PREPARATION AND QUALITY EVALUATION OF PEANUT MILK INCORPORATED YOGHURT

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

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Approval Letter

This *dissertation* entitled *Preparation and Quality Evaluation of Peanut Milk Incorporated Yoghurt* presented by Ashish Chhetri has been accepted as the partial fulfillment of the requirements for the B. Tech. degree in Food Technology.

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Abstract

The main objective of this study was to prepare yoghurt by using cow milk and different proportion of peanut milk with 2% starter culture of *Lactobacillus acidophilus* and *Streptococcus thermophilus* and evaluate the effects of peanut milk incorporation on yoghurt quality. Raw peanut of variety 'Jayanti' (Type-Spanish bunch) was collected from Sunsari district, Dharan and were soaked for 18 h and grinded with water (1:6 of kernel to water) to prepare peanut milk. Design expert® version 10 D-optimal design was employed for the formulating the recipe of yoghurt. The obtained seven formulations coded A (5%), B (10%), C (15%), D (20%), E (25%), F (30%) and G (0%) of peanut milk incorporated yoghurt were prepared in laboratory. The samples were subjected to sensory evaluation (color, taste, texture, aroma and overall acceptability) by quality scoring method for consumer acceptability and the sensory data were analyzed by two-way ANOVA (no blocking) using Genstat and means were compared using LSD at 5% level of significance.

From sensory evaluation, formulation D (20%) peanut milk incorporated yoghurt was found to be significantly ($p<0.05$) superior in sensory quality. The total solids, fat, acidity, protein, total ash, moisture content, lactose content, antioxidant activity and pH of this formulation were found to be 19.63%, 3.45%, 0.66%, 3.72%, 0.97%, 80.7%, 2.92%, 74.46% and 4.2 respectively. The shelf life of this product was estimated in terms of acidity and total plate count and the shelf life was found to be 1 day at room temperature and 6 days under refrigeration (5°C). The total cost of the best peanut milk incorporated yoghurt per 100 ml was found to be NRs. 12.05 (as of 2020).

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List of Abbreviations

Abbreviations	Full form
SMP	Skim milk powder
UHT	Ultra high temperature
HTST	High temperature short time
ANOVA	Analysis of variance
LSD	Least significant difference
CCP	Critical control point
CIP	Clean in process
LAB	Lactic acid bacteria
RDI	Recommended daily intake
DPPH	2,2-diphenyl-1-picrylhydrazyl
TPC	Total plate count
CFU	Colony forming unit

Part I

Introduction

1.1 General introduction

Fermented milk products such as yogurt have been consumed for several thousand years and the belief that they are beneficial to health is probably equally ancient. But it is only in recent years that scientific support for these beliefs has begun to build. Fermented milk products, like the milk from which they are made, are rich in protein, vitamins and minerals. However, in addition to these purely nutritional properties, there is increasing support for a number of other health advantages and also helps to preserve milk with an extended shelf life (Buttriss, 1997). Microorganisms employed as starters for production of cultured dairy foods are divided into two types, based on the optimum temperature ranges at which they operate. The lactic acid bacteria incubated at temperatures above 35°C are referred to as thermophilic bacteria and those incubated at 20-30°C are called mesophilic starters, which act in symbiosis with each other (Chandan *et al.*, 2008).

Yoghurt is a fermented product obtained through an anaerobic fermentation of lactose in milk by relevant microorganisms most of which are classified as pro-biotic (Tull, 1997). Yoghurt is a cultured dairy product produced by fermenting milk, with or without added non-fat dry milk (NFDM) with *Lactobacillus bulgaricus* and *Streptococcus thermophilus* bacteria. It usually contains 12-14% total milk solids and has soft, friable custard like consistency, and a clear and distinct acid flavor. Yoghurt is usually produced by heating the mix to 80-85°C for 30 min to pasteurize it and to modify the milk proteins so that they will provide the proper viscosity and gelation with a minimum of syneresis in the product (Adhikari, 2018).

Peanut (*Arachis hypogaea* L.) is a major source of edible oil and protein meal. It is considered to be highly valuable in human and animal nutrition. Peanuts may be consumed raw, roasted, pureed, or in a variety of other processed forms and constitute as a multimillion-dollar crop worldwide with numerous potential dietary benefits like high protein content and health promoting oils. It is therefore necessary to adequately research into the possibility of peanut processing and utilization in other edible products (Isanga and Zhang, 2007). It has been used as a major source of edible oil and protein

meal and considered highly valuable for human and animal nutrition in developing countries. Peanuts are rich source of multiple nutrients and their consumption is associated with various health benefits, including reduced cardiovascular disease risk. It has been reported that eating peanuts or peanut butter could provide the body with the daily requirements of many of the essential vitamins and minerals such as vitamin A, vitamin E, folate, magnesium, zinc, iron, calcium, and dietary fiber (Elsamani and Mohamed, 2014).

Therefore, consuming symbiotic foods that contain prebiotics (fibers) and probiotics (lactic acid bacteria) would offer added nutritional benefits that can help to boost overall health and well-being. Therefore, the objective of this study is to produce yoghurt enriched with peanut milk and to determine the physicochemical, microbial, sensory qualities and overall acceptability of the product.

1.2 Statement of the problem

Yoghurt is a dairy product made from milk that has been fermented with lactic acid. It is one among the world's most popular fermented milk products. In the human diet, yoghurt can be a rich provider of vital nutrients such as protein, milk fat and minerals. It could provide a major contribution to the daily calcium, potassium and magnesium requirements needed to keep the physiological process running smoothly (Teshome *et al.*, 2017). Because of its probiotic properties, yoghurt has medical applications. Probiotic foods can give a variety of health benefits, including improved immune system function and increased resistance to infection in the upper respiratory tract, common infections, and gastrointestinal diseases (Lollo *et al.*, 2013).

The addition of fruits and flavors to yoghurt has become very popular in recent years. In context of Nepalese market availability of fruits and flavor yoghurt is rare (Gupta, 2003). Therefore, addition of peanut milk can be beneficial to give the product variety and to improve the commercial value of yoghurt. Peanut milk incorporated yoghurt could be a better option to increase its utilization along with the improvement of yoghurt quality.

1.3 Objectives

1.3.1 General objective

The general objective of the dissertation work was to prepare yoghurt by the addition of peanut milk in different proportion.

1.3.2 Specific objectives

The specific objectives of this dissertation work were to:

a. Study the addition of different levels of peanut milk on the prepared yoghurt and to evaluate its sensory properties.
b. Analyze the peanut milk yoghurt for its proximate composition.
c. Study the shelf life of the yoghurt at room temperature and refrigeration.
d. Study the effect in the antioxidant property of yoghurt with addition of peanut milk.
e. Evaluate the cost of yoghurt.

1.4 Significance of the study

Yogurt is one of the most known and popular fermented dairy products due to its nutritional value and is consumed by most of the world's population. In modern technology of yoghurt manufacturing, the addition of fruit and flavoring is well popular (Kucukoner and Tarakci, 2003). Fruits and vegetables which are incorporated with yoghurt are great source of natural antioxidants, fibers (prebiotics), and also serve as flavoring and coloring agent. The photochemical antioxidants such as carotenoids, flavonoids and phenols etc. have potential health role in the reduction of platelet aggregation, blood pressure, cardiovascular of disease and a role in modulation of cholesterol synthesis and absorption (Abou El Samh *et al.*, 2013).

Peanut milk and peanut milk products have nutritional benefits for young and old people because of their extreme richness in protein, minerals and essential fatty acids such as linoleic and oleic acids, which are considered to be highly valuable in human nutrition. Peanut eating is not linked to weight gain and has been shown to benefit glucose metabolism, blood lipid levels, and overall cardiovascular health (Jones *et al.*, 2014). Peanuts are good food for infants suffering from various forms of malnutrition and for

individual with lactose intolerance allergies. Peanut milk does not contain any lactose and is therefore suitable for people with lactose intolerance (Yadav *et al.*, 2018). The current interest in peanut milk and peanut milk products is motivated by the fact that dairy and dairy products are always priced too high for the low-income earners. Another factor, no less important, is the growing awareness of the nutritional benefits of vegetable proteins in low cholesterol diets by health-conscious people. Also, peanut milk does not contain lactose; therefore lactose intoleranat patients can easily consume peanut milk yoghurt. Fermented products manufacturing revealed that hexanal, which is one of the compounds responsible for the unwanted beany flavor in peanut milk, completely disappeared as a result of fermentation (Lee and Beuchat, 1991). Also, incorporation of $NaHCO_3$ with soak peanut water helps to improve the flavor of extracted peanut milk (Beuchat and Nail, 1978). Preparation and fermentation of peanut milk may serve as one such effort that can increase the consumption of this valuable crop and hence improve protein availability and consumption (Isanga and Zhang, 2007).

1.5 Limitations of the study

Following were the limitations of the present study:

a. Best yoghurt could not be compared with commercial yoghurt.
b. Only one variety of peanut was taken for study.
c. Variation of sugar and skim milk could not be carried out.
d. Shelf life of yoghurt could not be compared with control.

Part II

Literature review

2.1 A brief history of yoghurt

Yogurt is a semisolid fermented product obtained by souring milk using a pure starter culture of *Lactobacillus bulgaricus* and *Streptococcus thermophilus* (Farinde *et al.*, 2009). It is a source of several essential nutrients, including protein, calcium, potassium, phosphorus, and vitamins B_2 and B_{12}, and serves as a vehicle for fortification. Yogurt is an ancient food that has gone by many names over the millennia: katyk (Armenia), dahi (India), zabadi (Egypt), mast (Iran), leben raib (Saudi Arabia), laban (Iraq and Lebanon), roba (Sudan), iogurte (Brazil), cuajada (Spain), and matsoni (Georgia, Russia, and Japan). It is believed that milk products were incorporated into the human diet around 10000–5000 BC, with the domestication of milk-producing animals (cows, sheep, and goats, as well as yaks, horses, buffalo, and camels) (Fisberg and Machado, 2015).

Yogurt has been consumed since recorded time. It is not exactly known how yogurt was discovered, but it is assumed that it was by accident, perhaps by Mesopotamians in about 5000 BC (Fisberg and Machado, 2015). During this time, herdsman would milk goats and sheep and carry the milk with them in pouches made from an animal's stomach. These stomachs contained a natural enzyme, called chymosin, which forms a gel or coagulum when added to milk. Given the warm climate in this part of the world, the storage conditions available at the time, and natural starter culture in the milk – either yogurt or cheese was made. Fermentation probably began within a few hours. Most likely, these people noted that this soured milk product tended to keep longer and they grew to prefer the flavor of yogurt to that of fresh milk. These people also eventually realized the health benefits of eating yogurt, and much later, some observers wrote about living a longer and healthier life as a direct result of frequent consumption of the fermented products (Andrews, 2000).

Yogurt also traces its roots to the Caucasus Mountain region of Russia. The people of this rugged region were commonly nomadic and as subsistence used both the milk and meat of cows, sheep, goats, and yaks. The fermented milk product traditional to this region, kefir, is a liquid cultured product whose name translates to 'good feeling'. It also earned

the reputation as being a healing drink and was considered a ‘gift of the gods’. Kefir was widely consumed by all families, and the bacteria culture that was used to ferment this product was prized and guarded most closely. The broad popularity of kefir in Russia dates back to the early 1900s.The society was looking to popularize this product for its reputed health and aging benefits. The royal Caucasus family closely guarded the culture used to produce kefir (Tribby, 2008).

Historically, fermentation was used by humans for preservation of milk. It is thought to have originated in the Middle East area even before the Phoenician era. In Egypt, the consumption of traditional fermented milk such as laban rayeb and laban khad dates back to around 7000BC. The Vedas (Indo-Aryan treatises) also mention dadhi (modern-day yogurt) dating back to 5000 years BC. Dadhi or dahi is still a crucial component of the South Asian diet. It is produced in most Indian households and consumed daily. The word yogurt is believed to have been first used by the Turks in the 8th century, which appeared as yoghurut. It is thus assumed that the Turkish nomads in Asia made yogurt. Another legend, however, states that yogurt was first prepared or invented by the Balkan people. Sour milk, prokish, was prepared from sheep’s milk by the peasants of Thrace. South Asian regions (India, Pakistan, Nepal, and Bangladesh), as well as southwest Asia regions (Iran, Iraq, Balkans, Turkey, Syria) are among the largest producers and consumers of fermented milk products (including yogurt). It is believed that the invasion of Mongols, Tartars, and other Asian rulers to Russia and Europe also contributed to the spread of yogurt and fermented milk to other parts of the world (Chandan *et al.*, 2017).

Nobel laureate Elie Metchnikoff at the Pasteur Institute in Paris first proposed a scientific rationale for the beneficial health effects of the yogurt bacteria at the beginning of the last century. In his article entitled “The Prolongation of Life” from the use of yogurt bacteria, he hypothesized that *S. thermophilus* and *L. delbrueckii* ssp. *bulgaricus* control the infections caused by enteric pathogens and regulate toxemia, both of which play a major role in ageing and mortality. He also related the longevity and good health of the Bulgarian peasants to their high consumption of yogurt and other fermented milk products. It was later discovered that the common yogurt starter bacteria *S. thermophilus* and *L. delbrueckii* ssp. *bulgaricus* were unable to implant in the intestine. This observation provided a great impetus to the manufacture and consumption of yogurt (Prajapati and Nair, 2003).

2.2 Milk

Milk is a complex biological fluid, the composition and physical characteristics of which vary from species to species, reflecting the dietary needs of the young mammal. The major constituent of milk is water and contains varying quantities of lipids, proteins and carbohydrates which are synthesized within the mammary gland. Smaller quantities of minerals and other fat-soluble and water soluble components derived directly from blood plasma, specific blood proteins and intermediates of mammary synthesis are also present (Varnam and Sutherland, 2001).

The pH of fresh milk ranges from 6.5 to 6.7 with an initial acidity of 0.14 to 0.16%. pH and acidity measurements are often used as acceptance tests and quality of milk. These tests are used to monitor processes such as cheese-making and yoghurt making (Rafiq *et al.*, 2016). Approximately 80% of the milk proteins are caseins which consist of α-, β-, κ-, and ϒ caseins. The casein micelles and the fat globules give milk most of its physical characteristics, and give taste and flavor to dairy products. Processing of milk by its nature involves the imposition on a changing colloidal system. This is because the colloidal particles in milk alter their nature and behavior. For instance changing pH causes disintegration rearrangement of the micelles and if pH is low enough, new particles of iso-electric casein are formed. Also, heating to high enough temperatures causes the binding of serum proteins to the micelle to breakdown (Lucey, 2002).

