PREPARATION AND QUALITY EVALUATION OF ALMOND 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 Almond Milk Incorporated Yoghurt*** presented by **Samikshya Poudel** has been accepted as the partial fulfillment of the requirement for the **B. Tech. degree in Food Technology.**

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Abstract

This work was carried out to prepare almond milk incorporated yoghurt with 2 % starter culture of *Lactobacillus bulgaricus* and *Streptococcus thermophilus* and to evaluate its sensory and physiochemical properties. Raw almond of sweet variety (Californian almond) was collected and soaked for 18-20 h and grinded with water (1:4 of kernel to water) to prepare almond milk. Design expert® version 13 D-optimal design was employed for the formulation of yoghurt recipe. The obtained five formulations coded A (25 %), B (50 %), C (75 %), D (100%) and E (0 %) of almond milk incorporated yoghurt were prepared in laboratory. The samples were subjected to sensory evaluation (appearance, aroma, color, taste, texture and overall acceptance) 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, formation A (25 %) almond milk incorporated yoghurt was found to be significantly ($p<0.05$) superior in sensory quality. The moisture, protein, fat, acidity, and antioxidant activity of this formulation increased than the control yoghurt while ash, pH, lactose and total solid were found to be decreased. The shelf life of this product was estimated in terms of acidity and total plate count and the shelf life was found to be 2 days at room temperature and 6 days under refrigeration (5 °C). The total cost per 100 ml of the best yoghurt was found to be NRs 24.02 (as of 2023).

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List of Abbreviations

Abbreviation	Full form
ANOVA	Analysis of variance
AOAC	Association of Analytical Communities
CFU	Colony Forming Unit
CIP	Clean in process
DM	Dry Matter
DPPH	2,2-diphenyl-1-picrylhydrazyl
HDL	High Density Lipoprotein
LAB	Lactic Acid Bacteria
LDL	Low Density Lipoprotein
LSD	Least Significant Difference
MSNF	Milk Solid Not Fat
RDA	Recommended Dietary Allowances
RDI	Recommended Daily Intake
SMP	Skim Milk Powder
SNF	Solid Not Fat
TPC	Total Plate Count

Part I

Introduction

1.1 General Introduction

Yoghurt is a fermented food made by important microorganisms, the majority of which are pro-biotics, fermenting lactose in milk under anaerobic conditions (Tull, 1997) . Yoghurt is a cultured dairy product made by fermenting milk with *Lactobacillus bulgaricus* and *Streptococcus thermophilus* bacteria, either with or without the addition of non-fat dry milk (NFDM). It typically has a milk solids content of 12–14 %, a soft, friable custard-like consistency, and a clear, pronounced acid flavor. Yoghurt is often made by pasteurizing the mixture and changing the milk proteins such that they will offer the right viscosity and gelation with the least amount of syneresis in the final product at 80 to 85 °C for 30 min (Morr, 1985).

Fermented milk products like yogurt and soured milk contain bacteria from the *Lactobacilli* group. These bacteria have a cleansing and healing effect and are found naturally in the digestive tract. Therefore, including fermented products in the diet can help prevent certain yeasts and bacteria that may cause illness (Fellows and Hampton, 1992). Fermented milk products have been reported to have a beneficial effect on the human digestive system and are also linked to serum cholesterol control. Both milk protein and lactose are more easily digestible in fermented milk than in raw milk. Proteins are partially degraded by the action of the bacterial proteolytic system. As part of the lactose is converted to lactic acid and/or alcohol, the lactose content is lower than in the original milk. Lactic acid is responsible for the characteristic sour taste of fermented products. Because some strains of lactic acid bacteria also synthesize folate, yoghurt and fermented milks may contain more folate than original milk. Fermentation not only improves the digestibility of milk but also increases its shelf life and microbiological safety (Khadka, 2018).

Almonds (*Prunus amygdalus*) are commonly used as snacks and as ingredients in a wide range of processed foods, particularly bakery and confectionery products (Esfahlan *et al.*, 2010). Almonds have been shown to reduce colon cancer risk in rats (Davis and Iwahashi, 2001) and to increase HDL cholesterol while decreasing LDL cholesterol levels in humans (Hyson *et al.*, 2002). Almond seed proteins are typically high in acidic amino acids (aspartic and glutamic acids), have a ~29 % essential-to-total amino acid ratio (E/T), and are low in sulfur amino acids (methionine and cysteine). Phosphorus, calcium, potassium, magnesium, manganese, copper, zinc, and iron are all found in almond seeds. Tannins are astringent bitter-tasting polyphenols found in almond seeds that act as antioxidants, inhibiting and/or minimizing the production of destructive free radicals that play a role in the development and progression of cancer, atherosclerosis, and inflammation. Another antioxidant found in almond seeds is vitamin E, with a one-ounce serving (~24 seeds) providing 50 % of the RDA for vitamin E (15 mg/day for both male and female adults) (Monaghan, 2008).

1.2 Statement of the Problem

Yoghurt, as a fermented dairy product, is considered a probiotic carrier and is nutritionally rich in available protein, calcium, milk fat, potassium, and magnesium. Because most of the lactose in the milk precursor has been converted to lactic acid by the bacterial culture, people who are moderately lactose intolerant can enjoy yoghurt without adverse effects. Yogurt also has medical applications due to its probiotic properties, such as aiding in the treatment of a variety of gastro intestinal conditions and preventing antibiotic-associated diarrhea (Lourens-Hattingh and Viljoen, 2001). A plant-based diet that includes grains, legumes, seeds, nuts, fruits, and vegetables has gained popularity among consumers for a number of reasons, including their opposition to harming animals, health issues like lactose intolerance, desire for a healthier lifestyle, and environmental awareness (Aydar *et al.*, 2020).

The availability of fruits and flavor yoghurt in the Nepalese market is limited (Gupta, 2003). However, in developing countries where animal protein is insufficient and relatively expensive, research is being directed toward finding alternative protein sources from plant foods to meet protein demands (Nsofor and Maduako, 1992). As a result, almond milk can help to provide a variety of products while also increasing the commercial value of yoghurt.

