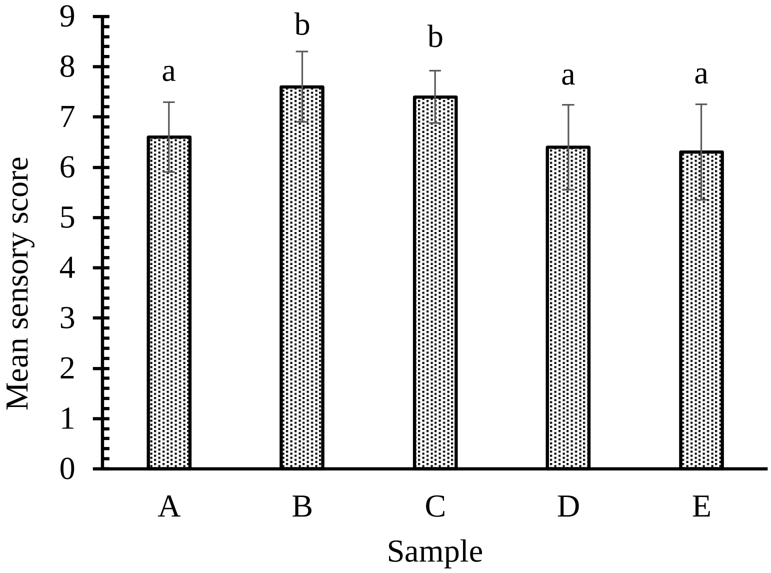

Fig. 4.2 Mean sensory score for flavor of cinnamon incorporated yoghurt

*Vertical error bars represent the value of standard deviation. Values of same subscript represents the samples were similar in terms of flavor.

According to Illupapalayam *et al.* (2014) the flovor of spices incorporated yoghurt was significantly different from the control (whole milk) yoghurt. The flavor of spiced incorporated yoghurt was preffered more than that of control sample. Similar results were obtained in our study interms of flavor. Similarly, Junaid *et al.* (2013) reported that there was significant difference in flavor of yoghurt incorporated with different fruit juice in small amount.

4.3.3 Texture

Regarding texture of cinnamon oleoresin incorporated yoghurt, the analysis shows that the mean sensory score for sample A, B, C, D, and E were found to be 7.4, 7.8, 7.6, 6.6, and 6.5 respectively. Statistical analysis shows that effect of different cinnamon portion on texture of the product was significant ($p<0.05$). B had scored higher sensory mean value for texture than other and were significantly different from other samples. While, D and E were not significantly different from each other. According to sensory evaluation following results were obtained. The mean sensory score for texture for different samples is shown in Fig. 4.3.

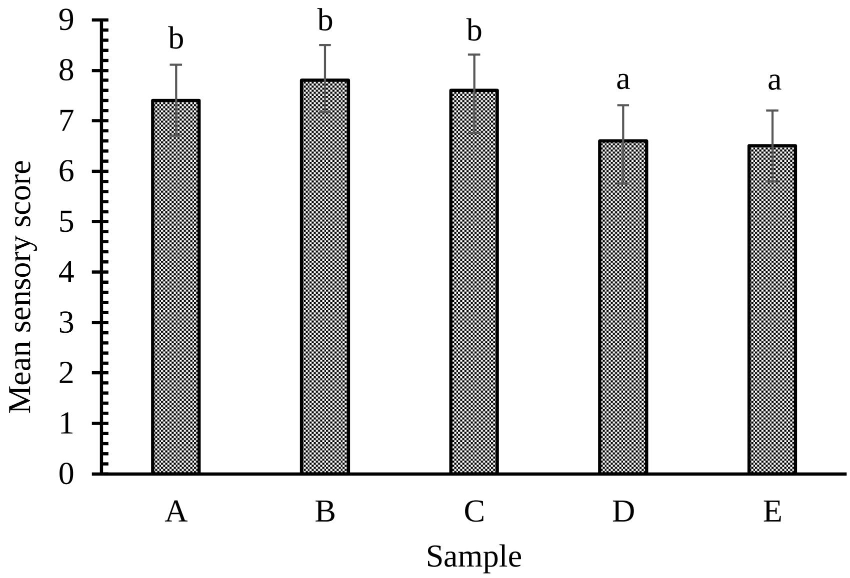

Fig. 4.3 Mean sensory score for texture of cinnamon incorporated yoghurt

*Vertical error bars represent the value of standard deviation. Values of same subscript represents the samples were similar in terms of texture.