2.3 Milk fermentation and biochemical changes

Food fermentation is one of the oldest known uses of biotechnology. Fermented food and beverages dated back many thousands of years and it continues to provide an important part of human diet supplying about 20-40% of food worldwide (Campbell-Platt, 1994). In recent years this method has been widely used to improve food quality, safety, nutritional values and palatability and to develop new food products. Microbial fermentation in food fermentation involves the breakdown of sugar and protein which results in the production of a large array of organic compounds that contribute to the flavor, preservation and outer appearance of the food product (Hugenholtz *et al.*, 2000). Milk fermentation is initiated by lactobacilli and streptococci bacteria which use nutrients in milk for their growth and alter the nutritional composition and physical appearance of milk (Urbiene and Leskauskaite, 2006). Milk fermentation can be defined as any modification of the chemical or physical

properties of milk or dairy products resulting from the activity of microorganisms or their enzymes. It occurs when bacteria break down milk sugars and other components of milk to give lactic acid, alcohols, carbon dioxide etc. Lactose, fat and citric acid comprise the important fermentable compounds of milk. Lactose a disaccharide, is the chief source of carbon while fat and citric acid provides hydrogen and oxygen source respectively (Widyastuti and Febrisiantosa, 2014).

Lactose is used by lactic acid bacteria (LAB) as the principal source of carbon for growth and energy. It is initially hydrolyzed by lactase into galactose and glucose (Greenberg and Mahoney, 1982) followed by subsequent glucose conversion to D- or Llactic acid via the glycolytic, Embden-Meyerhof-Parnas pathway (Hemme *et al.*, 1980). The lactic acid fermentation consists of two major pathways that include homolactic fermentation which produces lactic acid and heterolactic fermentation which produce equimolar amount of lactic acid, carbon dioxide and ethanol (Vakil and Shahani, 1970). Protein is degraded by proteolysis and increases the peptide and free amino acid content of fermented milk products (Alm, 1982). Lipids are sparingly hydrolyzed by LAB lipases which are more active towards lower but not higher molecular weight triglycerides (Collins *et al.*, 2003). Although lipases are present in *S. thermophilus* & *L. delbrueckii* subsp. *bulgaricus*, they have little effect on free fatty acid content of fermented milk products (Fernandes *et al.*, 1991). LAB require minerals and vitamins for growth (as mineral catalysis and mediators in the enzymatic reaction respectively) but their requirement is small and would not significantly alter the total content of fermented milk products. The bioavailability of some of the minerals may be changed due to pH changes caused by fermentation (Hayek *et al.*, 2019).

2.4 Probiotics

Probiotics are defined as ‘live microorganisms’ which when administered in adequate amounts confer a health benefit on the host (Ismail *et al.*, 2018). Probiotics are live microbes which influence the well-being of their host through their effect on the intestinal microflora. It was also called “a live microbial food ingredient that is beneficial to health”. Probiotic improves intestinal microbial balance and reduction in these bacteria which are naturally found in the human small intestine and large intestine increases the presence of potentially pathogenic microbes (Salminen *et al.*, 1998). Many probiotics are members of

the genera *Lactobacillus* and *Bifidobacterium*. Some probiotic strain with scientific documentation include: *Lactobacillus rhamnosus, Lactobacillus acidophilus, Lactobacillus paracasei, Lactobacillus plantarum, Lactobacillus delbrueckii* subsp. *bulgaricus, Bbifidobacterium lactis ,Bifidobacterium longum* and *Lactobacillus gasseri* (Kneifel, 2000). Over the past decade, considerable interest has developed in the use of probiotic organisms in food, pharmaceutical and feed products (Crittenden *et al.*, 2005).

Dairy products including yoghurt and cheese, due to the presence of lactose and peptides, are preferred medium for probiotics or health promoting bacteria. They provide the ideal food system for the delivery of these beneficial bacteria to the human gut, given the suitable environment to promote growth and support viability of these cultures (Ricke and Pillai, 1999). Functional food contains a proper balance of nutrients and non-nutrients such as dietary fibre and various bioactive compounds as well as probiotics which aid in the preventing and treatment of diseases. From health point of view probiotic bacteria (*Lactobacillus acidophilus* and bifidobacteria) are widely used as dietary adjuncts as these organisms are normal inhabitants of the intestine. One of the most important properties of probiotic bacteria is their ability to survive passage through a gastrointestinal tract and persist for a sufficient time in the gut so they can provide beneficial health effects (Huang and Adams, 2004). They have several health specific advantages and nutritive values such as preventing diet related diseases, coronary heart diseases, obesity, hypertension, and certain type of cancer, gastrointestinal diseases and osteoporosis (Guarner and Malagelada, 2003).

2.5 Health benefit of probiotics

Several reported health benefits of probiotic bacteria are reduced duration of diarrhea, antagonistic effects against pathogenic microorganisms, improved lactose digestion regulation of intestinal motility, and reduced activities of cancer-related enzymes, improved calcium resorption and provision of water soluble vitamins (Crittenden *et al.*, 2005). The action of probiotics on intestinal flora results in vital benefits, including protection against pathogens, development of the immune system and positive effects on colonic health and host nutrition. There is also evidence to suggest that certain species/strain of probiotics is anti-carcinogenic. Other important properties that have been attributed to probiotics include prevention and treatment of gastrointestinal disorders,

reduction of food intolerance, modulation of the host immune responses, and prevention of cancer and cardiovascular diseases (reduction of serum cholesterol and lipids) (Wollowski *et al.*, 2001). Multiple species or high numbers of probiotic organisms may be required to be administered simultaneously to achieve colonization, as shown in the treatment of pouchitis and in reducing the risk of urogenital infections. It is becoming more apparent that the more these intestinal-friendly bacteria are present in the colon, the lower are the chances of acquiring colon diseases (Reid *et al.*, 2003).

2.6 Yoghurt culture bacteria

The thermophilic LAB, *Streptococcus thermophilus* & *Lactobacillus delbrueckii* subsp. *bulgaricus* are used together as important starter microorganisms in the production of yoghurt and some kind of cheeses. Because both bacteria are able to grow alone in milk, this indirect positive interaction is called proto-cooperation (Rotar *et al.*, 2007). This positive relationship often has a beneficial effect on bacterial growth and on the production of lactic acid and aromatic compounds. Lactic acid production results in the lowering of pH and this makes product unsuitable for growth of spoilage or pathogenic microorganisms (Donkor *et al.*, 2007). *S. thermophilus* grows faster and produces both acid and carbon dioxide. The formate and carbon dioxide produced stimulates *L. bulgaricus* growth. On the other hand, the proteolytic activity of *L. bulgaricus* produces stimulatory peptides and amino acids for use by *S. thermophilus.* These microorganisms are ultimately responsible for the formation of typical yoghurt flavor and texture. The yoghurt mixture coagulates during fermentation due to the drop in pH. The streptococci are responsible for the initial pH drop of the yoghurt mix to approximately 5.0. The lactobacilli are responsible for a further decrease to pH 4.0. Lactic acid, acetaldehyde, acetic acid and diacetyl are the fermentation products that contribute to flavor (Chandan *et al.*, 2017). Different methods are used to improve the quality of lactic and probiotic starters like the use of effective cryoprotectants, the use of adequate freezing and storage conditions and the selection of more-resistant strain (Wang *et al.*, 2005).

2.7 Types of starter culture

2.7.1 Pure and mixed culture

A further sub-division is made into either pure cultures or mixed cultures. Pure culture consists of only one species of lactic acid bacteria, whereas mixed cultures consist of several species of lactic acid bacteria. Pure cultures may consist of one or more strains of the same species. Mixed cultures are the most common type in acidification with a mixed culture and on rare occasions on its own. DL cultures used to be cultivated as “dairy cultures” at individual dairies, often the same culture for decades (Dave and Shah, 1997).

2.7.2 Mesophilic and thermophilic culture

Mesophilic cultures have optimum temperature for growth between 20 to 30°C and include *Lactococcus* and *Leuconostoc*. These mesophillic lactic cultures are used in the production of many cheese varieties where important characteristics are:

1. Acid producing activity
2. Gas production, and
3. Production of enzymatic activity for cheese ripening, e.g., proteases and peptidases enzymes.

Thermophilic cultures have optimum temperature for growth between 37 to 45°C. Thermophilic cultures are generally employed in the production of yoghurt, acidophilus milk, swiss type cheese. Thermophilic cultures include species of *Streptococcus* and *Lactobacillus*. These cultures grow in association with milk and form the typical yoghurt starter culture. This growth is considered symbiotic because the rate of acid development is greater when two bacteria are grown together as compared to single strains (Dave and Shah, 1997).

2.7.3 Liquid culture

The liquid cultures are generally no longer distributed in commercial practice. To prepare a liquid culture the organisms are propagated in a suitable medium such as milk or whey and maintained in an active condition by periodic transfers. In general, a liquid culture contains about 10^9 organisms per ml of starter (Neilson and Ullum, 1989).

2.7.4 Powdered culture

Powdered cultures are manufactured by freeze-drying a liquid culture cultivated to a maximum bacterial count. Freeze drying means drying under vacuum. This is a gentle method which minimizes the reduction in the bacterial count during manufacture. Ordinary freeze-dried cultures must be re-inoculated into a mother culture before use (Neilson and Ullum, 1989).

2.7.5 Frozen culture

Deep frozen cultures are prepared by deep freezing a concentrated, liquid culture at the point of the bacteria growth at which the activity is at its highest. They are preserved by lyophilization in small vials. Super-concentrated, deep frozen cultures are made by adding growth-promoting substances to a milk substrate, continuously neutralizing the lactic acid formed by means of ammonium hydroxide, and finally concentrating the culture in a desludging centrifuge/bactofuge. The concentrate is pelletized by being frozen as individual drops in liquid nitrogen. The culture is stored at -196°C until it is dispatched to the dairies in foamed plastic boxes containing dry ice (Neilson and Ullum, 1989).

2.8 Preparation of starter culture

Culturing the two organisms together results in a symbiotic relationship since the growth rate and acid production by each organism are greater than in single culture. Optimum growth temperature for rod and coccus are 45°C and 40°C respectively. A ratio of 1:1 is generally accepted as ideal. Using 2% inoculum and incubation at 44°C for 2.5 h produces good yoghurt. *S. thermophilus* attains acidities of 0.85-0.95%, whereas *L. bulgaricus* attains acidities of 1.20-1.50% (Neilson and Ullum, 1989).

2.9 Metabolism charactistics of LAB in yoghurt

2.9.1 Carbohydrate metabolism and acid production

LAB needs a sugar for energy production and subsequent growth. Fermentation of lactose is called glycolysis or glycolytic pathway. LAB ferments lactose into pyruvic acid, which is then reduced to lactic acid. Thus, lactic acid is obtained as the sole product and this process is called homo-lactic fermentation (Tamime and Robinson, 1999). Lactic acid

reduces the pH of the milk and leads to a progressive solubilization of micellar calcium phosphate. This causes the demineralization of casein micelles and their destabilization, which generates the complete precipitation of casein in a pH range of 4.6-4.7 (Zourari *et al.*, 1992). The increase in lactic acid in yoghurt decreases pH. The decrease in pH causes acid coagulation of milk with a clot formation in the final semi-solid mass (Baglio, 2014).

2.9.2 Protein metabolism

Lactic acid bacteria (LAB) are characterized by their high demand for essential growth factors such as peptides and amino acids. However, milk does not contain sufficient free amino acids and peptides to allow the growth of LAB (Abu-Tarboush, 1996). Therefore, the degradation of milk proteins to peptides is catalyzed by proteolytic enzymes present in LAB. LAB possesses a complex system of proteinases and peptidases, which enable them to use milk casein as a source of amino acids and nitrogen. The first step in casein degradation is mediated by cell wall located proteases, which cleave casein to oligopeptides. Further degradation to smaller peptides and amino acids that can pass through the cell membrane is performed by peptidases (Wohlrab and Bockelmann, 1992). The hydrolysis of peptides to free amino acids and subsequent utilization of these amino acids is a central metabolic activity in LAB, and proteolysis has been identified as the key process influencing the rate of flavor and texture development in yoghurt (Bintsis, 2018).

2.9.3 Formation of flavor components

The three main pathways which are involved in the development of flavor in fermented food products are glycolysis (fermentation of sugars), lipolysis (degradation of fat) and proteolysis (degradation of proteins) (Smit *et al.*, 2005). Lactate is the main product generated from the metabolism of lactose and a fraction of the intermediate pyruvate can alternatively be converted to diacetyl, acetone, acetaldehyde or acetic acid which are important for typical yogurt flavor (Bintsis, 2018). The contribution of LAB to lipolysis is relatively little, but proteolysis is the key biochemical pathway for the development of flavor. Degradation of proteins yields small peptides and free amino acids, the latter of which can be further converted to various alcohols, aldehydes, acids, esters and sulphur compounds for specific flavor development in dairy products (Tamime and Robinson, 1999).

2.9.4 Synthesis of organic acid and vitamins

Dairy products have generally been considered an excellent source of B vitamins, riboflavin, niacin, vitamin B_6, and vitamin B_{12}. A greater loss of vitamins may occur during the processing of yogurt because vitamins are more sensitive to changes in environmental factors (Buttriss, 1997). Folate is the best example of a B vitamin that some LAB species synthesize. The folate content of yogurt can vary widely, ranging from 4 to 19 μg/100 g (Shahani and Chandan, 1979). In the metabolism of lactic acid bacteria, certain metabolic processes can synthesize a variety of organic acids like fumaric, succinic, benzoic, acetic, butyric, pyruvic, formic acid and also the content of citric, orotic, and hippuric acids increase significantly in supplemented yoghurt (Venica *et al.*, 2014).

2.9.5 Lipid metabolism

Milk sources contain lipids, free fatty acids, triacylglycerols, cholesterol and phospholipids. Milk fat is present as complex globules with structural properties distinct from other biological sources of fats (Lock *et al.*, 2008). The enzymatic metabolism of fat is limited during the manufacture of fermented food products. The degradation of milk fat releases free fatty acids and glycerol, monoacylglycerides or diacylglycerides. In addition, they react with alcohols or free sulphydryl groups to form esters and thioesters, respectively, or act as precursors of a number of other flavor compounds, such as lactones (Fox and Wallace, 1997).

2.9.6 Production of exopolysaccharides

Several Gram-negative and Gram-positive bacteria, including lactic acid bacteria, produce exocellular polysaccharides (Ruas-Madiedo *et al.*, 2002). Some strains of *S thermophilus* and *L bulgaricus* produce neutral exopolysaccharides. The slime secreted by a strain of *L bulgaricus* and *S thermophilus*, contains galactose, glucose, mannose and small amounts of rhamnose, xylose and arabinose. Production of polysaccharides improves viscosity and texture, increase resistance to mechanical handling and decrease susceptibility to syneresis (Cerning *et al.*, 1990).