Therefore, almond milk yoghurt may be a better option to increase its utilization along with the improvement of yoghurt quality.

1.3 Objective

1.3.1 General objectives

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

1.3.2 Specific Objectives

The specific objectives of this dissertation work were to:

1. To analyze the proximate composition of milk, almond and almond milk.
2. To study the effect of different levels of almond milk on the yoghurt and to evaluate its sensory properties.
3. To analyze the almond milk yoghurt for its proximate composition.
4. To study the effect in the antioxidant property of yoghurt with addition of almond milk.
5. To determine the shelf life of the yoghurt.
6. To evaluate the cost of yoghurt.

1.4 Significance of the study

Yoghurt is a well-known and widely consumed fermented milk product that is consumed by a large portion of the world's population due to its nutritional value. Fruit and flavoring are widely used in modern yoghurt manufacturing technology (Kucukoner and Tarakci, 2003). The popularity of yogurt as a food component is primarily determined by its sensory properties, the most important of which are aroma and taste (Khadka, 2018). Fruits and vegetables that are incorporated with yoghurt are high in natural antioxidants and fibers (prebiotics), and they also serve as a flavoring and coloring agent. The phytochemical antioxidants, which include carotenoids, flavonoids, and phenols among others, may play a role in modulating cholesterol synthesis and absorption, platelet aggregation, blood pressure, and cardiovascular disease (Abou El Samh *et al.*, 2013).

But in context of Nepalese market the availability of fruit and flavor yoghurt is quite rare in comparison to plain yoghurt. So, the almond milk yoghurt will give a new variety in yoghurt, which also upgrades the nutritional value of yoghurt. Almond milk contains nutrients that are necessary for daily consumption, including high-quality protein, lowglycemic carbohydrates, unsaturated fatty acids, vitamins, minerals, dietary fiber, phenolic acids, and flavonoids, which may act as probiotic bacteria stimulators (Kundu *et al.*, 2018). This plant-based milk is suitable for consumers who are lactose intolerant or allergic to cow milk, as well as those looking for plant-based beverages as an alternative to dairy milk (Maghsoudlou *et al.*, 2016).

Sensory evaluation of soy, almond, and peanut milk showed that probiotic almond beverage was the most preferred in terms of consistency, flavor, texture, and overall acceptability. According to this study, probiotic almond beverages could be successfully marketed to vegans and people who are lactose intolerant (He and Hekmat, 2015). Therefore, the almond milk can be a positive step towards increasing the commercial value of yoghurt along with almond.

1.5 Limitation of the study

The limitations of this dissertation work were:

1. Best yoghurt was not compared with commercial yoghurt.
2. Only one variety of almond was taken for study.
3. Variation of sugar and skim milk was not carried out.
4. Shelf life of yoghurt was not compared with control.
5. pH was not measure as the shelf life of yoghurt.

Part II

Literature review

2.1 Development of Dairy industry in Nepal

Nepal has a relatively short history of dairy development. It all started in Tushal (Kabhre) in 2009 B.S. In 1956, a modern milk processing plant was built in Lainchaur, Kathmandu, with financial aid from the New Zealand government of 500 liters capacity. DDC (Dairy Development Corporation) was established in 1969 under the Agriculture Development Act of 2001 to undertake an effective dairy development program. Similarly, in 1974, a modern milk processing plant with a capacity of 2000 liters were established in Biratnagar, Nepal's eastern area. Hetauda's 3000-liter capacity was established in 1974. In 1977, another plant with a capacity of 5000 liters was developed in Balaju, Kathmandu. Furthermore, the Pokhara milk supply system, the Lumbini milk supply scheme, and the Kohalpur and Surkhet milk supply schemes were formed. The Kathmandu dairy development program is also known as the central dairy since milk is supplied to the dairy from all of Nepal's dairies. In addition, the National Dairy development Board (NDDB) was established as a further step in the growth of dairy in Nepal. This body is in charge of developing policy, planning, and developing the dairy profession as a coordinator between the private and public sectors (Adhikari, 2018).

2.2 Milk

The mammary gland of milch animals produces milk, which is a lacteal secretion. It is made up of proteins, lipids, carbohydrates, and numerous other organic and inorganic salts that are dissolved or dispersed in water. Although there are a few phospholipids, sterols, fat-soluble vitamins A and D, carotene, and xanthophylls, the majority of lipids are made up of fat. Casein, lactalbumin, and lactoglobulin are the three types of protein found in milk. The carbohydrate in milk is lactose (Meyer, 1960).

Milk contains a variety of salts and minerals. There are plenty of vitamins, but vitamin C is limited. Milk contains a variety of enzymes, some of which appear to be secreted in the milk and others are formed by microorganisms (Meyer, 1960).

2.3 Milk Fermentation

Any change in the chemical or physical properties of milk or dairy products caused by the activity of microorganisms or their enzymes is considered milk fermentation. Lactic acid, alcohols, carbon dioxide, and other compounds are produced when bacteria break down milk sugars and other components. Milk's key fermentable compounds are lactose, fat, and citric acid. The primary carbon source is lactose, a disaccharide; fat and citric acid provide hydrogen and oxygen, respectively (Davies and Law, 1984). Milk fermentation either results in spoiled and degraded products or contributes to the flavor and texture of cheese and yoghurt that consumers prefer. Microbial cultures with known properties are added to the milk or dairy product substrate to ensure the desired fermentation (Yuliana and Rangga, 2010).

The activity of lactic acid bacteria, which play a crucial role in converting milk as a raw material into fermented milk products, has been used in the milk fermentation process. As starter cultures, various industrial strains of LAB are utilized in the milk fermentation industry. After being isolated, selected, and confirmed, lactic acid bacteria starter cultures were obtained from sequence activities. The ability of LAB to acidify milk (Mayra-Makinen and Bigret, 2004) and generate flavor and texture through the conversion of milk protein through their proteolytic activities (Frank and Marth, 1998) are their most significant properties. Minerals and vitamins are necessary for LAB growth (as mediators in the enzymatic reaction and mineral catalysis, respectively), but their consumption would not significantly alter the total content of fermented milk products. Changes in pH brought on by fermentation may alter the bioavailability of some minerals (Hayek *et al.*, 2019).