According to Illupapalayam *et al.* (2014) the texture of cardamom incorporated yoghurt was significantly different from the control (whole milk) yoghurt but cinnamon incorporated yoghurt was similar to control sample in terms of texture. This was different from our study. The texture of spice (cardamom) incorporated yoghurt was preffered more than that of control sample. Similarly, Junaid *et al.* (2013) reported that there was no significant difference in texture of yoghurt incorporated with different fruit juice in small amount.

4.3.4 Taste

Regarding taste of cinnamon oleoresin incorporated yoghurt, the analysis shows that the mean sensory score for sample A, B, C, D, and E were found to be 7, 8.4, 7.3, 6.2, and 6 respectively. Statistical analysis shows that effect of different cinnamon portion on taste of the product was significant ($p<0.05$). Sample B had scored higher sensory mean value for taste than other and were significantly different from other samples. While, A and C , D and E were not significantly different from each other. According to sensory evaluation following results were obtained. The mean sensory score for taste for different samples is shown in Fig. 4.4.

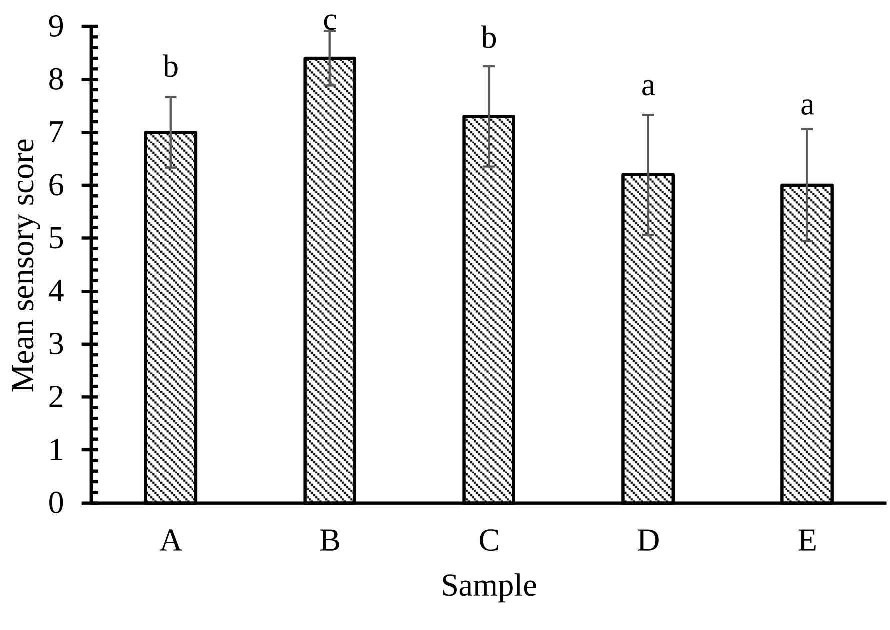

Fig. 4.4 Mean sensory score for taste of cinnamon incorporated yoghurt

*Vertical error bars represent the value of standard deviation. Values of same subscript represents the samples were similar in terms of taste.

According to Illupapalayam *et al.* (2014) the taste of spices incorporated yoghurt was significantly different from the control (whole milk) yoghurt but cinnamon incorporated yoghurt was similar to control sample in terms of taste. The taste of spices incorporated yoghurt was preffered more than that of control sample. This was similar to our study. Similarly, Junaid *et al.* (2013) reported that there was significant difference in taste of yoghurt incorporated with different fruit juice in small amount.

4.3.5 Overall acceptability

Regarding overall acceptability of cinnamon oleoresin incorporated yoghurt, the analysis shows that the mean sensory score for sample A, B, C, D, and E were found to be 6.9, 8.2, 7.3, 6.3, and 6.1 respectively. Statistical analysis shows that effect of different cinnamon portion on overall acceptability of the product was significant ($p<0.05$). Sample B had scored higher sensory mean value for overall acceptability than other and were significantly different from other samples. While, A and C , D and E were not significantly different from each other. According to sensory evaluation following results were obtained. The mean sensory score for overall acceptability for different samples is shown in Fig. 4.5.