2.9.7 Production of antimicrobial compounds

Lactic acid bacteria produce metabolites like hydrogen peroxide, organic acids that promotes significant inhibitory, antagonistic effect and an important target for pathogens (Gram-positives and Gram-negatives) and food spoilage microorganisms (Papadimitriou *et al.*, 2015). Yogurt is a rich source of bioactive peptides with antioxidant activity that are produced during fermentation (Nguyen and Hwang, 2016). Thiocyanate and hydrogen peroxide have a broad-spectrum antibacterial action on pathogens (Seifu *et al.*, 2005). Several mechanisms suggest that the inhibitory activity of LAB against pathogenic bacteria, especially Gram-negative pathogens include production of organic acids, hydrogen peroxide, inhibitory peptides and bacteriocins, and competition for colonization sites with pathogenic bacteria (Davoodabadi *et al.*, 2015).

2.10 Yoghurt

Yogurt is typically milk that has been fermented and acidified with viable and well-defined bacteria, creating a thickened, often flavored, product with an extended shelf life. It contains essential nutrients and is a vehicle for fortification (added probiotics, fibers, vitamins, and minerals). It is also easily modified by sweeteners, fruits, and flavors to affect consistency and aroma. Yogurt can also be produced from rice, soy, or nuts (Fisberg and Machado, 2015). Yoghurt is a fermented milk product prepared by fermenting cow milk with lactic acid bacteria *Streptococcus thermophilus* and *Lactobacillus bulgaricus*. Acidification of milk by lactic acid bacteria enhances the aggregation of milk proteins to form a yoghurt gel. When milk is fermented and in the process gets acidified, the internal structural properties of casein miscells are disrupted (Kwasi *et al.*, 2014). Acid induced milk gels are formed by the aggregation of casein particles as the pH of milk decreases and caseins approach their isoelectric point (pH 4.6) This product is dependent upon the fact that casein (major protein of milk) is insoluble at its isoelectric point (pH 4.6, where the net charge of the casein is 0). Lactic acid bacteria produce lactic acid which reduces the pH from the natural pH of milk (pH 6.5-6.6) to pH 4.6 and lower. Yoghurt is produced by lactic acid bacteria that grow best at about 40°C. Commercial production of yoghurt increased rapidly in Europe after Metchnikoff's (1908) findings that consumption of sour milk prolongs life. The typical yoghurt flavor is caused by lactic acid, which imparts an acidic and refreshing taste, and a mixture of various carbonyl compounds like acetone,

diacetyl, and acetaldehyde, the latter of which is considered the major flavor component (Tamime and Deeth, 1980). Among the various dairy products, yoghurt is unique with the presence of acetaldehyde which is relatively high in concentration and desirable as essential flavor component (Osundahunsi *et al.*, 2007). Yoghurt is rich nutritional sources such as fat, high biological value protein, calcium, zinc, potassium, magnesium, phosphorus, riboflavin (vitamin B_2), thiamine (vitamin B_1), vitamin B_6, vitamin B_{12}, niacin, folate etc (Matela *et al.*, 2019).

Yoghurt has also been used as the most popular vehicle for incorporation of probiotic organisms. The LAB must survive in the gastrointestinal tract to provide beneficial properties. When viable LAB is consumed through fermented milk, the dairy constituents offer excellent buffering capacity. Furthermore since LAB is present in yoghurt (pH 4-4.5) the cells may be conditioned to low pH environment and survivability may be high in gastric juice which has low pH (Dave and Shah, 1997).

2.11 Coagulum formation in yoghurt

The acid coagulation of milk is the basis for a wide diversity of cultured dairy products. Acidification directly impacts the stability of casein micelles, reducing their charge, dissolving some of the insoluble calcium phosphate crosslinks and modifying internal bonding between proteins. The formation of aggregates and ultimately gels occurs at some critical point when electrostatic repulsion is reduced and is not sufficient to overcome attractive forces, like hydrophobic interactions. Acid-induced milk gels increase in stiffness with time due to on-going bond formation between casein particles within the network. In gels made from heated milk, an increase in the loss tangent parameter is observed for a short period after gelation; this phenomenon is due to the loss of insoluble calcium phosphate crosslinks within the casein particles that are already forming the gel matrix. The texture and physical properties of acid-induced gels are dependent on the specific conditions used for gel formation including: the rate of acidification, temperature, extent of whey protein denaturation, protein content, and presence of polysaccharide stabilizer (Lucey, 2016).

The formation of yoghurt gel is the result of the following biological and physical action of milk. Starter in yoghurt utilizes lactose for its energy and produces lactic acid and other relevant compounds become inevitable. Gradual development of lactic acid

destabilizes the calcium caseinate phosphate complex. Aggregates of casein micelles and/or the individual micelles group together and partially coalesce as the pH approaches the isoelectric point (pH 4.6 to 4.7). It is most likely that α-lactalbumin and β-lactoglobulin interaction with the κ-casein (linked by –SH and –SS bridges) partially protects the micelles against complete destabilization or disruption. As a result the gel network or matrix consists of a regular structure, which entraps within all the outer constituents of the basic mix including the water phase (Tamime and Robinson, 1999).

2.12 Health benefits of yoghurt

Healthy reasons to eat yoghurt are accumulating especially with the continuing research findings on the consumption of yoghurt and prevention of diseases formation. These are briefly described in the following:

1. Many people who cannot tolerate milk either because of protein allergy or lactose intolerance can enjoy yoghurt. The culturing process makes yoghurt more digestible than milk.
2. The friendly bacteria in yoghurt reduces the conversion of bile into carcinogenic bile acids and this seems to deactivate harmful substances (such as nitrates and nitrites before they are converted to nitrosamines) before they can become carcinogenic (Commane *et al.*, 2005).
3. Consumption of yoghurt during antibiotic prescription will minimize the effects of the antibiotic removal of friendly bacteria in the intestines. The live bacterial cultures in yoghurt can help replenish the intestines with helpful bacteria before the harmful ones take over.
4. Yoghurt can decrease yeast infection and it has prevention of growth of pathogenic bacteria.
5. Yoghurt is a rich source of calcium. Because of the live active cultures in yoghurt increase the absorption of calcium, serving of yoghurt gets more calcium into the body than same volume of milk. Daily intake of yoghurt reduces the risk of osteoporosis (Smith *et al.*, 1985).
6. Yoghurt is the excellent source of protein. Besides being a rich source of proteins, the limited proteolysis of the milk proteins during fermentation makes these proteins easier to digest. For this reason proteins are often called “pre-digested

protein" and have beneficial uses for certain people who lack the digestive enzyme due to disease states (Savaiano and Levitt, 1984).

7. Fermented milk products are excellent sources of dietary minerals particularly calcium, phosphorous, magnesium and zinc.
8. Several LAB are capable of synthesizing B-vitamins and their concentration in fermented milk is generally high.
9. Yoghurt can reduce the blood cholesterol. This is because the live cultures in yoghurt can assimilate the cholesterol or because yoghurt binds bile acids (which has also been shown to lower cholesterol) or both.
10. Certain whey peptides are known to have biological activity such as opioid and bactericidal activity.
11. Several peptides arising from proteolysis of milk proteins have biological activity and have pharmalogical effects on the nervous system, cardiovascular system and digestive system including immuno-modulating properties (Meisel and Schlimme, 1990).

2.13 Method for improving body of yoghurt

Traditional yoghurt was made by heating milk in open pans, concentrating it in this way to two-third volume. The higher solids content would also give thicker or more viscous yoghurt. Sheep milk, if used, would also give thicker yoghurt because it is about 50% richer in solids than ordinary cow milk (Tamime and Robinson, 1999).

If yoghurt is made from non-concentrated or unfortified cow milk an attractive gel is obtained, but this is delicate and easily broken by vibration. SMP at 4-5% level is incorporated to overcome this difficulty. The easiest and cheapest way is to incorporate a carbohydrate gum such as carrageenan, alginate, agar, etc. at a level of about 0.3%. This is harmless but does not add to the nutritive value, the milk fat is normally homogenized (Tamime and Robinson, 1999).

Ropy strains of both *S. thermophilus* and *L. bulgaricus* can be used at same temperature (43ºC) for ordinary yoghurt. However, the lower the temperature and the longer the time of incubation, the higher will be the viscosity. Thus temperature of 30-32ºC with an incubation time of 12-15 h may be used (Chandan *et al.*, 2008).

2.14 Yoghurt production

Yogurt process and formulation variations are as numerous as the number of manufacturers. The finished yogurt will vary in regard to body and texture, depending upon the type of ingredients, processing, starter cultures, flavor, and packaging that is used. The processing of yogurts can be broken down into the following steps: blending, pasteurization, homogenization, culturing and cooling, packaging and storage. Each is extremely important in the process, and strict attention to detail must be taken (Tribby, 2008). The basic steps for yoghurt production are as follows:

2.14.1 Milk procurement

Milk intended for yoghurt production must be of the highest bacteriological quality. It should have low content of bacteria and substances which may impede the development of the yoghurt culture. Milk should not contain antibiotics, bacteriophages, residues of CIP solution or sterilizing agents. The milk must be very carefully analyzed at the dairy (Bylund, 1995).

2.14.2 Standardization and mix preparation

In most yoghurt formulation, standardization of milk fat and SNF contents is done to bring uniformity in the product quality. 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 centrifugation to separate fat from milk. For stirred yogurt manufacture, the solids content of the milk is usually increased to about 16% with 1-5% being fat and 11-14% solids-not-fat (SNF). This is accomplished either by evaporating off some of the water, or adding concentrated milk or milk powder, other ingredients. 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 milk substance is fermented until it becomes yogurt. Fruits and flavorings are added to the yogurt before packaging the yogurt by reducing the tendency for it to separate on storage. Yogurt mix should have a minimum SNF of 12% to increase the viscosity and also to increase the resistance to "wheying off". After the solids composition is adjusted, stabilizers are added and the milk is pasteurized (Tribby, 2008).

2.14.3 Homogenization

Yogurt mix homogenization aids in hydration of stabilizers, and the interaction of stabilizers with milk proteins. In the manufacture of yogurt and other dairy products, it is common to homogenize mixes at approximately 63°C (145°F), with a total pressure of between 7 and 10 MPa (1000 and 1500 psi) in the 1st stage and 3 MPa (500 psi) in the 2nd stage. Different types of homogenizers may be used but the same pressure conditions are applied. Homogenization is done to reduce fat globule size, so that it helps to produce smooth texture, increases viscosity of yoghurt, and prevent creaming during incubation (Tribby, 2008).

2.14.4 Pasteurization

Pasteurization of yogurt mixes can be accomplished by several different methods. As with any other dairy product, the purpose for pasteurization is to heat treat milk to eliminate pathogenic bacteria. Coliforms and staphylococci are heat sensitive and are usually destroyed at temperatures above 60ºC (Adegoke *et al.*, 1992). In addition, it is very important to denature the proteins to attain the highest level of functionality from the milk proteins. Pasteurization also aids in the hydration of the stabilizers and dry ingredients that were added during blending, as well as adding a pleasant cooked flavor. The three main types of pasteurization are low temperature long time (LTLT) i.e., 63°C for 30 min, high temperature short time (HTST) i.e., 72-75°C for 15 s, and ultra-high temperature (UHT) i.e., 125-138°C for 2-4 s (Ranieri *et al.*, 2009).

2.14.5 Cooling

After pasteurization and homogenization, the yogurt mix is cooled to the optimum setting temperature. The milk is cooled to 42-45°C, which is the optimum temperature for the activity of yoghurt starter culture (Bylund, 1995).

2.14.6 Starter addition

The yoghurt starter consists of *Streptococcus thermophilus* and *Lactobacillus bulgaricus* in the ratio of 1:1. The symbiotic relationship between two organisms, at a given ratio is synergistic. Although they can grow independently, the rate of acid production is much

higher when used together than either of the two organisms grows individually. The symbiosis is responsible for typical yoghurt flavor and texture (Donkor *et al.*, 2007).

2.14.7 Filling into cups

For the production of set type yoghurt, the inoculated mix is filled in cups before incubation. The cups are lidded, loaded in incubator racks and transferred to incubation chamber preset at 43°C (Tribby, 2008).

2.14.8 Incubation

After addition of the culture, the cup-set yogurt is moved to the incubation room where it will be left until the pH reaches pH 4.4-4.6. At isoelectric pH (4.7) of casein, the colloidal casein micelles collapse, thereby precipitating into curd. This usually takes yogurt between 5 and 6 h depending upon regional differences and variations in solids levels, and heat treatments. Product should be checked for pH after 3 h of ripening. The best temperature for yoghurt production is 43°C, with an incubation time of 2.5 to 3.5 h (Tamime and Robinson, 1999).

2.14.9 Cooling and storage

When the coagulum is well set and optimum pH (typically 4.5) is reached, it is time to start cooling. The product is cooled to 18-20°C within 30-45 min. Final cooling is normally down to 5°C, which takes place in the cool store, where the products are held to await distribution (Bylund, 1995).

2.15 Types of yoghurt

2.15.1 Set type yoghurt

In set type yoghurt, the milk is fermented in the retail cartons, giving a continuous gelled structure in the final product. They are fairly thick and have a flat surface with any fruit or flavorings at the base. The yoghurt cups are filled and transferred to the incubation chamber at 42°C. After 3 h, the cups are cooled to 15-20°C by means of cold air in the chamber or in the cooling tunnel (Pant, 1992).

2.15.2 Stirred type yoghurt

It is soured in tank after which the product is stirred, cooled and packed. Stirred yoghurt has distinct consistency, thick and smooth, and should make good eating, rather than drinking. From 0.5 to 0.7% stabilizer is added in order to impart gel structure, to ensure a smooth body and texture and to prevent wheying off or syneresis after packaging. The stirred type may be plain, fruit and flavored and this form of yoghurt is more popular (Tamime and Robinson, 1999).

2.15.3 Drinking type yoghurt

The storage of product and handling of the coagulum are similar to stirred yoghurt but fruit syrup is used and the coagulum is homogenized after fermentation. Three different types can be produced, firstly the coagulum is set, heated and the product has shelf-life of three weeks at 10°C, secondly pasteurization of yoghurt at 75°C for a few seconds, followed by cooling and packing gives the product a few weeks shelf-life at 10°C and thirdly UHT drinking yoghurt is heated at 110°C for 4 s, cooled and filled into sterilized container under aseptic conditions. The latter type has shelf-life of several months at ambient temperature (Pant, 1992).