2.4 Advantages of Milk Fermentation

The most important advantages of fermented food are:

i. Preventing milk from spoiling due to the accumulation of lactic acid and other antibacterial metabolites during fermentation.

ii. The digestibility of fermented products, particularly proteins, is improved, which may be beneficial for people with digestive disorders.

iii. In some circumstances, the fermentation process may reduce the bulk of the raw material, giving the finished product a longer shelf life. For instance, conventional fermented milk-cereal mixtures are typically dried and are portable (Vedamuthu, 1982)

iv. Fermented milk products contain antibiotics produced by microorganisms used as culture, which have a negative effect on harmful microorganisms in the intestine and limit their growth.

v. Some fermented milk products are beneficial in the nutritional treatment of certain diseases such as dysentery, gastritis, anemia, kidney stones, and so on.

Fermented milk, such as yoghurt, has the ability to increase weight more effectively than milk feeding (Hargrove and Alford, 1978).

2.5 Probiotics

Probiotics are defined as 'live microorganisms' that confer a health benefit on the host when administered in sufficient amounts (Ismail *et al.*, 2018). Probiotics are live microbes that influence the health of their hosts by influencing the intestinal microflora. Probiotics improve intestinal microbial balance, and a decrease in these bacteria found naturally in the human small and large intestines 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, Bifidobacterium lactis, Bifidobacterium longum* and *Lactobacillus gasseri* (Kneifel, 2000). Probiotic bacteria (*Lactobacillus acidophilus and bifidobacteria*) are widely used as dietary adjuncts because they are natural inhabitants of the intestine. One of the most important properties of probiotic bacteria is their ability to survive passage through the gastrointestinal tract and persist in the gut for long enough to provide health benefits.

Probiotic bacteria have been linked to a reduction in diarrhea duration, antagonistic effects against pathogenic microorganisms, improved lactose digestion, regulation of intestinal motility, reduced activities of cancer-related enzymes, improved calcium resorption, and the provision of water-soluble vitamins (Crittenden *et al.*, 2005). The action of probiotics on intestinal flora results in vital benefits such as pathogen protection, immune system development, and positive effects on colonic health and host nutrition. There is also evidence that certain probiotic species/strains are anti-carcinogenic (Chhetri, 2021).

2.6 Starter Culture

A starter culture is a product that contains a high concentration of lactic acid bacteria, which can cause milk to acidify. Starter cultures are typically created in special starter culture laboratories, but they can also be cultured and propagated in dairy (Gandhi, 2006).

The most common yoghurt starter culture is a symbiotic blend of *Streptococcus salivarius* subsp. *thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus*. Although they can grow independently, the rate of acid production is much higher when they are used together than when the two organisms are grown separately. *S. thermophilus* grows more quickly and produces acid and carbon dioxide. The formate and carbon dioxide produced stimulate the growth of *L. bulgaricus. L. bulgaricus*' proteolytic activity, on the other hand, generates stimulatory peptides and amino acids for use by S. thermophilus (Desai *et al.*, 1994). These microorganisms are ultimately responsible for the flavor and texture of yoghurt. Because of the pH drop, the yoghurt mixture coagulates during fermentation. The streptococci are responsible for the yoghurt mix's initial pH drop to around 5.0. Lactobacilli are responsible for decreasing the pH to 4.0. Fermentation products that contribute to flavor are lactic acid, acetaldehyde, acetic acid, and diacetyl (Adhikari, 2018).

2.7 Types of Starter Culture

2.7.1 Pure and mixed culture

A further classification is made into pure cultures and mixed cultures. A pure culture is made up of only one type of lactic acid bacteria, whereas a mixed culture is made up of several types of lactic acid bacteria. Pure cultures can be made up of one or more strains from the same species. Acidification with a mixed culture is the most common type, and on rare occasions on its own. DL cultures were once cultivated as "dairy cultures" at individual dairies, with the same culture often being cultivated for decades (Dave and Shah, 1997).

2.7.2 Mesophilic and thermophilic culture

Mesophilic cultures, which include *Lactococcus* and *Leuconostoc*, grow best at temperatures ranging from 20 to 30 °C. These mesophilic lactic cultures are used in the production of many cheese varieties that have the following important characteristics:

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

Thermophilic cultures grow best at temperatures ranging from 37 to 45 °C. Thermophilic cultures are commonly used in the production of yoghurt, acidophilus milk, and Swiss cheese. Thermophilic cultures include *Streptococcus* and *Lactobacillus* species. These cultures combine with milk to form the typical yoghurt starter culture. This growth is considered symbiotic because the rate of acid development is faster when two bacteria are grown together than when grown alone (Dave and Shah, 1997).

2.7.3 Liquid culture

Liquid cultures are no longer widely distributed in commercial practice. The organisms are multiplied in a suitable medium, like milk or whey, and kept active by routine transfers, in order to prepare a liquid culture. A liquid culture usually contains about 109 organisms per ml of starter (Neilson and Ullum, 1989).

2.7.4 Powdered culture

Powdered cultures are created by freeze-drying a liquid culture that has been cultivated to a high bacterial count. Drying under vacuum is referred to as freeze drying. This is a gentle method that reduces the bacterial count during manufacturing. Ordinary freeze-dried cultures need to be reinoculated into a mother culture before use (Neilson and Ullum, 1989).

2.7.5 Frozen culture

Deep frozen cultures are made by deep freezing a concentrated liquid culture at the point of bacterial growth, where activity is at its peak. Lyophilization is used to preserve them in small vials. Super-concentrated, deep frozen cultures are created by adding growth factors to a milk substrate, continuously neutralizing the lactic acid formed via ammonium hydroxide, and then concentrating the culture in a desludging centrifuge/bactofuge.

Pelletization occurs when the concentrate is frozen as individual drops in liquid nitrogen. The culture is kept at -196 °C until it is shipped to the dairies in foamed plastic boxes with dry ice (Neilson and Ullum, 1989).