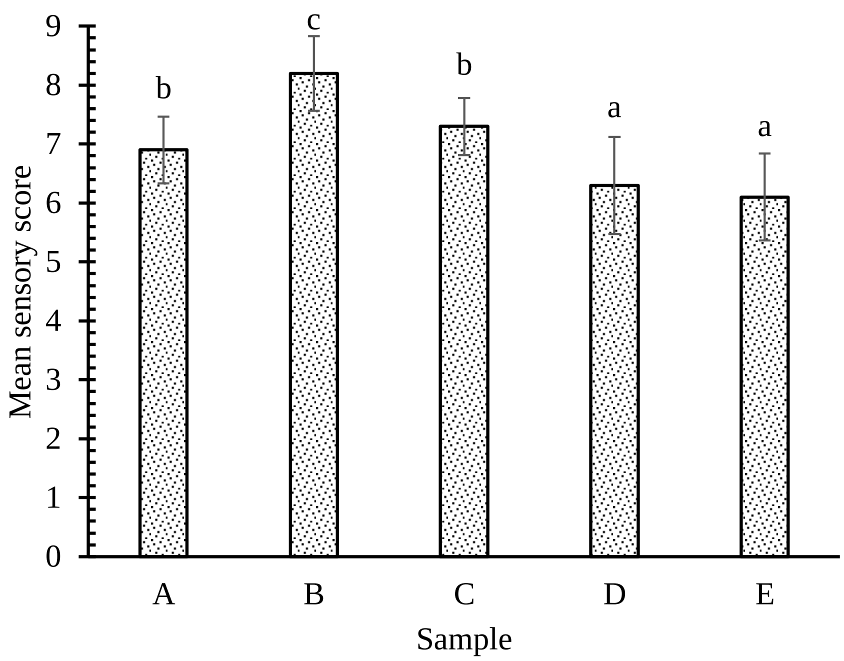

Fig. 4.5 Mean sensory score for overall acceptability of cinnamon incorporated yoghurt

*Vertical error bars represent the value of standard deviation. Values of same subscript represents the samples were similar in terms of overall acceptability.

According to Illupapalayam *et al.* (2014) the overall acceptability of spices incorporated yoghurt was significantly different from the control (whole milk) yoghurt but cinnamon incorporated yoghurt was similar to control sample in terms of overall acceptability. The overall acceptability of spices incorporated yoghurt was preffered more than that of control sample. This was similar to our study.

On overall B sample was found to be superior in terms of color, flavor, texture, taste and overall acceptability. Appearance and taste appears to be the important factor for consumer acceptability. In the present study, yogurts containing cinnamon at 0.1 ml per 100 ml of milk indicates the most preference by consumers in terms of taste.

4.4 Physicochemical composition of yoghurt

The proximate composition of cinnamon oleoresin incorporated yoghurt was similar to that of control sample. The physicochemical composition of yoghurt obtained were presented in Table 4.2.

Table 4.2 Physicochemical composition of yoghurt

Parameter	Sample B	Control
Fat (%)	3.43^{a} (0.05)	3.1^{a} (0.1)
TS (%)	16.02^{a} (0.43)	14.79^{a} (0.76)
Acidity (% as lactic acid)	0.6^{a} (0.026)	0.77^{b} (0.045)
pH	4.71^{a} (0.021)	4.70^{a} (0.015)
Ash (%)	0.6^{a} (0.2)	0.75^{a} (0.047)
Lactose (%)	3.52^{a} (0.12)	3.85^{a} (0.09)
Protein (%)	4.64^{a} (0.59)	4.18^{a} (0.59)
Polyphenol content (mg/g)	103.36^{b} (6.22)	17.88^{a} (5.76)

Values are the mean of triplicates and values in parenthesis represent standard deviation.

The lactose content of yoghurt from whole milk was similar to that reportd by Bhagiel *et al.* (2015). Protein content of control sample found in our study was similar to that reported by Mohammad and El-Zubeir (2011). Fat and Total solid of control sample found in our study was similar to that reported by Hofi *et al.* (1994). pH and acidity of control sample was similar to Salji and Ismail (1983). The lactose content and pH decreases and acidity increases during fermentation. This may be due to the fermentation of lactose into lactic acid (Zourari *et al.*, 1992). The fat content, TS and protein increases during fermentation. This may be due to the presence of microbial cells in fermented products they are rich in protein and fat.

The incorporation of cinnamon did not change the proximate composition of yoghurt which was also reported by Illupapalayam *et al.* (2014). Due to the antimicrobial activity of spices the acidity developed in cinnamon yoghurt was found to be less which was also illustrated by Asimi *et al.* (2013). The polyphenol content of cinnamon yoghurt was significantly higher from the control sample. This may be due to the incorporation of cinnamon (Illupapalayam *et al.*, 2014).

4.5 Storage stability of cinnamon oleoresin incorporated yoghurt

The storage stability of cinnamon oleoresin incorporated yoghurt was studied in terms of pH, acidity, syneresis and microbial count at room temperature and refrigerated condition for 10 days.