2.15.4 Frozen yoghurt

The yoghurt base is produced in the conventional way. The milk should be subjected to UHT treatment, before fermentation with starter culture and produced natural stirred yoghurt, then 65-80% yoghurt base, 20-35% fruit syrup base are mixed and 0.85% stabilizer and emulsifier are added. The product is then frozen in an ordinary ice cream freezer (outlet temperature -6°C). Finally, the yoghurt is packed at 0 to -6°C and dispatched (Tamime and Robinson, 1999).

2.15.5 Dried yoghurt

Yogurt powder is produced by fermenting non-fat milk using standard yogurt cultures until attain the desirable pH followed by a step of drying, most probably by freeze-drying. In addition, blended yogurt powder is manufactured by blending cultured non-fat milk, cultured whey, cultured whey protein concentrate, cultured dairy solids, nonfat dry milk

and lactic acid which are similar to the flavor and functionality to that of the traditional yogurt powder (Kumar and Mishra, 2004).

The main intension of manufacturing yogurt powder is to store the product in a stable and readily utilizable state, and it can be utilized to replace fresh yogurt for beverage and dip, and in confectionary industry as a coating material for coating of dried fruit, nuts, pretzels, cereal and other snack items (Krasaekoopt and Bhatia, 2012).

2.15.6 Therapeutic yoghurt

The fact that most strains of *S. thermophilus* and *L. bulgaricus* do not survive in the intestinal tract, may be limiting factor if yoghurt is used for antibiotic therapy and/or any other medical purpose. However, the inclusion *Lactobacillus acidophilus* and *Bifidobacterium bifidum* as yoghurt starter culture may contravene some existing definitions of yoghurt; the resultant milk product is reported to be of excellent therapeutic value e.g. lactose-hydrolyzed yoghurt is beneficial for lactose intolerant patient (Tamime and Robinson, 1999).

2.16 Syneresis

Syneresis in yoghurt is a physical separation of water from the curd of milk. The bonding type of water and mobility of water molecules are relevant to yoghurt manufacturing process with regard to sensory evaluation, stability, texture and food processing. The unique microstructure of yoghurt means that all the liquid (whey) is immobilized within its body. No consumer would accept yoghurt from which whey separates easily. If, the milk is not heated at about 90°C for a time long enough (about 15 min), larger pores may develop in the yoghurt body in some areas and larger clusters of casein micelles may develop in other areas. The whey then starts showing in the containers during storage. Some yoghurt manufacturers use small additions of various thickening agents such as starch gel, various plant gums or pectin to the milk to improve the retention of water in yoghurt (Amatayakul *et al.*, 2006).

Syneresis of the acid-induced casein network in yoghurt occurs during storage, and is related to the amount of total solid and casein content in milk, incubation temperature, and rate of acidification and presence of stabilizers that interact with the casein network. The firmness of yoghurt is affected by homogenization, pH, denaturizing of β-lactoglobulin

(whey proteins) and adjacency to casein micelles (Lucey *et al.*, 1998). Yoghurt is usually prepared from homogenized milk to improve stability. This process coats the increased surface of fat globules with casein, enabling the fat globules to participate as a copolymer with casein to strengthen the gel network and reduce syneresis (Keogh and O'kennedy, 1998).

2.17 Addition of fruit and flavoring in yoghurt

Fruit yogurt is one of the most favorable types of yogurt in the current market. Flavored yogurts are prepared by adding sugar and fruit flavorings to plain yogurt. Yogurt is mainly pigmented and flavored by adding fruit juices or pulp from which provide natural color and flavor as well as bioactive compounds (Nguyen and Hwang, 2016). The production of fruit yogurt is very similar to that of the normal yogurt. The first three steps, i.e., standardization, homogenization, and heat treatment are similar. Addition of fruit may be after heating and before fermentation in set-style fruit yogurt, or after fermentation in stirred-style fruit yogurt. The enhanced color, taste, and texture of fruit yogurt are generally more appealing to a vast majority of customers. Fruit flavorings and preparations are prepared according to the variety of yogurt (Chandan *et al.*, 2017). The protocols approved for fruit yogurt state the minimum fruit level as 5%, with an exception for passion fruit (3.5%). Sundae-style yogurt is prepared by layering 15%–18% of fruit puree or syrup on the bottom of the containers and pouring warm inoculated mix over the puree or syrup, followed by sealing the containers and incubating. The temperature of the inoculated milk usually is 46°C. However, the temperature drops to 42°C when the fruit is covered. The fermentation is carried out at this temperature for 4 h followed by refrigeration. The fruit in the product is mixed with the yogurt gel by consumers before consumption (Chandan *et al.*, 2017).

An increasing demand can be seen for fruit yogurts. Incorporation of fruits endorses the healthy image of yogurt. Addition of fruit preparations, fruit flavors, fruit purees, and flavor extracts enhances versatility of taste, color, and texture for the consumer. Yogurts are available in a vast array of flavors including fruit (apple, apricot, black cherry, black currant, blue berry, lemon, mandarin, raspberry, strawberry, peach), chocolate, vanilla, caramel, ginger, etc. The key to the increase in sales of yoghurt is a continuous evaluation and modification of the product to match consumer expectations (Teshome *et al.*, 2017).

Moreover, with the aim of enriching yoghurt with antioxidant molecules, the influence of fruit pulp addition has been evaluated on the nutrient composition and structure of milk yoghurt (Gaglio *et al.*, 2019).

2.18 Shelf life of yoghurt

Shelf-life of a product may be defined as the number of days after production that can be consumed while still remaining safe, retaining its quality appeal and meeting consumer expectations. In other words, it should remain microbiologically safe and organoleptically acceptable within its stated shelf-life (Ahmed, 2011). Acidity is one of the major indices for consumer's acceptability of plain yogurt since acid and flavor development go hand in hand in this fermented product of bacterial symbiosis (Salji and Ismail, 1983). The range of acidity for the best consumption of yoghurt is (0.6-0.9%) as reported by (Ahmed, 2011).

The majority of short shelf-life yoghurts are "lives" i.e. the culture organisms are still viable. Although their metabolic rate at 7°C is relatively low, there is nonetheless some activity. This can be determined during shelf-life by pH measurement, by titrable acidity determination, and by taste (Akpan *et al.*, 2007).

At chill temperature of about 5°C yoghurt has a shelf-life of approximately 10 days, after which the bacterial growth, although restricted, will increase the level of acidity to such an extent as to impair the flavor, eventually rendering it unpalatable to most people. Ultimately the bacteria are destroyed and the yoghurt becomes separated into curds and whey. Yoghurt is particularly susceptible to attack by yeasts and molds; great care is needed to ensure that the starter is free from these organisms and they do not gain access during packaging (Tamime and Deeth, 1980). Depending on the standards of hygiene observed during the manufacture of yoghurt, and the microbiological quality of the ingredients and packaging materials, the shelf-life of yoghurt is around 3 weeks under refrigerated condition (Goodluck *et al.*, 2014).

2.19 Peanut

2.19.1 History of peanut cultivation

Peanut cultivation is believed to have originated in Bolivia and surrounding countries in South America. Any warm, temperate region of the world has the capability of growing

healthy, edible seeds. North and South American natives grew peanuts for some time before its written history. The seeds and techniques used by natives were taken to Europe during early colonization of the United States. During the 16th century, peanut growth and cultivation techniques quickly spread throughout Africa, Asia, and the Pacific Islands. There is no record which could tell us that when and where peanut was first introduced in Nepal. Elderly people in the villages told that peanut arrived in Nepal from India-with Nepali men who were travelling between India and Nepal for employment and the Indian nationals who came to work in Nepal's terai villages (Shrestha, 2002).

Peanut reached East Asia from South America and from there it came to India-entering the country through the east coast of Madras along with the Spaniards. The fact that peanut came to East Asia with the Spanish people would allow to tentatively conclude that peanut must have been certainly entered Nepal through our terai in the hands of migrant farmers. Elderly Nepali villagers note that this crop can be grown in marginal lands also and with much less inputs than what is required for many other crops and that must have been one of the reasons for the initial adoption (Kattel *et al.*, 2002).

2.19.2 Varieties of groundnut grown in Nepal

NARC has been a source of seed for the high yield varieties of peanut and they supply the seeds to any farmer who is eager to adopt their 'improved seeds'. According to NARC, some varieties of peanut found in Nepal are listed in the Table 2.1

Table 2.1 Varieties of groundnut grown in Nepal

SN	Name of released variety	Year of release	Origin	Yield potential Mt/ha	Maturity (Days)	Recommendation Domain
1	Baidehi	2005		3.3	115	Terai, Inner terai
2	Rajharsi	2005		2.8	115	Terai, Inner terai
3	Jyanti	1996		2.2	115	Terai, Inner terai & Mid hills
4	Jyoti	1996		2.0	137-153	Terai, Inner terai & Mid hills
5	Janak	1989	India	2.5	145	Terai, Inner terai & Mid hills
6	B-4	1990	India	1.5	140	Terai, Inner terai & Mid hills

Source: NARC (2014)

2.19.3 Peanut growth

Peanut seed germination occurs when the soil temperature reaches 15.55ºC and the soil moisture is adequate for the seed to absorb 50% of its weight in water. With adequate moisture, a radical sprouts from the germinating seed a few days after planting. Food reserves are maintained in the cotyledon until the shoots emerge from the soil and begin to accumulate sunlight via photosynthesis. Peanuts are considered to be self-pollinating, with natural cross-pollination rates of less than 1% (Shrestha, 2002). Peanuts are also called ground nuts because they develop in the soil (Kamuhu *et al.*, 2019). The fruit of the peanut is a pod with 1 to 5 seeds that develop underground after the elongated pod with ovarian structure penetrates the soil 3-6 cm. Peanut plants produce bright-yellow complete flowers

with male and female parts located in the axils of the leaves. Flowers normally appear 4-6 weeks after planting and plants continue to flower through the growing season. Depending on the variety, peanuts require anywhere from 100 to 150 days from planting to reach full maturity (Sanders *et al.*, 2000). Peanut has a unique feature different from other legume plants. Flowering and fertilization occur above ground, while the following processes of pod formation and development proceed in the soil. The zygote divides only few times to develop into pre-embryo and then further embryo developmental process stops when the gynoecium is exposed to light condition or normal day/night period. After fertilization, embryo-containing ovary elongates and grows downward to the ground, forming a peg-like structure. The elongating ovary pushes the tip of the ovary penetrated into the soil where embryo and pod development are initiated (Zhang *et al.*, 2018).

2.19.4 Antioxidants in peanut

The antioxidants are the substances that can prevent the oxidation of easily oxidizable substances. They have the ability to trap the free radicals produced as a result of different metabolic processes and protect the lipids, proteins and nucleic acids from the oxidative damage. The flavonoid dihydroquercetin, extracted from peanut kernels exhibited antioxidant activity. Methanolic extracts of peanut exhibits marked antioxidative activity, and antioxidative component is identified as luteolin. Total phenolic compounds in peanut helps to retard fat rancidity and to improve the stability of lipid peroxidation (Yen *et al.*, 1993). It is also a significant source of resveratrol, a chemical compound that is reported to have a number of beneficial health effects, such as anti-cancer, antiviral, neuroprotective, anti-aging, anti-inflammatory and life prolonging effects (Shiriki *et al.*, 2015). The antioxidant components in vegetable oils are composed of hydrocarbons, carotenes, tocopherol, phytosterols, and triterpenes; the minor constituents of various vegetable oils are associated with medicinal qualities and thus can be useful in preventing or delaying the onset of chronic diseases and promoting health (Ciou *et al.*, 2021). Antioxidants function as free radical scavengers, reducing agents, complexers of pro-oxidant metals and quenchers of singlet oxygen. Dietary polyphenols offer an indirect protection by activating endogenous defense systems and by modulating the cellular signaling processes such as NF-κB activation, AP-1 DNA binding and glutathione biosynthesis (Janu *et al.*, 2014).

2.19.5 Nutrition of peanut

From a nutritional standpoint, peanuts contain many of the essential vitamins and minerals necessary for proper health. Peanuts also contain roughly 50% fat, the majority being unsaturated. In comparison to other nuts, such as pecans and walnuts, peanuts contain less total fat (Maga, 1991). Peanut containing high (>70%) oleic acid (18:1), a monounsaturated fatty acid, may also be useful in dietary regimes designed to reduce blood cholesterol levels in postmenopausal women, without resulting in problems associated with oxidation of low density lipoproteins (O'Byrne *et al.*, 1997). Peanut are the great source of proteins (Albuquerque *et al.*, 2015). The vitamin and mineral content present in peanut as % RDI in one ounce serving of dry roasted peanuts are given in Table 2.2.

Table 2.2 Vitamins and minerals present in peanuts and their uses in the body

Vitamins and Minerals	% RDI in one ounce serving of dry roasted peanuts	Uses in the Body
Vitamin E	25%	Vital antioxidant, which protects Vitamin A and the body's cells and tissues from damage. It is important for the immune system and may aid in the prevention of tumour growth.
Niacin	19%	Necessary for maintenance of healthy skin, the nervous system, and digestive tract.
folate	10%	Important for development of new cells in the body, particularly during periods of growth and during pregnancy.
Thiamine (B_1)	8%	Needed to ensure normal functioning of the nervous system, appetite, and digestion.
Pyridoxine (B_6)	4%	Produces and breaks down proteins in the body and manufactures red blood cells used to transport oxygen in the body.

Cont'd

Table 2.2. Cont'd

Riboflavin (B_2)	2%	Releases energy from the food we eat, helps skin stay healthy, and assists in the normal functioning of the eye.
Magnesium	12%	Important in the building of bones and teeth, creation of protein, transmission of nerve impulses, and maintenance of body temperature.
Copper	10%	Important for the formation of haemoglobin, health of bones, blood vessels, and nerves.
Phosphorous	10%	Component of all soft tissues that is fundamental to growth, maintenance and repair of bones and teeth.
Potassium	10%	Needed to ensure water balance in the body and creation of protein. It also helps release energy from nutrients and aids in nerve impulse transmission.
Iron	4%	Transport and distribution of oxygen in body's cell

Cont'd

Table 2.2. Cont'd

Calcium	2%	Needed for the development and maintenance of healthy bones and teeth
Zinc	6%	Aids in the formation of protein, wound healing, blood formation, taste perception, appetite, night vision, and general growth and maintenance of all tissues

Source: Baker (2002)

Peanuts are nutrient dense foods and also contain a high fat content half of which is unsaturated, which includes monounsaturated fatty acids (oleic) and polyunsaturated fatty acids (Kamuhu *et al.,* 2019). Raw peanut contains low moisture content, this makes the shelf-life to be long and contribute to the stability of Arachis hypogeal and prevent rancidity of the oil (Ayoola *et al.,* 2012). Peanuts contain high levels of fiber, with naturally low sodium, are cholesterol free, and represent a good source of folic acid. Peanuts also have chemical characteristics that parallel recent discoveries in nutrition that have been found to be beneficial to human health. Recently, resveratrol has received attention from the research community due to possible health benefits. Resveratrol was found in relatively moderate levels in muscadine grapes several years ago. Peanuts contain moderate levels of resveratrol, betastigmasterol, and behenic acid (Sanders *et al.*, 2000). The by-products of edible peanuts, such as the skin, contain behenic acid, which is used in cosmetics and shampoos (Baker, 2002).