2.8 Preparation of Starter Culture

Since each organism produces more acid and grows faster when cultured together than when cultured separately, the two organisms develop a symbiotic relationship. For rod and coccus, the ideal growth temperatures are 45 °C and 40 °C, respectively. The ideal ratio is usually considered to be 1:1. Good yoghurt can be made by using a 2 % inoculum and incubating it at 44 °C for 2.5 h. While *L. bulgaricus* achieves acidities of 1.20–1.50 %, *S. thermophilus* only reaches acidities of 0.85–0.95 % (Chhetri, 2021).

2.9 Yoghurt

Typically, yogurt is milk that has been acidified and fermented by bacteria that are both viable and well-defined, resulting in a thickened, frequently flavored product with a long shelf life. It is a vehicle for fortification (added probiotics, fibers, vitamins, and minerals) and contains essential nutrients. Sweeteners, fruits, and flavors can also easily alter its consistency and aroma (Fisberg and Machado, 2015). By fermenting cow milk with the lactic acid bacteria *Streptococcus thermophilus* and *Lactobacillus bulgaricus*, yogurt, a fermented milk product is prepared. Milk proteins aggregate more readily to form a yoghurt gel after being acidified by lactic acid bacteria (Kwasi *et al.*, 2014). The pH of milk is naturally 6.5–6.6. Lactic acid produced by lactic acid bacteria lowers the pH to 4.6 and below. Yogurt is made by lactic acid bacteria that grow at temperatures around 40 °C. The distinctive yogurt flavor is brought about by lactic acid, which gives an acidic and reviving taste, and a combination of different carbonyl compounds such as diacetyl, acetone, and acetaldehyde, the latter of which is the main flavor component (Tamime and Deeth, 1980). Protein with a high biological value, fat, calcium, zinc, potassium, magnesium, phosphorus, riboflavin (vitamin B2), thiamine (vitamin B1), vitamin B6, vitamin B12, niacin, folate, and other nutrients are abundant in yogurt (Matela *et al.*, 2019).

Yogurt is also the most popular vehicle for incorporating probiotic organisms. For the LAB to offer its beneficial properties, it must remain in the gastrointestinal tract. The dairy components in fermented milk have a magnificent buffering capacity when viable LAB is consumed. Additionally, since LAB is found in yoghurt (pH 4-4.5), the cells may have been conditioned to a low pH environment and may have a high rate of survivability in gastric juice (Dave and Shah, 1997).

2.9.1 A brief history of yoghurt

Yogurt's history goes back over 6000 years. The word "yogurt" is thought to have evolved from the Turkish word "jugurt" (Rasic and Kurmann, 1978). It is assumed that the limited availability of milk in the Middle East due to the dry desert environment led to the development of a yogurt-like product. It was thought to be consumed in Turkey as a preserved milk product (Tamime and Robinson, 1999). Dahi is the name given to yogurt in South Asia, and it has a soft coagulum, a lumpy texture, and a mild acidic flavor (Khadka, 2018). Most regulatory agencies around the world consider yogurt to be a fermented milk product that contains digested lactose and specifically defined, viable bacterial strains, typically *Streptococcus thermophilus* and *Lactobacillus bulgaricus.* It serves as a vehicle for fortification and is a good source of protein, calcium, phosphorus, potassium, and vitamins B2 and B12 (Fisberg and Machado, 2015). Fermented dairy products have been around since the dawn of civilization. Ancient Indian Sanskrit texts known as the Vedas describe the nutritional benefits of the fermented milk product known as Dahi, which is comparable to modern yoghurt. The Bible provides additional evidence for the existence of soured milk as a food in ancient times (Chandan and Shahani, 1995).

A common fermented milk product known as yogurt has gained widespread consumer acceptance as a nutritious food. It contains a high concentration of nutrients in relation to its energy and fat content, making it a nutrient-dense food. Yogurt, in particular, can supply the body with significant amounts of calcium in a bioavailable form. Furthermore, yoghurt has numerous health benefits in addition to providing basic nutrition, such as improved lactose tolerance, a possible role in body weight and fat loss, and a variety of health attributes associated with probiotic bacteria (Mckinley and Michelle, 2005).

2.9.2 Types of yoghurt

2.9.2.1 Set type yoghurt

Set type yoghurt has a continuous gelled structure because the milk is fermented in the retail cartons during production. They have a flat surface, a fair thickness, and any fruit or flavorings are at the base. The yogurt cups are filled, then moved to the incubation chamber, which is set at 42 °C. The cups are cooled to 15-20 °C after three h using cold air in the chamber or cooling tunnel (Pant, 1992).

2.9.2.2 Stirred type yoghurt

It is soured in a tank before being stirred, cooled, and packed. Stirred yoghurt has a distinct consistency, being thick and smooth, and should be eaten rather than drunk. A stabilizer of 0.5 to 0.7 % is added to impart gel structure, ensure smooth body and texture, and prevent wheying off or syneresis after packaging. This type of yoghurt is more popular because it can be plain, fruity, or flavored (Tamime and Robinson, 1999).

2.9.2.3 Drinking type yoghurt

The coagulum has been "broken" prior to cooling in this type of yoghurt as well. The agitation used to "break" the coagulum in yoghurt is severe. Coagulum reformation is unlikely to occur (Aswal *et al.*, 2012).

2.9.2.4 Frozen yoghurt

Incubation and inoculation procedures for frozen yoghurt are the same as for stirred yoghurt. As with ice cream, cooling is accomplished by pumping through a whipper, chiller, or freezer. The size and distribution of the produced ice crystals, as well as the whipper and freezer, have a significant impact on the finished product's texture (Aswal *et al.*, 2012).

2.9.2.5 Concentrated yoghurt

Similar to stirred yoghurt, this type of yoghurt undergoes the same inoculation and fermentation processes. After "breaking" the coagulum, some of the water is boiled off to concentrate the yoghurt. To lower the temperature needed, this is frequently done under vacuum. Heating low pH yogurt can frequently cause the protein to become completely denatured, resulting in rough and gritty textures. This is frequently referred to as "strained yoghurt" because the liquid that is released from the coagulum upon heating was traditionally "strained" off in a process similar to making soft cheese (Aswal *et al.*, 2012).