4.5.1 pH

The pH of sample incorporated with cinnamon oleoresin was found to be decreased at both room temperature and refrigerated condition from 4.5-3.06 and 4.5-3.8 respectively and that of control sample at room temperature and refrigerated condition was found to decrease from 4.22-3.04 and 4.22-3.49 respectively during the storage period of 10 days. The decreasing rate was found to be more at room temperature than that in refrigerated condition. Incase of control sample the rate of decreasing pH was high than that of cinnamon incorporated sample. The graph for pH is shown in Fig. 4.6.

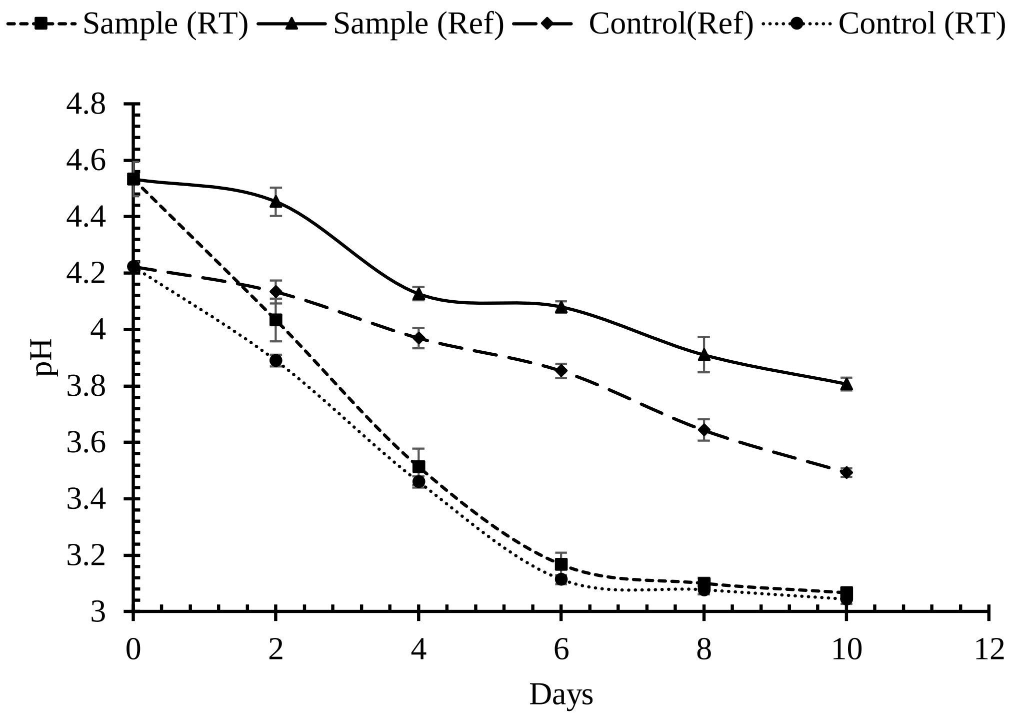

Fig. 4.6 pH changes during the storage period of yoghurt

* Vertical error bars represent the standard deviation.

Salji and Ismail (1983) reported the higher decreasing rate in pH as increase in temperature. This was similar to the result obtained in our study. The decrease in pH may be due to the the formation of lactic acid by the fermentation of lactose present in milk (Zourari *et al.*, 1992). But this decreasing rate was lower than that of control sample. This may be due to antimicrobial activity present in cinnamon (Asimi *et al.*, 2013). The incorporation of cinnamon decreases the rate of decline of pH this may be due to the interference of cinnamon in fermentation as cinnamon is antimicrobial in nature.

4.5.2 Acidity

The acidity of sample incorporated with cinnamon oleoresin was found to be increased at both room temperature and refrigerated condition from 0.6-1.23% and 0.6-0.87% as lactic acid respectively and that of control sample at room temperature and refrigerated condition was found to increase from 0.76-1.38% and 0.76-1.05% as lactic acid respectively during the storage period of 10 days. The increasing rate was found to be more at room temperature than that in refrigerated condition. The increasing rate of acidity was high for control sample than that of cinnamon incorporated sample. The graph for acidity is shown in Fig. 4.7.