2.19.6 Peanut milk

Plant-based or non-dairy milk alternatives are a fast-growing segment in the functional and specialty beverage development category of newer food products around the world. Cow milk allergy, lactose intolerance, calorie concerns, the incidence of hypercholesterolemia, and a growing desire for vegan diets have all motivated consumers to seek out cow milk

substitutes. Plant-based milk substitutes are becoming increasingly popular as a low-cost alternative for impoverished people in developing countries and in areas where cow's milk is scarce (Sethi *et al.*, 2016).

Among various oilseeds, peanut is also a promising raw material for the preparation of plant based milk. Peanut milk has been extensively utilized in developing countries by low income earner group, undernourished children, vegetarians and by people who are allergic to cow's milk (Diarra *et al.*, 2005). Peanut milk is highly healthful because of their extreme richness in protein, minerals and fatty acids such as linoleic and oleic acids (Abou-Dobara *et al.*, 2016). Many volatile compounds are present in peanuts. Peanuts are regarded nutritious because they contain various bioactive components that have been linked to disease prevention. Peanuts are high in proteins, lipids, fibers, vitamins, minerals, antioxidants, phytosterols, and other nutrients that may help to improve blood lipid levels, blood sugar levels, and longevity (Wien *et al.*, 2014). Peanut contains 24.1% protein, 50.9% fat (Shiriki *et al.*, 2015), 17.43% carbohydrates (Yadav *et al.*, 2018), 9.8% crude fiber (Kamuhu *et al.*, 2019).

Part III

Materials and methods

3.1 Materials

The materials collected for the preparation of peanut milk 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 Kamdhenu Dairy Development Limited.

3.1.2 Peanut

Out of several varieties of peanuts, 'Jayanti' (Type-Spanish bunch) variety was selected because of high oil content and purchased from local market of Dharan. The variety 'Jayanti' was confirmed from the seller.

3.1.3 Milk solid not fat

Skim milk powder was used as the source of MSNF and it was bought from Kamdhenu Dairy Development Limited.

3.1.4 Sweetener

Sugar was used as 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 Kamdhenu Dairy Tharahara.

3.1.6 Containers

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

3.1.7 Equipment and chemicals

Following equipment and chemicals were used in the present study (Table 3.1 and 3.2).

Table 3.1 List of equipment used

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

Table 3.2 List of chemicals used

Chemicals	
Sodium bicarbonate	DPPH
Culture medium (Plate count agar)	Boric acid
Starter culture	Oxalic acid
40% Formaldehyde	Methanol
Saturated potassium oxalate	Mixed indicator solution
Sodium hydroxide	Sulphuric acid

3.2 Method

3.2.1 Preparation of peanut milk

Peanuts were soaked for 18 h in 1% $NaHCO_3$ (1:3 ratio kernels to 1% $NaHCO_3$). After soaking, peanuts were dehusked. The dehusked kernels were washed with water and ground with hot water in a ratio of 1:6 (kernels to water) in the grinder. The slurry formed was sieved by muslin cloth and peanut milk was produced. Sodium bicarbonate ($NaHCO_3$) was used to the removal of beany flavor in the final product, and to help soften the peanuts.

Flow chart for the preparation of peanut milk is shown in Fig. 3.1

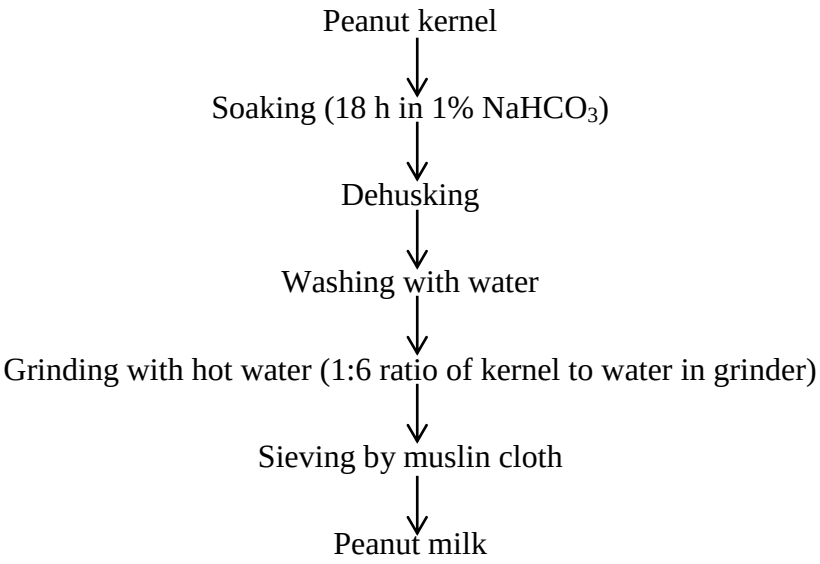

Fig. 3.1 Preparation of peanut milk

Source: Yadav *et al.* (2018)

3.2.2 Preparation of set type peanut milk incorporated yoghurt

The standardized and pasteurized milk from Kamdhenu Dairy Development Limited was taken for the preparation of yoghurt. The milk was mixed with 4% SMP (Skim milk powder) 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, seven formulations of the samples were made by adding 0, 5, 10, 15, 20, 25 and 30% of peanut milk per 100 ml yoghurt mix. Then the starter culture is added at the rate of 2% to each formulation. Then the mix is placed in plastic cups. About 1 L of yoghurt was prepared for each formulation. 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.

Flowchart for the preparation of peanut milk incorporated yoghurt is shown in Fig. 3.2

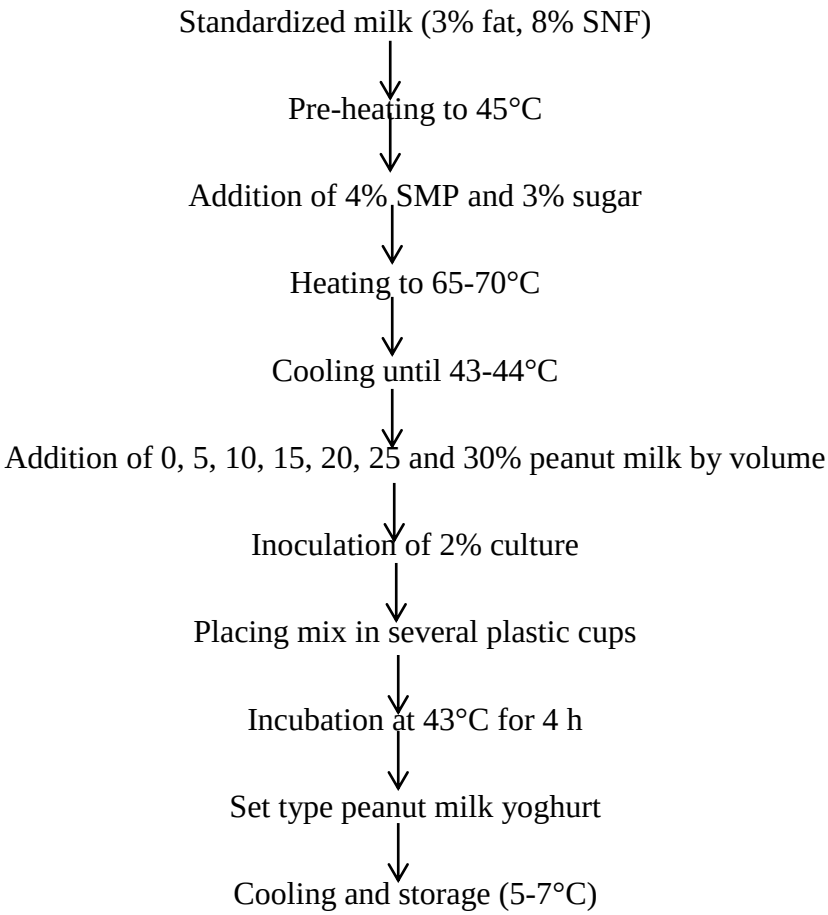

Fig. 3.2 Preparation of peanut milk incorporated yoghurt

Source: Biswas (2013)

3.2.3 Chemical analysis of raw peanut

3.2.3.1 Protein

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

3.2.3.2 Fat

Fat content was determined by Gerber method as described by Kharel (1999).

3.2.3.3 Ash

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

3.2.3.4 Moisture

Moisture content was determined as per the method described by Ranganna (1986).

3.2.3.5 Carbohydrate

Total carbohydrate contents of sample was calculated by difference, that is the percentage of moisture, ash, protein, and fat was subtracted from 100% (Pearson, 1976).

%carbohydrate = 100 – (moisture + protein + crude fat + crude fiber + ash)

3.2.3.6 Crude fiber

Crude fiber was determined by the method given by Ranganna (1986).

3.2.3.7 Free radical scavenging capacity

The antioxidant activity of peanut oil was determined with some modifications in the method described by Shad *et al.* (2012) The oil sample (0.1 g) was dissolved in n-hexane, the volume was made up to 10 ml and 40 µM DPPH solution prepared in n-hexane was added to the sample. The absorbance was read at 517 nm after 30 min of incubation in the dark. Finally, percentage scavenging activity was determined using following equation.

$$\text{Scavenging activity (\%)} = (1 - A_{sample} / A_{blank}) \times 100$$

3.2.4 Chemical analysis of peanut milk

3.2.4.1 Acidity

Acidity was determined by titrimetric method as Pearson (1976).

3.2.4.2 Fat

Fat content in milk was determined by Gerber method as described by Kharel (1999).

3.2.4.3 Protein

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

3.2.4.4 Ash

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

3.2.4.5 pH

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

3.2.4.6 Total Solid (TS)

Total solid was determined by subtracting the moisture from the 100 according to Ranganna (1986).

3.2.4.7 Moisture

Moisture content was determined as per the methods described by Ranganna (1986).

3.2.4.8 Carbohydrate

Total carbohydrate was calculated by difference, that is the percentage of moisture, ash, protein, and fat was subtracted from 100% (Pearson, 1976).

3.2.5 Chemical analysis of milk

3.2.5.1 Acidity

Acidity was determined by titrimetric method as Pearson (1976).

3.2.5.2 Fat

Fat content in milk was determined by Gerber method as described by Kharel (1999).

3.2.5.3 Protein

Protein content was determined by formal titration method as described by Kharel (1999).

3.2.5.4 Ash

The ash content will be determined as described by Ranganna (1986).

3.2.5.5 pH

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

3.2.5.6 Moisture

Moisture content was determined as per the methods described by Ranganna (1986).

3.2.5.7 Total solid (TS)

Total solid was determined by subtracting the moisture from the 100 according to Ranganna (1986).

3.2.5.8 Lactose

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

3.2.6 Design expert

Using Design expert version 10, five formulations are designed under mixed condition which are then coded alphabetically as given in Table 3.3

Table 3.3 Sample formulation in coded form

Sample	Milk	Peanut milk
A	95%	5%
B	90%	10%
C	85%	15%
D	80%	20%
E	75%	25%
F	70%	30%
G	100%	0%

3.2.7 Analysis of yoghurt

3.2.7.1 Sensory evaluation

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

3.2.7.2 Physical analysis

3.2.7.2.1 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:

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

3.2.7.3 Chemical analysis

3.2.7.3.1 Fat

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

3.2.7.3.2 Lactose

Lactose content was determined by the Lane and Eynon method as described in Ranganna (1986)

3.2.7.3.3 pH

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

3.2.7.3.4 Titrable acidity

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

3.2.7.3.5 Protein

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

3.2.7.3.6 Ash

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

3.2.7.3.7 Moisture

Moisture content was determined as per the method described by Ranganna (1986).

3.2.7.3.8 Total solid (TS)

Total solid was determined by subtracting the moisture from the 100 according to Ranganna (1986).

3.2.7.3.9 Total carbohydrate

Total carbohydrate contents of samples will be calculated by difference, that is the percentage of moisture, ash, protein, and fat was subtracted from 100% (Pearson, 1976).

3.2.7.3.10 Radical scavenging activity

The DPPH radical scavenging activities of yogurt samples were determined using the method of Yoon *et al.* (2019) with slight modifications. First, one gram of yoghurt was diluted in 95% ethanol. The sample was thoroughly mixed and centrifuged at 440×g for 20 min at 4°C. 100 μM of DPPH solution was prepared in methanol and added 100 μL of samples (1:1 ratio). The mixture was covered with aluminum foil and kept in the dark at room temperature for 30 min. After incubation for 30 min, DPPH radical scavenging activity of yoghurt was assessed spectrophotometrically by the DPPH cation de-colorization assay at 517 nm. The DPPH radical scavenging activity was calculated according to the following equation:

$$\text{Scavenging activity (\%)} = (1 - A_{sample} / A_{blank}) \times 100$$

3.2.7.4 Microbiological examination

Total Plate Count (TPC) was determined by pour plate technique on Plate Count Agar (PCA) medium (incubated at 30°C/48 h). Coliform count was determined by pour plate technique on MacConkey medium (incubated at 37°C/48 h) (AOAC, 2005).

3.2.7.5 Statistical 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. and t-Test: Two-Sample Assuming Equal Variances was carried out using Microsoft Excel 10 to evaluate the significant difference between the syneresis of the two samples. The data obtained from chemical analysis of control and best yoghurt were subjected to t-Test for statistical analysis

Part IV

Results and discussions

Peanut milk incorporated yoghurt was prepared at CCT, Dharan, in a laboratory for the present study. The peanut milk incorporated yoghurt samples were prepared by incorporating 0, 5, 10, 15, 20, 25 and 30% peanut milk. The milk was mixed with 4% SMP and 3% sugar at 45°C. Heating of milk was further continued to 65-70°C for certain period. After that the heated milk was cooled to around 43-44°C. Seven formulations of the samples were made by adding 0, 5, 10, 15, 20, 25 and 30% of peanut milk per 100 ml yoghurt mix. Then the starter culture is added at the rate of 2% to each formulation. 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.

4.1 Chemical composition of raw peanut

The chemical composition of the raw peanut collected from local market of Dharan is presented in the Table 4.1.

Table 4.1 Chemical composition of raw peanut

Parameters	Values
Protein (% dry basis)	23.48±1.04
Fat (% dry basis)	49.47±0.61
Moisture (%)	4.2±0.40
Ash (% dry basis)	2.125±0.11
Crude fiber (% dry basis)	6.46±0.22
Carbohydrates (by difference)	14.75±1.42
Antioxidant activity (DPPH % inhibition)	91.7±2.42

*Values in the table are arithmetic mean of triplicate samples. Figure in the parentheses indicates standard deviation.