2.9.2.6 Flavored yoghurt

Typically, the flavors are added at or right before filling the pots. Fruit or berries are frequently used as additives; they are typically pureed or whole fruits in syrup. These additives frequently contain up to 50 % sugar, but as the trend toward healthy eating gains momentum, many producers offer versions of their products with less sugar and fat. Yogurts with low or no sugar content are frequently sweetened with aspartame or saccharin (Aswal *et al.*, 2012).

2.9.3 Raw materials used for yoghurt preparation

Milk, sugars, stabilizers, fruits, flavors, and a bacterial culture (*Lactobacillus bulgaricus*) are all common ingredients used to make yogurt. These microorganisms interact with the milk during fermentation to transform it into a curd. Additionally, the organisms as well as acetaldehyde change the flavor of the milk, giving it the characteristics yoghurt flavor. Lactic acid is the main byproduct of the fermentation process. The completion of yoghurt fermentation is determined by acid level which is mostly 3-4 h. To create yoghurt with a variety of flavors and textures, the suppliers of these yogurt cultures provide different combinations of the two bacterial types (Khadka, 2018).

Various ingredients may be added to the yogurt to change some of its characteristics. Yogurt can be made sweeter by adding sucrose (sugar) at a rate of about 7 %. Artificial sweeteners like aspartame or saccharin are used in yogurts with fewer calories. To provide a texture with more smoothness, cream may be added. Stabilizers like food starch, gelatin, locust-bean gum, guar gum, and pectin can be added to yogurt to improve its consistency and shelf stability. These ingredients are used because they don't significantly affect the flavor of the final product. Although they are not necessary, some marketers choose not to use stabilizers in order to maintain a more authentic image for their yogurt (Khadka, 2018).

Numerous fruits are added to yogurt to enhance taste and offer a wide range of flavors such as strawberries, blueberries, bananas, and peaches but almost any fruit can be added. Other flavorings are added besides fruits. These can be things like mint, coffee, chocolate, and vanilla. Recently, manufacturers have used both natural and artificial flavorings to produce a variety of yogurts (Khadka, 2018).

2.9.4 Process of yoghurt production

The body and texture of the finished yogurt will vary depending on the ingredients, processing, starter cultures, flavor, and packaging used. Yogurt processing is divided into:

blending, pasteurization, homogenization, culturing and cooling, packaging, and storage. Each process requires strict attention to detail (Tribby, 2008).

2.9.4.1 Milk procurement

Milk of the highest bacteriological quality must be used for yoghurt production. It should contain low bacterial content and other things that could prevent the yoghurt culture from growing. Antibiotics, bacteriophages, leftover CIP solution, and sterilizing agents shouldn't be present in milk. The dairy must conduct a very thorough analysis of the milk (Bylund, 1995).

2.9.4.2 Standardization and mix preparation

To ensure consistency in the product quality, milk fat and SNF contents are standardized in most of yoghurt formulations. Before being used to make yogurt, the milk's composition is altered at the plant where it is received. Usually, the total solids are increased while the fat content is decreased during this standardization process. By separating the fat from the milk using centrifugation, the fat content is decreased. The milk's solids content is increased to 16 % for the purpose of making yogurt, with 1–5 % of the solids constituting fat and 11–14 % being solids-not-fat (SNF). Either some of the water is evaporated to achieve this, or additional ingredients like concentrated milk or milk powder are added. Adding more solids to the yogurt increases its nutritional value, makes it simpler to make a firmer yogurt, and increases the stability of the milk substance as it ferments into yogurt. Before the yogurt is packaged, fruits and flavorings are mixed in to lessen the likelihood that it will separate while being stored. SNF should be at least 12 % in yogurt mixture in order to increase viscosity and "wheying off" resistance. Stabilizers are added after the solid composition is adjusted, and the milk is then pasteurized (Tribby, 2008).

2.9.4.3 Homogenization

The process of homogenizing yoghurt mixtures helps to hydrate stabilizers and facilitate their interaction with milk proteins. It is typical to homogenize mixtures during the production of yogurt and other dairy products at a temperature of about 63 °C (145°F), with a total pressure of between 7 and 10 MPa (1000 and 1500 psi) in the first stage and 3 MPa (500 psi) in the second stage. The same pressure conditions are used regardless of the type of homogenizer. In order to produce a smooth texture, increase the viscosity of the yoghurt, and prevent creaming during incubation, homogenization is done, which reduces the size of the fat globules (Tribby, 2008).

2.9.4.4 Pasteurization

Yogurt mixtures can be pasteurized using a variety of techniques. The objective of pasteurization is to heat-treat milk in order to eradicate pathogenic bacteria. Since they are heat-sensitive, coliforms and staphylococci usually die at temperatures above 60 °C (Adegoke et al., 1992). In order to get the most functionality out of the milk proteins, it is also essential to denature the proteins. Additionally, pasteurization imparts an appealing cooked flavor and helps hydrate the stabilizers and dry ingredients that were added during blending. The three primary pasteurization methods are low temperature long time (LTLT), which involves 63 °C for 30 min, high temperature short time (HTST), which involves 72–75 °C for 15 s, and ultra-high temperature (UHT), which involves 125-238 °C for 2-4s (Ranieri *et al.*, 2009).

2.9.4.5 Cooling

After being homogenized and pasteurized, the yogurt mixture is cooled to the ideal setting temperature. The milk is cooled to 42–45 °C, the ideal temperature for the yoghurt starter culture to function (Bylund, 1995).

2.9.4.6 Starter addition

Streptococcus thermophilus and Lactobacillus bulgaricus compose the yogurt starter in a 1:1 ratio. At a certain ratio, the symbiotic relationship between two organisms is synergistic. The rate of acid generation is substantially larger when the two organisms are utilized together than when each grows alone, despite the fact that they can both grow independently. The symbiosis is in charge of giving yoghurt its characteristic texture and flavor (Donkor *et al.*, 2007).

2.9.4.7 Filling into cups

Before incubation, the inoculated mix is poured into cups to make set-type yoghurt. The cups are lidded before being loaded into incubator racks and transported into an incubator chamber that is preheated to 43 °C (Tribby, 2008).