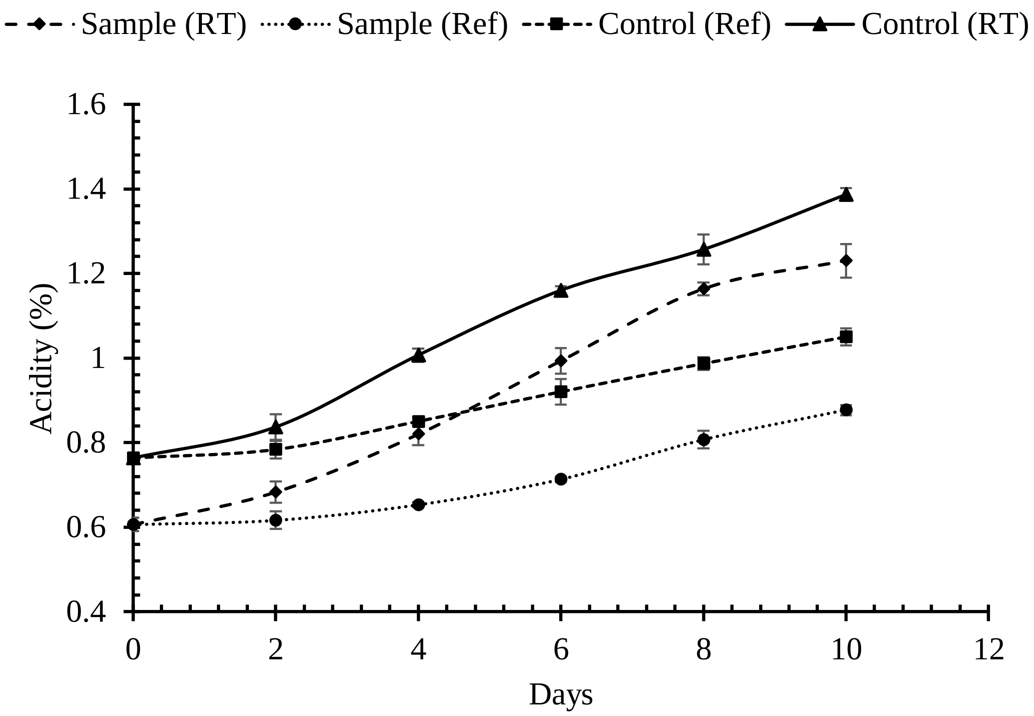

Fig. 4.7 Acidity changes during the storage period of yoghurt

* Vertical error bars represent the standard deviation.

Salji and Ismail (1983) reported the higher increasing rate in acidity as increase in temperature. This was similar to the result obtained in our study. The increase in acidity may be due to the the formation of lactic acid by the fermentation of lactose present in milk (Zourari *et al.*, 1992). But this increasing rate was lower than that of control sample. This may be due to antimicrobial activity present in cinnamon (Asimi *et al.*, 2013). The incorporation of cinnamon decreases the rate of increase in acidity this may be due to the interference of cinnamon in fermentation as cinnamon is antimicrobial in nature.

4.5.3 Syneresis

The syneresis of cinnamon oleoresin incorporated yoghurt at room temperature and refrigerated temperature were found to be increased from 22.61-40.60% and 22.61-31.97% respectively and that of control sample at room temperature and refrigerated condition was found to increase from 18.62-32.44% and 18.62-29.99% respectively during 10 days of storage period. The increasing rate of syneresis was higher incase of room temperature than refrigerated condition. The increasing rate of syneresis was higher for cinnamon incorporated sample than that of control sample. The graph for syneresis is shown in Fig. 4.8.