Raw peanut contains low moisture content, this makes the shelf-life to be long and contribute to the stability of Arachis hypogeal and prevent rancidity of the oil (Ayoola *et al.*, 2012). The moisture content of raw peanut (4.2%) was comparable to the result obtained by Yadav *et al.* (2018) i.e. (5.25%). The fat content of raw peanut (49.47%) was similar to the data obtained by Shiriki *et al.* (2015) i.e. (50.9%). The crude fiber content of raw peanut was found to be (6.46%). The result was less than data provided by Kamuhu *et al.* (2019) in the Valencia variety of peanut i.e. (9.8%) but was more than data obtained by Ayoola *et al.* (2012) in the raw peanut i.e. (2.83%).

The crude protein of raw peanut was found to be (23.38%) was similar to the result obtained by Shiriki *et al.* (2015) i.e. (23.0%) and by Kamuhu *et al.* (2019) in red Valencia variety i.e. (22.02%). The carbohydrate content in raw peanut (14.75%) was less than data obtained by Yadav *et al.* (2018) i.e. (17.43%). The antioxidant activity of peanut oil was found to be 91.7% DPPH inhibition. The result was comparable to the data obtained by Janu *et al.* (2014) i.e. 95% DPPH inhibition of 1000 mg/ml groundnut oil.

4.2 Chemical composition of milk and peanut milk

The proximate composition of the peanut milk and dairy milk collected from Kamdhenu Dairy Development Limited is presented in the Table 4.2

Table 4.2 Chemical composition of milk and peanut milk

Chemical composition	Milk	Peanut milk
Protein (%, db)	3.2±0.2	4.69±0.28
Fat (%, db)	2.94±0.05	3.86±0.15
Lactose (%, db)	4.56±0.28	0
Ash (%, db)	0.62±0.1	0.27±0.03
Acidity (% as lactic acid)	0.13±0.05	0.09±0.01
Moisture (%)	88.5±0.53	88.13±0.35
Total solid (%, db)	12.78±0.38	11.75±0.68
pH	6.6±0.15	6.73±0.11

*Values in the table are arithmetic mean of triplicate samples. Figure in the parentheses indicates standard deviation.

The composition of milk in Table 4.2 has little variation over the composition of milk analyzed by Dahal (2009). The milk analyzed by Dahal is produced from Kamdhenu Dairy Co-operative (KDDC). This variation in composition of milk may be due to the species, nutritional aspect of animal, stage of lactation and feeding of animals. The variation may also be due to different processing standard and specification of dairies.

The protein content of peanut milk (4.69%) was comparable to the result obtained by Yadav *et al.* (2018) i.e. (3.68%) and Isanga and Zhang (2007) i.e. (3.76%). The fat content of peanut milk was found to be (3.86%) which was more than data obtained by Yadav *et al.* (2018) i.e. (2.16%) but less than data obtained by Isanga and Zhang (2007) i.e. (6.8%). The ash content of peanut milk (0.27%) was similar to data obtained by Yadav *et al.* (2018) i.e. (0.24%) and Isanga and Zhang (2007) i.e. (0.27%). The acidity of peanut milk was found to be i.e. (0.09%) which was similar to the result obtained by Abou-Dobara *et al.* (2016) i.e. (0.08%). The moisture content of peanut milk (88.13%) was similar to the data obtained by Yadav *et al.* (2018) i.e. (89.20%) and more than data obtained by Isanga

and Zhang (2007) i.e. (86.71%). The total solid of peanut milk (11.75%) was comparable to the result obtained by Abou-Dobara *et al.* (2016) i.e. (13.29%) and Yadav *et al.* (2018) i.e. (10.8%). The pH of the peanut milk (6.73) is similar to result obtained by Albuquerque *et al.* (2015) i.e. (6.70).

4.3 Sensory evaluation of peanut milk yoghurt

Sensory evaluation of all seven formulation of the product which were carried out by a group of ten semi-trained panelist evaluating aroma, color, texture, and overall acceptance of prepared peanut milk yoghurt. The Analysis of Variance (ANOVA) was carried out using least significant difference (LSD) at 5% level of significance as post-hoc test.

4.3.1 Aroma

Regarding aroma of peanut milk incorporated yoghurt, the analysis shows that the mean sensory score for sample A, B, C, D, E, F and G were found to be 6.2, 6.7, 7, 7.7, 6.3, 6 and 6.5 respectively. Statistical analysis showed that the variation in proportion of peanut milk had significant effect in aroma of yoghurt ($p<0.05$). LSD at 5% level of significance indicated that the sample D was significantly different from rest of the sample and also had the highest score. It may be due to the appropriate amount of peanut milk in the yoghurt. The aroma score of A, E, F, G showed that they were significantly different than B, C, and E.

Beuchat and Nail (1978) showed that fermenting peanut milk gives peanut flavor though increasing volume of peanut milk in yoghurt may give slightly beany flavor. Sample D was given highest score by the optimum number of panelist.

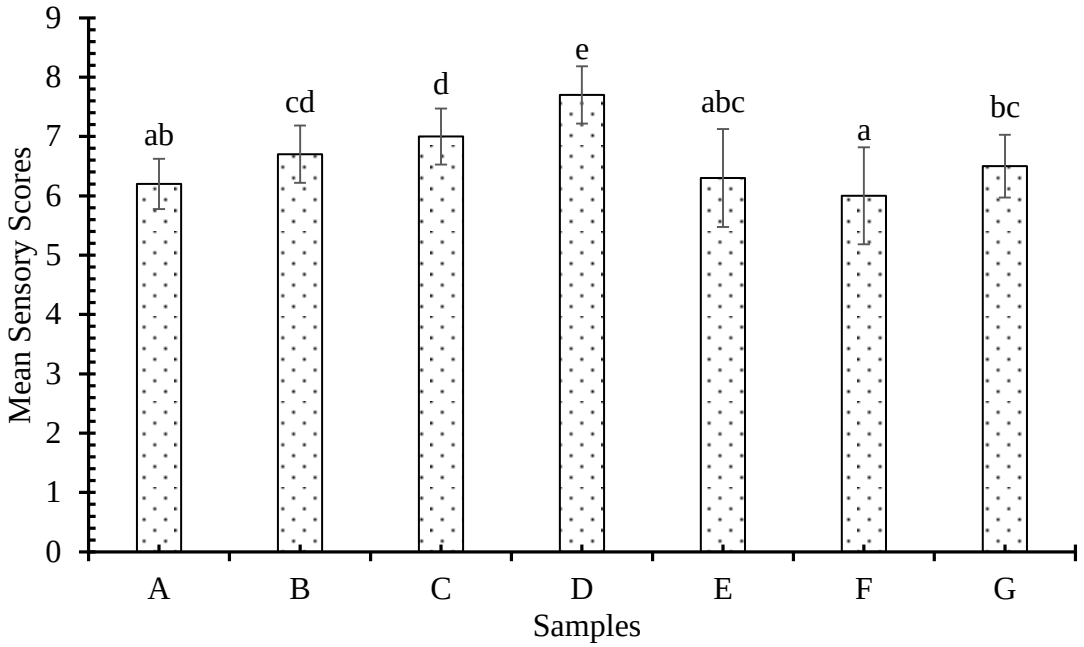

Fig. 4.1 Effect of peanut milk on aroma of yoghurt

Fig. 4.1 represents the mean sensory scores for aroma of peanut milk yoghurt. Values on the top of the bars bearing similar superscript are not significantly different at 5% level of significance. Vertical error bar represent ±standard deviation of scores given by panelist.

4.3.2 Color

The mean color score for samples A, B, C, D, E, F, and G were found to be 7, 6.7, 6.5, 7, 6.4, 6.3 and 6.9 respectively. Statistical analysis showed that the variation in proportion of peanut milk had significant effect in color of yoghurt ($p<0.05$). LSD at 5% level of significance indicated that sample A, D, and G were not significantly different from each other but were significantly different from sample C, E, and F.

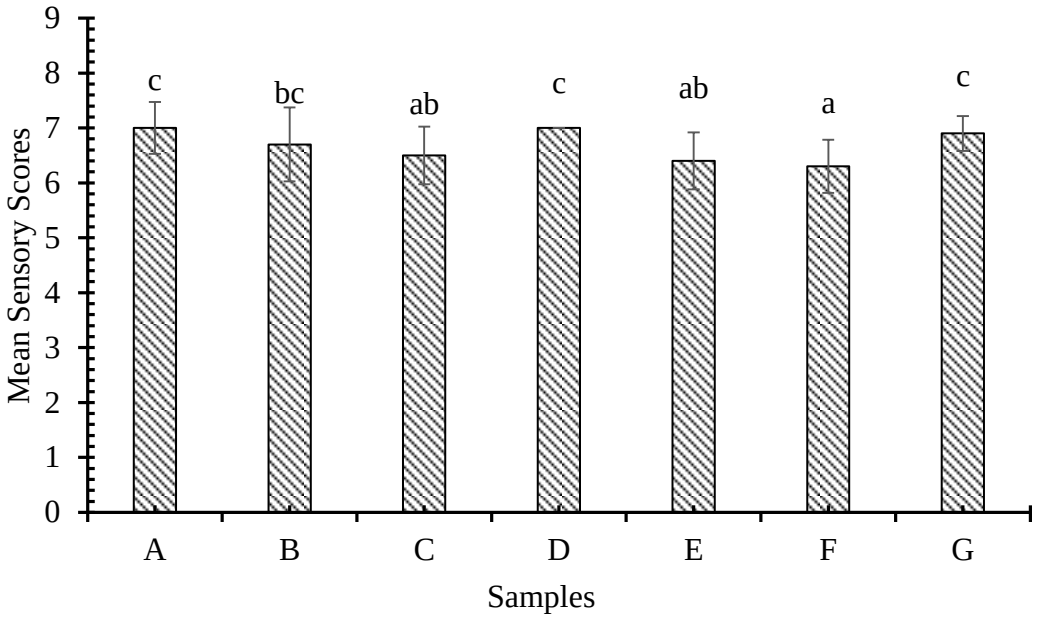

Fig. 4.2 Effect of peanut milk on color of yoghurt

Fig. 4.2 represents the mean sensory scores for color of peanut milk yoghurt. Values on the top of the bars bearing similar superscript are not significantly different at 5% level of significance. Vertical error bar represent ±standard deviation of scores given by panelist.

Kwasi *et al.* (2014) reported that whiteness of the yoghurt decreases with addition of peanut milk, making the product yellowish-white in color. Color of the product is the important sensory attribute that informs consumer acceptability toward the product. Sample A and D were given highest score by the panelist.

4.3.3 Taste

Regarding taste of peanut milk incorporated yoghurt; the analysis showed that the mean sensory score of sample A, B, C, D, E, F and G were found to be 7, 7.1, 7.4, 8.4, 6.2, 5.6, and 7.4. Statistical analysis showed that the variation in proportion of peanut milk had significant effect in taste of yoghurt ($p<0.05$). LSD at 5% level of significance indicated that the sample D was significantly different from rest of the sample and also had the highest score. The sample A, B, C, and G were not significantly different to each other but were significantly different with D, E, and F.

Among seven samples, sample D got the high mean score due to optimum acceptance of panelist.

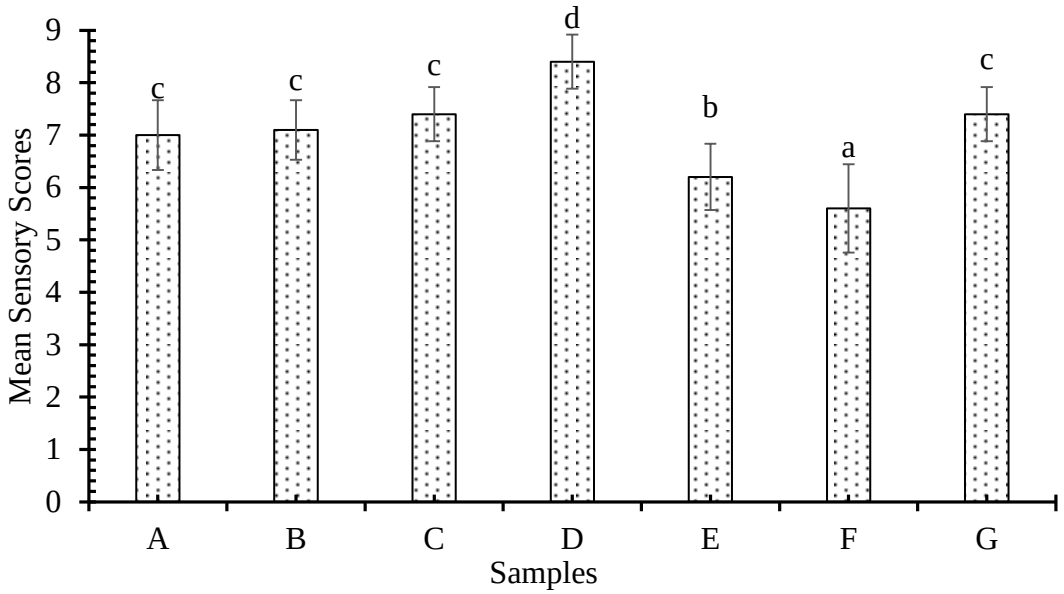

Fig. 4.3 Effect of peanut milk on taste of yoghurt

Fig. 4.3 represents the mean sensory scores for taste of peanut milk yoghurt. Values on the top of the bars bearing similar superscript are not significantly different at 5% level of significance. Vertical error bar represent ±standard deviation of scores given by panelist.

Elsamani and Mohamed (2014) stated that the fresh yoghurt prepared by adding different of skim milk powder was significantly higher in flavor and taste than peanut milk yoghurt. Anyway fermentation of peanut milk highly optimizes its sensory attributes as stated by Lee and Beuchat (1991) that hexanol which is responsible for beany flavor in peanut disappers after fermentation.

4.3.4 Texture

The average sensory of texture were found to be 7.1, 7, 7.2, 7.6, 6, 5.6 and 7.2 for samples A, B, C, D, E, F, and G respectively. Statistically there was significant effect on texture of yoghurt due to variation of proportion of peanut milk ($p<0.05$). LSD at 5% level of significance between the samples indicated that the samples A, B, C, D, and G were significantly different than samples E and F. Sample D had highest score due to the optimum acceptance of panelist.