2.9.4.8 Incubation

The cup-set yogurt is transferred to the incubation room after the culture has been added, where it will remain until the pH is between pH 4.4 and 4.6. Collapsing colloidal casein micelles cause curd to precipitate at the isoelectric pH of casein (4.7). Depending on regional variables, variations in solids levels, and heat treatments, this typically takes yogurt between 5 and 6 h. After 3 h of ripening, the product's pH should be evaluated. A temperature of 43 °C and an incubation period of 2.5 to 3.5 h are ideal for the production of yoghurt (Tamime and Robinson, 1999).

2.9.4.9 Cooling and storage

It is time to begin cooling once the coagulum has properly set up and the ideal pH has been obtained (usually 4.5). The product is cooled for 30-45 min at 18–20 °C. Final cooling often occurs at 5 °C in the cool store, where the products are kept while they wait to be distributed (Bylund, 1995).

2.10 Syneresis

In yoghurt, syneresis is the physical separation of water from the milk curd. Yogurt has a distinct microstructure that causes all of the liquid (whey) to be trapped within its structure. Yogurt whose whey separates readily wouldn't be consumed. Larger holes and larger clusters of casein micelles may form in the yoghurt body if the milk isn't heated at around 90 °C for a sufficient amount of time (about 15 min) (Amatayakul *et al.*, 2006). During storage, the acid-induced casein network undergoes syneresis. It is related to the quantity of total solids and casein content in milk, the acidification rate, the incubation temperature, and the presence of stabilizers that interact with the casein network. Homogenization, denaturization of β-lactoglobulin (whey proteins), pH, and adjacency to casein micelles all affect yoghurt firmness (Lucey *et al.*, 1998).

Syneresis, or spontaneous whey separation on the top layer of set yogurt, is considered a defect in yoghurt. This issue can be minimized or removed by raising the milk solids content to 15 % (Tamime and Deeth, 1980). During storage, the whey starts appearing in the container. To increase the milk's ability to hold onto water, some yoghurt producers add small amounts of various thickening agents, like starch gel, various plant gums, or pectin (Amatayakul *et al.*, 2006).

2.11 Benefits of yoghurt

Yogurt consumption is becoming increasingly beneficial, especially in light of growing evidence linking it to a lower risk of developing various diseases. According to Mika (2021), the health benefits of yoghurt are:

i. Nutrient rich

The most obvious advantage of consuming yogurt is that it is a well-rounded and overall nutritious product that should be included in one's diet. It contains a variety of healthful components, including calcium and vitamin B as well as several lesser-known nutrients such as phosphate and potassium, both of which are essential. The nutrition of yoghurt is shown in the Table 2.1.

Table 2.1 Nutrition per 100 grams of whole-milk plain yoghurt

Nutrient	Amount
Calories	61
Water	88 %
Protein	3.7 g
Carbohydrate	4.7 g
Sugar	4.7 g
Fiber	0 g
Fat	3.3 g

Source: Arnarson (2019)

ii. Good source of protein

Yogurt can supply a lot of protein to bodybuilders and any individual who is concerned about their health. In fact, 200 g of yogurt includes 12 g of protein, which implies that consuming yogurt on a daily basis can help the body create greater muscle mass. Furthermore, protein may enhance metabolism, allowing to burn more calories throughout the day and maintain a healthy weight (Mika, 2021).

iii. **Solve the problem of constipation**

Many people nowadays consume unhealthy meals that are abundant in highly processed products, chemicals, and preservatives. That's why many of us have trouble going to the bathroom and frequently suffer from irritating constipation. The biggest problem is a lack of fiber in diets, but so is a lack of probiotics. Fortunately, yogurt helps to balance out the bacteria in our stomach, allowing us to digest meals more effectively and making it easier to go to the toilet (Mika, 2021).

iv. **Boost immune system**

Consuming yogurt on a regular basis, may significantly enhance immune system as well as its ability to defend body from hazardous bacteria and viruses. Furthermore, yogurt may be used as a nutritious snack item, making it one of the simplest and most effective ways to naturally improve immune system. Consuming yogurts with high amounts of probiotics can help control inflammation as well as minimize the risk of getting various illnesses and conditions. This is especially critical during flu season, when millions of individuals get infected each year (Mika, 2021).

v. **Helps in digestion**

Consuming some yogurt before or after a meal may solve digestive issues. Yogurt is frequently considered to aid digestion since it includes a high concentration of probiotics. Probiotics are a type of healthy and useful bacteria found in the gut. Probiotics serve to balance out bacteria in the digestive system, keeping everything running smoothly and in order. Yogurts that have high concentrations of CFUs (specified on the yogurt label) should be consumed to get the most out of them (Mika, 2021).

2.12 Shelf life of yoghurt

A product's shelf-life is the number of days after manufacture that it may be consumed while being safe, maintaining its quality appeal, and matching customer expectations. In other words, it should be microbiologically safe and organoleptically acceptable for the duration of its shelf life (Ahmed, 2011). Since acid and flavor development go hand in hand in this fermented product of bacterial symbiosis, acidity is one of the primary criteria for consumer acceptability of plain yogurt (Salji and Ismail, 1983). According to Ahmed (2011), the ideal acidity range for yoghurt consumption is (0.6-0.9 %).

Yogurt has a shelf life of around 10 days at a chilled temperature of around 5 °C. After 10 days, although the bacterial growth is restricted, it will elevate the degree of acidity to such a level that it will damage the flavor, ultimately making it unpalatable to most people. The bacteria are eventually eliminated, and the yoghurt separates into curds and whey. Yogurt is especially vulnerable to yeast and mold attack; great care must be taken to ensure that the starter is free of these organisms and that they do not obtain access during packaging (Tamime and Deeth, 1980). The shelf life of yoghurt is around 3 weeks when refrigerated, depending on the hygiene standards observed during the manufacturing process and the microbiological quality of the ingredients and packaging materials (Goodluck *et al.*, 2014).