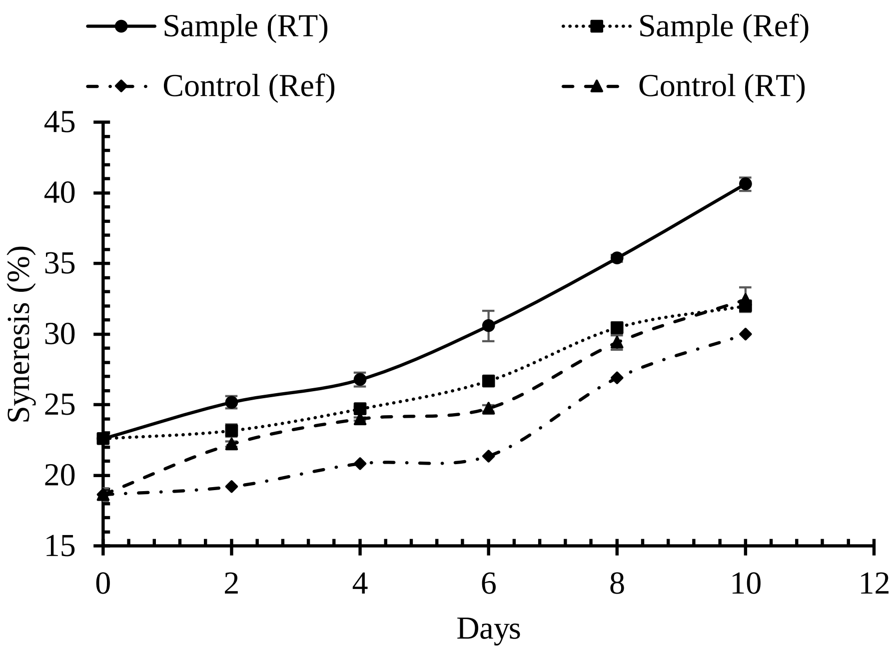

Fig. 4.8 Syneresis changes during the storage period of yoghurt

* Vertical error bars represent the standard deviation.

Dönmez *et al.* (2017) found that there was increase in syneresis rate with the incorporation os 2% green tea in yoghurt but also found decrease in syneresis of yoghurt incorporated with green coffee. This was similar to that of our study. This may be due to the presence of polyphenol content presence in cinnamon as the binding mechanisms were both hydrophobic and hydrophilic interactions. The formation or precipitation of protein-polyphenol complex was modeled by many researchers (Dönmez *et al.*, 2017). So, the presence of polyphenols may be both increase or decrease by polyphenols.

4.5.4 Microbial count

The TPC of room temperature and refrigerated condition of cinnamon oleoresin incorporated sample were found to be increased from 3.8×10^4 -8.4×10^4 cfu/ml and 3.8×10^4 -6.6×10^4 cfu/ml respectively and that of control sample at room temperature and refrigerated condition was found to increase from 4.1×10^4 -8.0×10^4 cfu/ml and 4.1×10^4 -7.3×10^4 cfu/ml during the 10 days storage period. The TPC was found to be increased more at room temperature that that in refrigerated condition. The TPC was higher for control sample than that of cinnamon incorporated sample. The graph for microbial count is shown in Fig. 4.9.

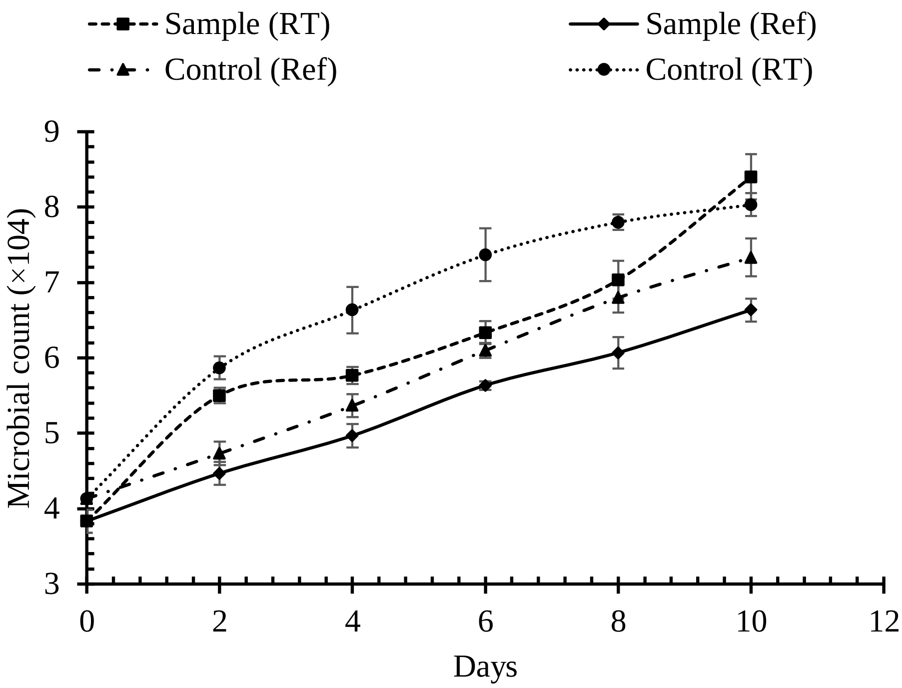

Fig. 4.9 Microbial count during the storage period of yoghurt

* Vertical error bars represent the standard deviation.

The increase in TPC of yoghurt is due to the production of lactic acid bacteria which increases with the increase in time. The results are in agreement with Ahmed (2011). Similar results was obtained by Goodluck *et al.* (2014) for the consumable range of total baterial count as in the range of (3.0×10^3-10.5×10^4 cfu/ml). The TPC was lower than that of control yoghurt. This may be due to antimicrobial activity of cinnamon (Asimi *et al.*, 2013).