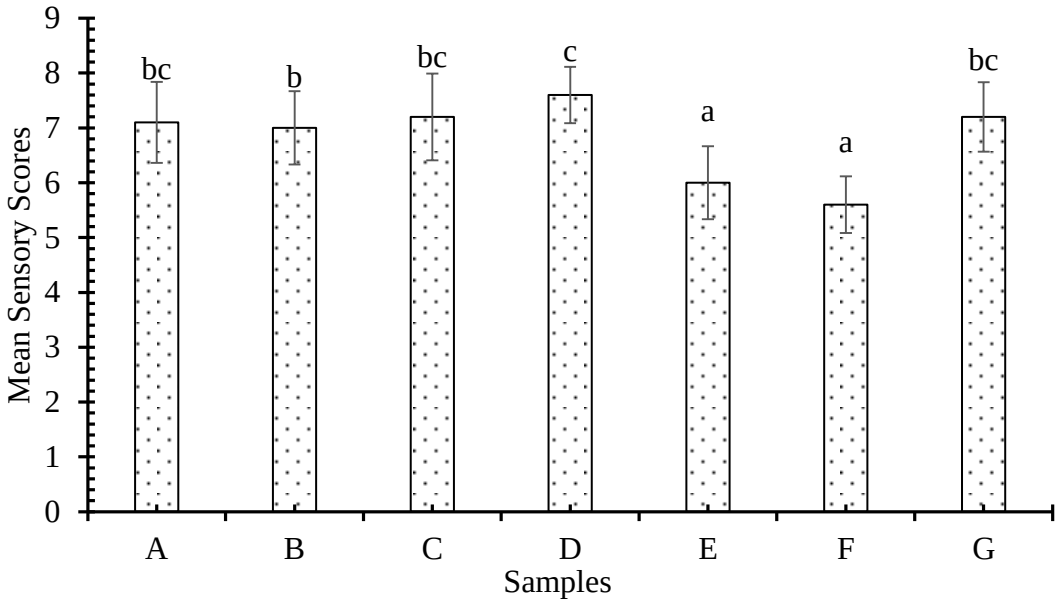

Fig. 4.4 Effect of peanut milk on texture of yoghurt

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

Isanga and Zhang (2009) showed the similar result that peanut milk yoghurt had higher texture scores than cow milk yoghurt. The superior sensory texture may be attributed due to the high-fat content of peanut milk yoghurt which makes the yoghurt creamier.

4.3.5 Overall acceptability

Regarding overall acceptability of peanut milk incorporated yoghurt; the analysis showed that the mean sensory score for sample A, B, C, D, E, F, and G were found to be 6.8, 6.9, 7.1, 7.8, 6.3, 5.8, and 6.9. Statistical analysis showed that effect of different peanut milk portion on overall acceptability of the product was significant ($p<0.05$). LSD showed that sample A, B, C and G were not significantly different to each other but were significantly different to sample D, E and F. Sample D was significantly different to sample E and F and had highest mean score. Among seven samples, sample D got the high mean score due to optimum acceptance of panelist.

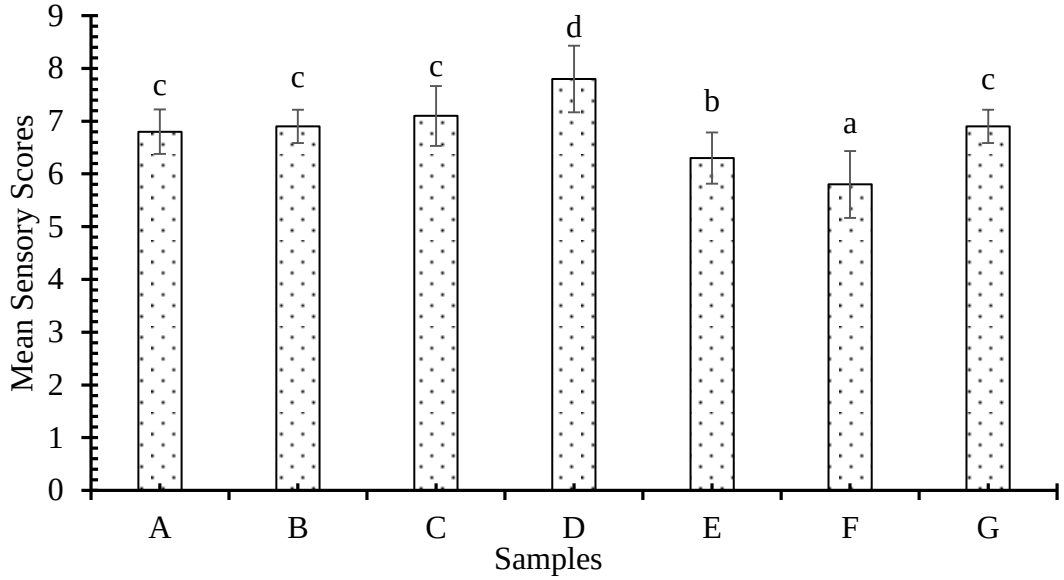

Fig. 4.5 Effect of peanut milk on overall acceptability of yoghurt

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

There was significant difference between the samples D with respect to other samples but there was no significant different between other samples except sample E and F. The overall acceptability of Sample D was higher due to the improvement in color, taste and texture with respect to other samples. Sample E and F got lowest score may be due to incorporation of high volume of peanut milk in yoghurt. The dominance of beany flavor in sample E and F may have effect on taste and overall acceptability of yoghurt as stated by Ismail *et al.* (2018).

From the sensory evaluation of the product conducted on the attributes like aroma, color, taste, texture and overall acceptability, the product containing 20% peanut milk and 80% cow milk by volume was rated as best in all attributes.

4.4 Chemical analysis of best product

The standardized and pasteurized milk from Kamdhenu Dairy development Limited was taken for the preparation of yoghurt. The milk was mixed with 4% SMP (Skim milk powder) 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, seven formulations of the samples were made by adding 0, 5, 10, 15, 20, 25, and 30% of peanut milk per 100 ml yoghurt mix. Then the starter culture is added at the rate of 2% to each formulation. 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.

Thus from the statistical analysis, the best product was found to be sample D yoghurt containing 20% peanut milk and 80% cow milk. Chemical analysis of sample D and control yoghurt (100% cow milk) was done. The value of the chemical analysis are shown in Table 4.3

Table 4.3 Chemical analysis of product

Parameters	Product G (Control)	Product D (Best)
Acidity (% Lactic acid)	0.62^{a}±0.03	0.66^{a}±0.02
Ash (%, db)	0.96^{a}±0.01	0.97^{a}±0.01
Fat (%, db)	2.78^{a}±0.07	3.45^{b}±0.05
Moisture	80.13^{a}±0.38	80.7^{a}±0.31
Protein (%, db)	2.93^{a}±0.20	3.72^{b}±0.12
pH	4.58^{a}±0.10	4.2^{b}±0.1
Lactose (%, db)	3.85^{a}±0.10	2.92^{b}±0.14
Total solid (%, db)	20.33^{a}±0.85	19.63^{b}±0.15
Antioxidant activity (% DPPH inhibition)	56.96^{a}±2.67	74.46^{b}±0.98

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

The lactose content of yoghurt from whole milk (control) was similar to that reported by Gaglio *et al.* (2019) i.e. 3.37%. Fat, total solid and moisture of control yoghurt found in our study was comparable to the data reported by Matela *et al.* (2019) i.e. 2.24%, 20.84% and 79.16% respectively. Acidity and pH of control sample in our study was similar to the result obtained by Salji and Ismail (1983). Protein content of control yoghurt was similar to the data reported by Farinde *et al.* (2009) i.e. 3%. The antioxidant activity of control was found to be 56.96% DPPH inhibition. The result was comparable to the data obtained by Nguyen and Hwang (2016) i.e. 59.47% DPPH inhibition.

The protein and fat content of sample D (best) was found to be higher than that of control sample. This may be due to high protein and fat content in the peanut milk compared to dairy milk. Similar result was obtained by Isanga and Zhang (2009). However, the lactose cotent of control sample was higher than best sample. This is because peanut milk does not contain lactose Sethi *et al.* (2016). Total solid content of control sample was higher than best yoghurt. This is because higher total solid content of cow milk than peanut milk. Acidity of best sample was found to be increased slightly but was not significantly different. Desai *et al.* (1994) reported that addition of fruit juice/pulp increases percent acidity. The radical scavenging capacity was found to be increased in best sample due to incorporation of peanut milk which shows high antioxidant activity.

4.5 Physical analysis

The physical analysis of the control yoghurt and best product was performed. The values obtained for the syneresis are shown in Table 4.4

Table 4.4 Syneresis of yoghurt

Samples	Syneresis (%)
Control yoghurt	23.39^{a}±0.94
Best yoghurt	24.16^{a}±0.85

t-Test was carry out to evaluate the significant different between the two samples. There were no significant difference between control and best product ($P<0.05$) as shown in Appendix D.10. The results are similar to the work done by Adhikari (2018).

4.6 Shelf-life of the product

Best product which was found best with respect to aroma, color, taste, texture and overall acceptability hence was used for further study. Hence it was subjected for chemical analysis with respect to acidity and microbial count in laboratory.

4.6.1 Acidity of yoghurt at room temperature

The acidity of yoghurt increased from 0.65% to 1.05% within 1 day of storage under room temperature. The increase in acidity of the yoghurt could be due to the acid production by the fermentation of lactose present in the milk by culture bacteria during storage period (Zourari *et al.*, 1992). The bacterial culture *Lactobacillus bulgaricus* and *Streptococcus thermophilus* have optimum temperature for growth between 37 to 45°C so they may have high rate of metabolic activity at room temperature (Dave and Shah, 1997). As the temperature increases, molecules move faster, enzymes speed up metabolism and cells rapidly increase in size. Therefore, the rate of acid production was high at room temperature due to temperature effect. Moreover increase in value of acidity is due to the addition of peanut milk which increases availability of higher nutrients for the production of lactic acid. Yoghurt sample remain suitable for consumption up to 1 day. The results are in agreement with Ahmed (2011) within the range of (0.6-0.9%) acidity in yoghurt.

4.6.2 Total plate count of yoghurt at room temperature

TPC of yoghurt increased from 4×10^4 to 12×10^4 CFU/ml within 1 day of storage under room temperature. The increase in TPC of yoghurt was due to the production of lactic acid bacteria which increases with the addition of peanut milk. Similar results was obtained by Goodluck *et al.* (2014) for the consumable range of total bacterial count as in the range of (3.0×10^3 – 10.5×10^4 CFU/ml). The bacterial culture *Lactobacillus bulgaricus* and *Streptococcus thermophilus* have optimum temperature for growth between 37 to 45°C so they may have high growth rate at room temperature due to temperature effect. As the temperature increases, molecules move faster, enzymes speed up metabolism and cells rapidly increase in size. (Dave and Shah, 1997). There were no colonies of coliform found. They were destroyed during pasteurization of milk. Yoghurt sample was suitable for consumption up to 1 day at room temperature. The results are in agreement with Goodluck *et al.* (2014).

4.6.3 Acidity of yoghurt under refrigeration

The acidity of chilled yoghurt increased very slowly from 0.65% to 0.97 % within 6 days of storage under refrigeration. The increase in acidity of yoghurt could be due to the acid production by culture bacteria during storage period owing to their activity even at low temperature (Salji and Ismail, 1983). The results are in agreement with Ahmed (2011) within the range of (0.6-0.9%) acidity in yoghurt. Acidity in yogurt samples also increased in refrigeration temperature but not as rapid as at room temperature due to temperature effect. The bacterial culture *Lactobacillus bulgaricus* and *Streptococcus thermophilus* have optimum temperature for growth between 37 to 45°C (Dave and Shah, 1997). Cold stress, which takes place during the cooling and freezing steps and during frozen storage, is the main cause of loss of bacterial activity. Therefore at the low temperature, metabolic rate of micro-organism descreases (Wang *et al.*, 2005). Yoghurt samples were suitable for consumption up to 6 days.

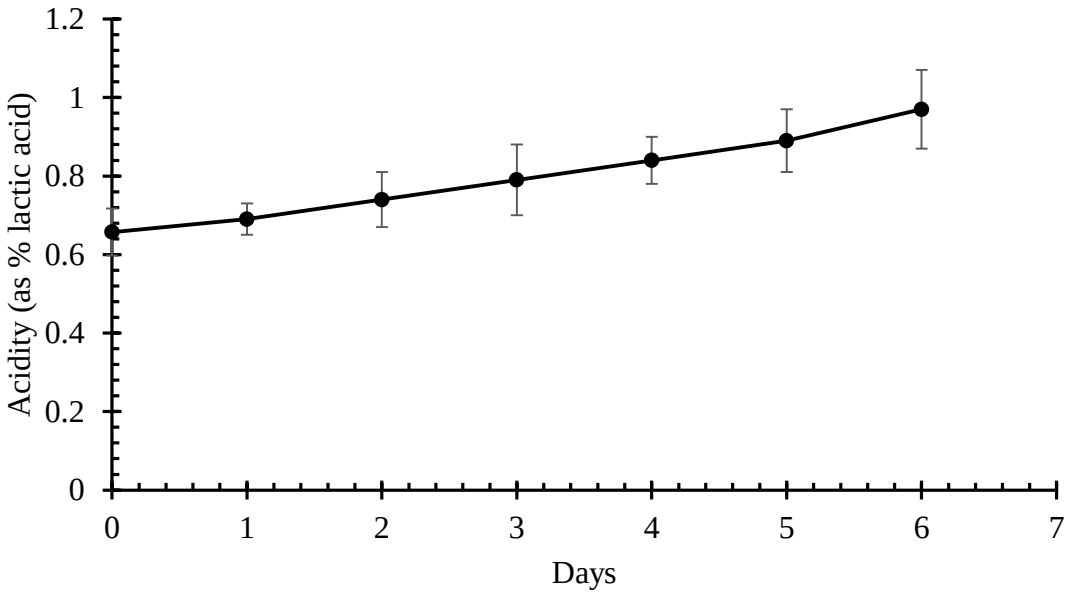

Fig. 4.6 Changes in acidity under refrigeration with respect to the number of days

In Fig. 4.6 vertical error bars represents the standard deviation

4.6.4 Total plate count of yoghurt under refrigeration

TPC of yoghurt increased slowly from 4×10^4 to 11.6×10^4 CFU/ml within 6 days of storage under refrigeration. The increase in TPC of yoghurt is due to the production of culture bacteria even at low temperature. The rate of increase was not as that of room temperature due to the

fact that the rate of increase in lactic acid bacteria decreases in low temperature (Salji and Ismail, 1983). The bacterial culture *Lactobacillus bulgaricus* and *Streptococcus thermophilus* have optimum temperature for growth between 37 to 45°C (Dave and Shah, 1997). Cold stress, which takes place during the cooling and freezing steps and during frozen storage, is the main cause of loss of bacterial activity. Therefore at the low temperature, growth and metabolic rate of micro-organism descreases (Wang *et al.*, 2005). Similar result was obtained by Goodluck *et al.* (2014) for the consumable range of total bacterial count as in the range of (3×10^3 – 10.5×10^4 CFU/ml). There were no colonies of coliform found. They were destroyed during pasteurization of milk. Yoghurt samples were suitable for consumption up to 6 days.