2.13 Fruit and flavored yoghurt

Yogurt with various fruits, flavorings, and aroma additions is quite popular; however, some markets are clearly showing an interest in natural yoghurt. Fruit and berries in syrup, processed, or as a purée are typical additives. Typically, 15 % contains fruit, with 50 % of it being sugar (Bylund, 1995). A typical composition for fruit flavored yoghurt is shown in Table 2.2

Table 2.2 Composition of fruit flavored yoghurt

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

Source: Bylund (1995)

In set-type fruit yogurt, fruit may be added after heating but before fermentation, while it is added after fermentation in stirred-type fruit yogurt. Fruit flavorings and preparations are prepared according to the variety of yogurt (Chandan *et al.*, 2017). In addition to fruit flavors (apple, apricot, black cherry, black currant, blue berry, lemon, mandarin, raspberry, strawberry, peach), yogurt is also offered in a wide variety of other flavors, such as chocolate, vanilla, caramel, ginger, etc. Yogurt sales have increased as a result of constant evaluation and enhancement to meet consumer expectations. Dry Matter (DM) content of the final yoghurt is raised by the additives (Teshome *et al.*, 2017).

2.14 Almond

Almond seeds are members of the Rosaceae family, which also includes *Pomoideae* (apples and pears), *Prunoideae* (apricot, cherry, peach, and plum), and *Rosoideae* (blackberry and strawberry) fruits. There are two kinds of almond seeds (*Prunus amygdalus*): sweet almonds (*Prunus amygdalus 'dulcis'*) are primarily used for culinary purposes, and bitter almonds (*Prunus amygdalus 'amara'*) are primarily utilized in the production of oils and flavorings. Almond seeds have 51 % fat, 21 % protein, 20 % carbohydrate, and 12 % fiber. Almond seeds are popular because of their sweet flavor and crunchy texture (Monaghan, 2008).

Scientific classification

Order	*Rosales*
Family	*Rosaceae*
Subfamily	*Prunoideae*
Genus	*Prunus*
Sub-genus	*Amygdalus*
Species	*P. dulcis*
Local name	Badaam

Source: Hussain *et al.* (2021)

According to the Almond Board of California (ABC), the bulk of almond seeds consumed (50 %) are utilized as an ingredient in manufactured foods such as confectionery, cereal, ice cream, and pastries. The remaining is bought at retail for consumer snacking, in-home baking and cooking (25 %), or consumed in food service (25 %). Almond seeds are commonly used in nougat, marzipan, cookies, ice cream, butters, snacks (roasted and/or salted mixed nuts) and as a topping for desserts, salads and vegetables. Tannins, the astringent bitter-tasting polyphenols are found in almond seeds, mainly in in the skin (hull) (Monaghan, 2008).

2.14.1 Origin and habitat

Almond seeds are members of the Rosaceae family, which is native to Central Asia but is now grown worldwide in hot-arid Mediterranean climates (Barreca *et al.*, 2020). Almond seeds, which are thought to be native to the Middle East, were domesticated as early as 3000 BC, and maybe much earlier, as wild almond seeds were discovered in Greek archaeological sites dating back to 8000 BC. In the mid-1700s, Spanish missionaries (particularly Franciscan Padres) are credited for introducing the almond seed to California and it became the world's number one country in almond seed production at around 1960 (Monaghan, 2008).

The almond tree grows well in subtropical regions ranging from 30 to 40 °N and 750 to 3500 mm above sea level. Summer heat and cold winter temperatures both have a negative impact on fruit growth and development. The ideal temperature for tree budding and flowering is 24 °C. Almond trees are grown in a variety of soils, ranging from sandy loam to sandy clay, although light, fertile, deep, and well-drained soils are favored. The pH of the soil has no effect on the growth and propagation of trees. However, it is thought that soil with a pH of 6.5 is ideal to grow almond trees (Hussain *et al.*, 2021).

2.14.2 Morphological Characteristics

Despite being referred to as nuts, almonds are botanically drupes belonging to the family *Rosaceae* and sub-genus *amygdalus*. Prunes have a corrugated shell surrounding the seed, which differ them from other *amygdalus* species. Small to medium-sized, upright almond trees grow to a height of 4 to 10 meters when fully developed. The wooden, brown truck has scales that are 30 cm wide. The almond is made up of three separate parts: the interior kernel or meat, the middle shell component, and an outside green shell cover or hull. Almonds are classified as either hard or soft shelled based on their texture (Hussain *et al.*, 2021).

The immature shoots are initially green, but after being exposed to the sun, they turn purplish. As the flowers are self-incompatible, insect pollinators are required to promote cross-pollination with other cultivars. The developing fruit (a drupe) looks like a peach until it ripens; as it ripens, the leathery outer covering, or hull, breaks open, folds outward, and releases the pit. Almonds, contrary to popular belief, are not true nuts (a sort of dry fruit), but rather seeds encased in a hard fruit covering. Sweet almonds are widely cultivated in selected favorable areas, although nut crops remain uncertain wherever frosts are expected to occur during flowering (Petruzzello, 2022).

2.14.3 Varieties of almond

Varieties of almond is shown in Table 2.3.

Table 2.3 Varieties of almond

Variety	Characteristics
Sweet	Sweet almonds are the most well-known edible kind, consumed as nuts and used in cooking or as a source of almond oil or almond powder.
Bitter	Bitter almond oil is used to make flavoring extracts for foods and liqueurs, although prussic (hydrocyanic) acid needs to be eliminated first.

Source: Petruzzello (2022)

2.14.4 Nutrition of Almond

It is a healthy food, with 100 g serving provides more than 20 % of the recommended intake of riboflavin, niacin, vitamin E, calcium, iron, magnesium, manganese, phosphorus, and zinc. The same serving size is a good source (10-19 % RDI) of thiamine, vitamin B6 and folate, as well as choline and the important mineral potassium. They are also high in dietary fibre, monounsaturated and polyunsaturated fats, all of which may help decrease LDL cholesterol. Almonds, like other nuts and seeds, contain phytosterols such as beta-sitosterol, stigmasterol, campesterol, sitostanol, and campestanol, which have been linked to cholesterol-lowering characteristics (Berryman *et al.*, 2011). Nutritional composition is shown in Table 2.4.