On overall all the parameters pH, acidity, syneresis and microbial count were found better at refrigerated condition than that in room temperature. So, shelf life of yoghurt can be extended in refrigerated condition better than in room temperature. Also, due to antimicrobial activity the use of cinnamon can extend the shelf life of the yoghurt as compared to control yoghurt. The shelf life of product at refrigerated condition was found to be more than 10 days and at room temperature was about 5 days.

Part V

Conclusions and recommendation

5.1 Conclusion

Yoghurt was prepared by incorporating cinnamon and the storage stability of the prepared yoghurt was studied. On the basis of result obtained from physicochemical analysis of yoghurt following conclusions were drawn:

- From sensory analysis, yoghurt incorporated 0.1 ml of cinnamon per 100 ml milk was found to be superior product among prepared five samples.
- The total solid, fat, acidity, protein, total ash, lactose content pH and polyphenol content of cinnamon flavored yoghurt were found 16.02%, 4.43%, 0.6%, 4.64%, 0.6%, 3.52%, 4.71 and 103.36 mg/g sample respectively.
- The TPC of cinnamon yoghurt at refrigerated condition was less than at room temperature. On overall the TPC was less in cinnamon incorporated yoghurt than in control sample.
- The shelf life of product was higher in refrigerated condition than in room temperature. So, shelf life can be extended by refrigerating the product thus formed.

5.2 Recommendation

- Flavored yoghurt can be prepared by incorporating cinnamon in large scale.
- Other spices can also be used to improve the flavor of plain yoghurt.
- Stabilizers can also be used to improve the syneresis effect of yoghurt.

Summary

Yoghurt is more nutritious than many other fermented milk products because it contains a high level of milk solids in addition to nutriments developed during the fermentation process. Yoghurt is one of the most popular dairy products consumed worldwide and is made principally on the bacterial fermentation of milk. Yoghurt is produced when a lactic acid bacterial starter culture is used to ferment the sugar Lactose found in milk to lactic Acid which then acts on the protein to give yogurt its characteristic texture and form. Dairy yoghurt is produced using a mixed culture of *Lactobacillus bulgaricus* and *Streptococcus thermophilus* bacteria. Flavor of yoghurt can be improved by incorporating fruits or spices.

The standardized and pasteurized milk from Kamadhenu dairy was taken for the preparation of yoghurt. The milk was heated to 45°C and mixed with 4% SMP (Skim milk powder) and 3% sugar. Heating of milk was further continued till the temperature reached to around 65-70°C for certain period to pasteurize the milk so that the microorganisms like yeast and mold can be destroyed. After that the heated milk was cooled to around 43-44°C. After cooling, five formulations of the samples were made by adding 0, 0.1, 0.2, 0.3, and 0.4 ml of cinnamon per 100 ml milk.Then the starter culture is added at the rate of 2% to each formulations.Then the mix is placed in plastic cups.The yoghurt mix was then kept in an incubator which was maintained at a temperature of about 43°C and was kept for 3.5-4 h until the coagulum is formed.

Sensory evaluation of five products was carried out. The sensory evaluation revealed that the product containing 0.1 ml cinnamon incorporated yoghurt was found to be best whose total solid, fat, acidity, protein, total ash, lactose, pH and polyphenol content were found to be 16.02%, 4.43%, 0.6%, 4.64%, 0.6%, 3.52%, 4.71 and 103.36 mg/g respectively. The best product obtained from the sensory analysis and the control sample was stored for 10 days and its shelf life was estimated in terms of acidity, pH, syneresis and total plate count. The shelf life was found to 10 days at refrigerated conditions and 5 days at room temperature. This shelf life of the product was higher than that of control samples both in case of room temperature and refrigerated conditions.

From the overall analysis of the result it is clear that good quality falvored yogurt could be prepared by adding cinnamon in milk. Antimicrobial activity is present in cinnamon

which helps to increase the shelf life as well as cinnamaldehyde, flavor compound of cinnamon, helps to enhance the flavor of yoghurt.