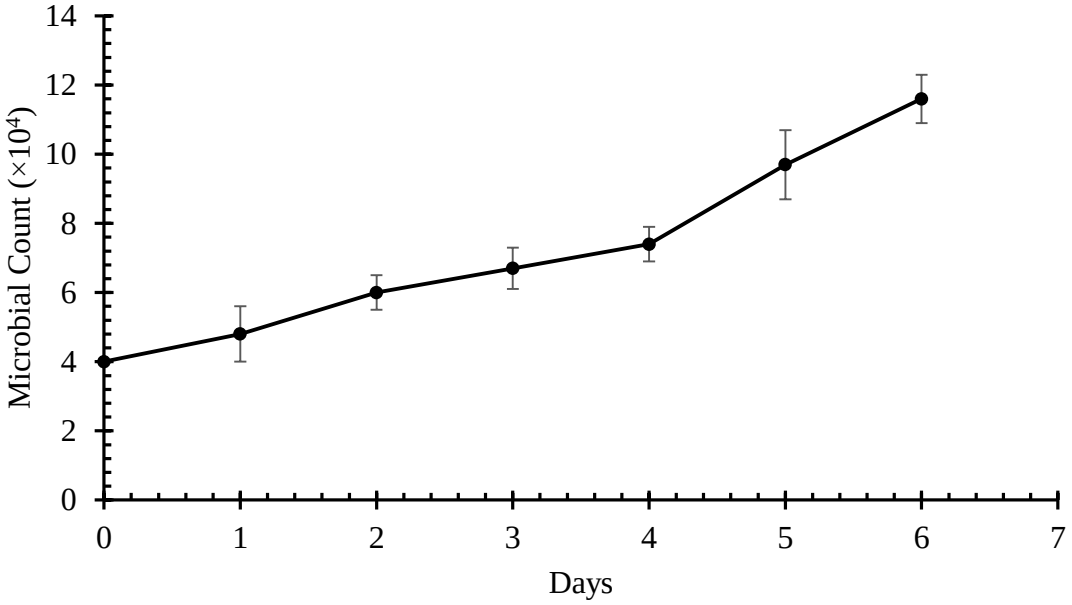

Fig. 4.7 Changes in TPC under refrigeration with respect to the number of days

In Fig. 4.7 vertical error bars represents the standard deviation

4.7 Cost evaluation

The total cost of the best peanut milk incorporated yoghurt was calculated. It is shown in the appendix B. The price for 100 ml peanut milk incorporated yoghurt was found to be NRs. 12.05 (as of 2020) which was cheaper than commercial yoghurt.

Part V

Conclusion and recommendations

5.1 Conclusion

On the basis of the work conducted, the following conclusion can be concluded.

- From the sensory evaluation of the product conducted on the attributes like aroma, color, taste, texture all overall acceptability, the product containing 20% peanut milk and 80% cow milk by volume was rated as best in all attributes.
- The protein, fat, acidity, total solid, lactose, ash, moisture, pH and antioxidant activity of best product were found 3.72%, 3.45%, 0.66%, 19.63%, 2.92%, 0.62%, 80.7%, 4.2 and 74.46% respectively.
- The shelf life of product was higher in refrigerated condition (6 days) than in room temperature (1 day). So, shelf life can be extended by refrigerating the product thus formed. The TPC and acidity of yoghurt in refrigerated condition was less than room temperature. Coliform was not tested in the product.
- Antioxidant activity also seemed to be increased (74.46% DPPH inhibition) compared to control yoghurt (56.96% DPPH inhibition). The nutritional quality of peanut yoghurt seemed to be enhanced in the case of fat and protein content.
- The cost of peanut milk incorporated yoghurt thus prepared was found to be NRs. 12.05 (for 100 ml) which is more nutritious and cost effective than the commercial yoghurt.

5.2 Recommendations

- Optimization of pH, incubation time and temperature for the preparation of peanut milk yoghurt can be carried out.
- Yoghurt can be prepared by blending different proportion of sugar, stabilizer and MSNF.
- Peanut milk based ice cream or energy drink can be prepared and quality studied.

Part VI

Summary

Peanut milk incorporated yoghurt is a cultured dairy product produced by fermenting milk and peanut milk, with or without added non-fat dry milk (NFDM) with *Lactobacillus bulgaricus* and *Streptococcus thermophilus* bacteria.

The standardized and pasteurized milk from Kamdhenu Dairy development Limited was taken for the preparation of yoghurt. The milk was mixed with 4% SMP (Skim milk powder) 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, seven formulations of the samples were made by adding 0, 5, 10, 15, 20, 25, and 30% of peanut milk per 100 ml yoghurt mix. Then the starter culture is added at the rate of 2% to each formulation. 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 seven products was carried out. The sensory evaluation revealed that the yoghurt containing 20% peanut milk and 80% milk by volume was found to be best whose protein, fat, acidity, total solid, lactose, ash, moisture, pH and antioxidant activity were found 3.72%, 3.45%, 0.66%, 19.63%, 2.92%, 0.62%, 80.7%, 4.2 and 74.46% respectively. Shelf life of the best product was estimated in terms of acidity and total plate count and the shelf life was found to be 1 day at room temperature and 6 days at refrigeration.

From the overall analysis of the result it is clear that good quality yogurt could be prepared by adding peanut milk with cow milk. As peanut has a potential role in combating malnutrition; its consumption should be increased especially in the developing countries. Fermentation of peanut milk may serve as one such effort that can increase consumption of this valuable crop. It is expected that consumer will be interested to consume this type of yogurt day by day. From commercial standpoint, production of maximum volume yogurt from low volume of cow milk by integrating peanut milk can make this enterprise profitable.

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Appendices

Appendix-A

Sensory evaluation card

Name: …….. Date: …….

Product: Peanut milk 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

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

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

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

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

Sample	Aroma	Color	Taste	Texture	OA
A					
B					
C					
D					
E					
F					
G					

Comments if any: ……

Signature……..

Appendix B

Table B.1 Cost evaluation of 100ml of 20% peanut milk incorporated yoghurt

Particulars	Quantity (g)	Rate (Rs.)	Amount (Rs.)
Milk	80 (ml)	76/liter	6.08
Peanut	6	200/kg	1.2
SMP	4	625/kg	2.52
Sugar	3	80/kg	0.24
Overhead cost (20%)			2.008
Total			12.05

Appendix C

ANOVA results of sensory analysis

Table C.1 ANOVA (no blocking) for aroma of Peanut milk incorporated yoghurt

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.	l.s.d
Sample	6	19.94	3.32	10.79	<.001	0.49
Panelist	9	5.77	0.64	2.08	0.047	0.59
Residual	54	16.63	0.31			
Total	69	42.34				

Table C.2 ANOVA (no blocking) for color of Peanut milk incorporated yoghurt

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.	l.s.d
Sample	6	5.08	0.84	4.83	<.001	0.37
Panelist	9	4.51	0.50	2.86	0.008	0.44
Residual	54	9.48	0.18			
Total	69	19.08				

Table C.3 ANOVA (no blocking) for overall acceptance of Peanut milk incorporated yoghurt

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.	l.s.d
Sample	6	23.60	3.93	15.36	<.001	0.45
Panelist	9	1.77	0.19	0.77	0.645	0.54
Residual	54	13.82	0.26			
Total	69	39.20				

Table C.4 ANOVA (no blocking) for taste of Peanut milk incorporated yoghurt

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.	l.s.d
Sample	6	48.88	8.14	23.02	<.001	0.53
Panelist	9	4.98	0.55	1.57	0.150	0.63
Residual	54	19.11	0.354			
Total	69	72.98				

Table C.5 ANOVA (no blocking) for texture of Peanut milk incorporated yoghurt

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.	l.s.d
Sample	6	31.68	5.28	14.04	<.001	0.54
Panelist	9	6.58	0.73	1.95	0.065	0.65
Residual	54	20.31	0.37			
Total	69	58.59				

Appendix D

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

	Product G	Product D
Mean	0.62	0.66
Variance	0.009	0.000246333
Observations	3	
Hypothesized Mean Difference	0	
df	3	
t Stat	-2.165646825	
P(T<=t) one-tail	0.059470957	
t Critical one-tail	2.353363434	
P(T<=t) two-tail	0.118941914	
t Critical two-tail	3.182446305	

Table D.2 t-test (two-sample assuming unequal variance) for ash of best sample with control

	Product G	Product D
Mean	0.96	0.97
Variance	0.00003333	0.00023333
Observations	3	3
Hypothesized Mean Difference	0	
df	3	
t Stat	-1.06066017	
P(T<=t) one-tail	0.183336089	
t Critical one-tail	2.353363434	
P(T<=t) two-tail	0.366672178	
t Critical two-tail	3.182446305	

Table D.3 t-test (two-sample assuming unequal variance) for fat of best sample with control

	Product G	Product D
Mean	2.78	3.45
Variance	0.0058333	0.00253333
Observations	3	
Hypothesized Mean Difference	0	
df	3	
t Stat	-12.560768	
P(T<=t) one-tail	0.00054396	
t Critical one-tail	2.35336343	
P(T<=t) two-tail	0.00108792	
t Critical two-tail	3.18244630	

Table D.4 t-test (two-sample assuming unequal variance) for moisture of best sample with control

	Product G	Product D
Mean	80.13	80.7
Variance	0.143333	0.09
Observations	3	3
Hypothesized Mean Difference	0	
df	4	
t Stat	-2.0318886	
P(T<=t) one-tail	0.05598590	
t Critical one-tail	2.13184678	
P(T<=t) two-tail	0.11197180	
t Critical two-tail	2.77644510	

Table D.5 t-test (two-sample assuming unequal variance) for protein of best sample with control

	Product G	Product D
Mean	2.93	3.72
Variance	0.04333333	0.0074333
Observations	3	3
Hypothesized Mean Difference	0	
df	3	
t Stat	-6.0729316	
P(T<=t) one-tail	0.00448123	
t Critical one-tail	2.35336343	
P(T<=t) two-tail	0.00896247	
t Critical two-tail	3.18244630	

Table D.6 t-test (two-sample assuming unequal variance) for pH of best sample with control

	Product G	Product D
Mean	4.58	4.16
Variance	0.0103	0.003333333
Observations	3	3
Hypothesized Mean Difference	0	
df	3	
t Stat	6.13140538	
P(T<=t) one-tail	0.00436171	
t Critical one-tail	2.35336343	
P(T<=t) two-tail	0.00872350	
t Critical two-tail	3.18244630	

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

	Product G	Product D
Mean	3.85	2.92
Variance	0.006533	0.020133
Observations	3	3
Hypothesized Mean Difference	0	
df	3	
t Stat	9.8641395	
P(T<=t) one-tail	0.0011077	
t Critical one-tail	2.3533634	
P(T<=t) two-tail	0.0022154	
t Critical two-tail	3.1824463	

Table D.8 t-test (two-sample assuming unequal variance) for total solid of best sample with control

	Product G	Product D
Mean	20.33	19.63
Variance	0.372333	0.02333
Observations	3	3
Hypothesized Mean Difference	0	
df	2	
t Stat	1.40312152	
P(T<=t) one-tail	0.14784130	
t Critical one-tail	2.91998558	
P(T<=t) two-tail	0.29568260	
t Critical two-tail	4.30265273	

Table D.9 t-test (two-sample assuming unequal variance) for antioxidant activity of best sample with control

	Product G	Product D
Mean	56.96	74.46
Variance	7.123	0.97
Observations	3	3
Hypothesized Mean Difference	0	
df	3	
t Stat	-10.65235291	
P(T<=t) one-tail	0.000884086	
t Critical one-tail	2.353363435	
P(T<=t) two-tail	0.001768173	
t Critical two-tail	3.182446305	

Table D.10 t-test (two-sample assuming equal variance) for syneresis of best sample with control

	Product G	Product D
Mean	23.39	24.16
Variance	0.6330	0.0948
Observations	3	3
Pooled variance	0.0435166	
Hypothesized Mean Difference	0	
df	4	
t Stat	-1.54974014	
P(T<=t) one-tail	0.098067286	
t Critical one-tail	2.131846786	
P(T<=t) two-tail	0.196134572	
t Critical two-tail	2.776445105	

Color plates

Plate 1 Peanut milk extraction

Plate 2 Incubation

Plate 3 Peanut milk incorporated yoghurt

Plate 4 Sensory evaluation

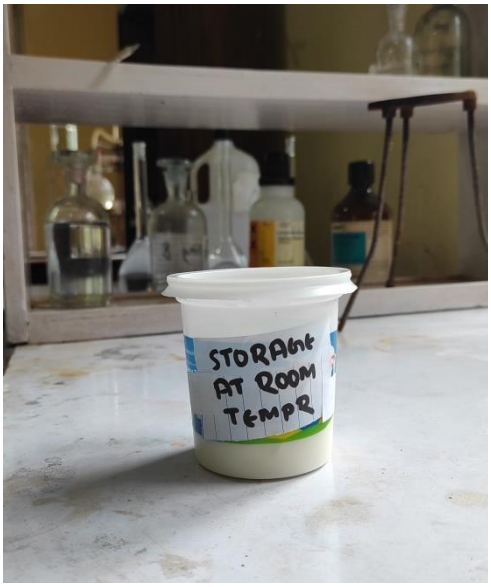

Plate 5 Storage at room temperature

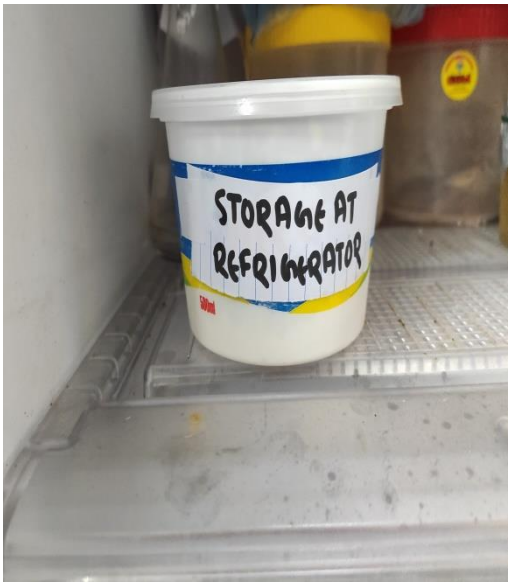

Plate 6 Storage at refrigerator

Plate 7 Spectrophotometric determination of Antioxidant activity