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Appendix

Appendix A

3. **Sensory evaluation Card (specimen)**

(Hedonic Rating test)

Name: …....................................   Date: ……………………………

Product: *Cinnamon incorporated yoghurt*

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

Quality Description

9 = Like extremely	6 = Like moderately	3 = Dislike moderately
8 = Like very much	5 = Neither like nor dislike	2 = Dislike very much
7 =Like moderately	4 = Dislike slightly	1 = Dislike extremely

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

Parameters	Sample A	Sample B	Sample C	Sample D	Sample E
Color					
Flavor					
Taste					
Texture					
Overall acceptability					

Comments if any: ……………………………………………………………………………

Signature ________________

Appendix B

Table B.1 ANOVA (no blocking) for color of yoghurt

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.	l.s.d
Sample	4	3.08	0.77	1.81	0.0148	0.5917
Panelist	9	13.78	1.5311	3.6	0.003	0.8368
Residual	36	15.32	0.4256			
Total	49	32.18				

Table B.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	4	14.32	3.58	6.68	<.001	0.664
Panelist	9	6.42	0.7133	1.33	0.255	0.939
Residual	36	19.28	0.5356			
Total	49	40.02				

Table B.3 ANOVA (no blocking) for texture of yoghurt

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.	l.s.d
Sample	4	14.08	3.52	7.76	<.001	0.6107
Panelist	9	8.98	0.9978	2.2	0.045	0.8636
Residual	36	16.32	0.4533			
Total	49	39.38				

Table B.4 ANOVA (no blocking) for taste of yoghurt

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.	l.s.d
Sample	4	36.88	9.22	16.02	<.001	0.688
Panelist	9	15.38	1.7089	2.97	0.01	0.973
Residual	36	20.72	0.5756			
Total	49	72.98				

Table B.5 ANOVA for overall acceptability of yoghurt

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.	l.s.d
Sample	4	28.32	7.08	22.6	<.001	0.5077
Panelist	9	8.32	0.9244	2.95	0.01	0.718
Residual	36	11.28	0.3133			
Total	49	47.92				

Appendix C

Table C.1 t-test for fat content

	Sample	Control
Mean	4.433333	3.9
Variance	0.003333	0.01
Observations	3	3
Hypothesized Mean Difference	0	
df	3	
t Stat	8	
P(T<=t) one-tail	0.002038	
t Critical one-tail	2.353363	
P(T<=t) two-tail	0.004077	
t Critical two-tail	3.182446	

Table C.2 t-test for TS

	Sample	Control
Mean	16.02333	14.79667
Variance	0.183633	0.585233
Observations	3	3
Hypothesized Mean Difference	0	
df	3	
t Stat	2.423046	
P(T<=t) one-tail	0.046956	
t Critical one-tail	2.353363	
P(T<=t) two-tail	0.093912	
t Critical two-tail	3.182446	

Table C.3 t-test for protein content

	Sample	Control
Mean	4.64	4.176667
Variance	0.3493	0.356633
Observations	3	3
Hypothesized Mean Difference	0	
df	4	
t Stat	0.955152	
P(T<=t) one-tail	0.196795	
t Critical one-tail	2.131847	
P(T<=t) two-tail	0.39359	
t Critical two-tail	2.776445	

Table C.4 t-test for lactose content

	Sample	Control
Mean	3.516667	3.853333
Variance	0.014633	0.008133
Observations	3	3
Hypothesized Mean Difference	0	
df	4	
t Stat	-3.86466	
P(T<=t) one-tail	0.009038	
t Critical one-tail	2.131847	
P(T<=t) two-tail	0.018075	
t Critical two-tail	2.776445	

Table C.5 t-test for ash content

	Sample	Control
Mean	0.6	0.746667
Variance	0.04	0.002233
Observations	3	3
Hypothesized Mean Difference	0	
df	2	
t Stat	-1.23613	
P(T<=t) one-tail	0.170945	
t Critical one-tail	2.919986	
P(T<=t) two-tail	0.341889	
t Critical two-tail	4.302653	

Table C.6 t-test for pH

	Sample	Control
Mean	4.716666667	4.703333
Variance	0.000433333	0.000233
Observations	3	3
Hypothesized Mean Difference	0	
df	4	
t Stat	0.894427191	
P(T<=t) one-tail	0.210824128	
t Critical one-tail	2.131846786	
P(T<=t) two-tail	0.421648255	
t Critical two-tail	2.776445105	

Table C.7 t-test for acidity

	Sample	Control
Mean	0.6	0.77
Variance	0.0007	0.0021
Observations	3	3
Hypothesized Mean Difference	0	
df	3	
t Stat	-5.564556201	
P(T<=t) one-tail	0.00572569	
t Critical one-tail	2.353363435	
P(T<=t) two-tail	0.011451381	
t Critical two-tail	3.182446305	

Color plates

P.1 Yoghurt sample

P.2 Sensory Analysis

P.3 Analysis of sample

P.4 Microbial analysis of sample