Table 2.4 Nutritional composition of almonds

	Nutrients	Per 100 g serving
Macronutrients	Water	4.41 g
	Energy	2420 kJ
	Protein	21.2 g
	Total Fat	49.9 g
	Carbohydrate	21.6 g
	Ash	2.97 g
Minerals	Dietary Fiber	12.5 g
	Calcium	269 mg
	Iron	3.71 mg
	Magnesium	270 mg
	Phosphorus	481 mg
	Potassium	733 mg
	Sodium	1 mg
	Zinc	3.12 mg
	Copper	1.03 mg
	Manganese	2.18 mg
Vitamin	Thiamin	0.205 mg
	Riboflavin	1.14 mg
	Niacin	3.62 mg
	Pantothenic acid	0.471 mg
	B6	0.137 mg
	E (α-Tocopherol)	25.6 mg
	Saturated fats	3.8 g
Lipids	Monounsaturated fats	31.6 g
	Polyunsaturated fats	12.3 g

Source: USDA. (2018)

2.14.5 Health benefits of almond

According to Zelman (2022b), the health benefits of almonds are:

i. **Lower cholesterol**

Low-density lipoprotein (LDL), a bad kind of cholesterol, is reduced and high-density lipoprotein (HDL), a healthy form, is increased when almonds are consumed. Additionally, almonds are anti-inflammatory and antioxidant, which can help prevent heart disease.

ii. **Keep a healthy weight**

Almonds, despite having a lot of calories, can actually lower the risk of weight gain and obesity, provided that attention is given to portion control. As a result of the protein and fiber in the nuts, people feel fuller more quickly, allowing to consume less calories while still satiating the hunger

iii. **Lower blood pressure**

Almonds aid in the reduction of systolic blood pressure that provides additional protection against heart disease

iv. **Provide essential vitamins, minerals, and fiber**

Almonds are a great source of magnesium, vitamin E, and dietary fiber. Almonds are a nutritious and satisfying snack in one serving.

v. **Makes bone stronger**

Calcium and phosphorus in almonds improve bone health and protect from fractures.

vi. **Better blood sugar Control**

Almonds appear to help prevent blood sugar rises after meals, which is important for diabetics.

vii. **Better gut health**

Almonds may be able to maintain the immunity of GI (gastrointestinal) tract and overall health, according to recent studies. A stronger physique and a greater immune system are results of a healthy gut.

2.14.6 Almond milk

Almond milk is one of the most popular almond products because it has an important feature: it is lactose-free because it is not derived from animals. Therefore, it's an excellent milk substitute for those who are lactose intolerant. It is also suitable for vegans. Others adore its nutty flavor. Almond milk can be consumed on its own or use it in recipes that call for animal or plant-based milk. Aside from its delicious taste, almond milk has some fairly significant health benefits. Almond milk is rich in vitamin E, an antioxidant that is beneficial for immune system and blood vessels. Almond milk is a good source of magnesium, which is necessary for muscle function, blood sugar regulation, blood pressure control, and the formation of bone, protein, and DNA. It provides bone-building calcium and is limited in calories and carbohydrates (Zelman, 2022a).

PART III

Materials and methods

3.1 Materials

The materials collected for the preparation of almond 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 Almond

Almond was collected from local market of Singhadevi Chowk Dharan.

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 Baraha Department store of Dharan.

3.1.5 Starter culture

Starter culture a liquid culture containing *L. bulgaricus* and *S. thermophilus* was collected from the Kamdhenu Dairy Tarahara.

3.1.6 Containers

Plastic cup as ice cream packaging materials were bought from local market of Singhadevi Chowk Dharan. The size of cup was 150 ml and plain in design.

3.1.7 Equipment and chemicals

Equipment and chemicals required were utilized from Central Campus of Technology laboratory which are given in Appendix B.1 and B.2.

3.2 Methods

3.2.1 Experimental procedure

3.2.1.1 Recipe formulation

Design expert version 13, one factorial design was used to design the formulation of milk and almond milk. Five formulations were designed under mixed condition which were then coded alphabetically as given in Table 3.1.

Table 3.1 Sample formulation in coded form

Sample	Milk (%)	Almond milk (%)	Sugar (%)	SMP (%)	Culture (%)
A	75	25	3	4	2
B	50	50	3	4	2
C	25	75	3	4	2
D	0	100	3	4	2
E	100	0	3	4	2

3.2.1.2 Preparation of almond milk

Raw almonds were soaked in water in a ratio of 1:3 (w/w) for 18–20 h. Then, the water was discarded, and the almonds were blotted dry to remove any surface water. The almonds were deskinned by hand and mixed with water at a ratio of 1:4 by weight. This mixture was then ground in the grinder. The resulting slurry was filtered through two layers of muslin cloth and almond milk was produced.

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

Raw Almond

↓

Soaking in water

↓

Blotting dry to remove surface water

↓

De-skinning

↓

Mixing (almond: water= 1:4)

↓

Grounding

↓

Filtration through muslin cloth

↓

Almond milk

Fig. 3.1 Preparation of Almond milk

Source: Devnani *et al.* (2020)

3.2.1.3 Preparation of set type almond milk incorporated yoghurt

To make yoghurt, standardized and pasteurized milk of 3 % fat and 8 % SNF from Kamdhenu Dairy Development Limited was taken. At 45 °C, 4 % Skim milk powder (SMP) and 3 % sugar were added to the milk. The milk was heated for a little longer until it reached a temperature of about 65 to 70 °C. The heated milk was then cooled to a temperature of 43–44 °C. Five formulations of the samples were created after cooling, using 0, 25, 50, 75and 100 % of almond milk per 100 ml of yoghurt mix. The starter culture was then added to each formulation at a rate of 2 %. The mixture was then put in plastic cups. After that, the yoghurt mix was stored in an incubator that was kept at a temperature of roughly 43 °C for 3.5–4 h for control yoghurt and 7-8 h for almond milk incorporated yoghurt till the coagulum formed. The yoghurt was then immediately cooled to 5-7 °C and stored in a refrigerator at that temperature.

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

Standardized milk (3 %fat, 8 % SNF)

↓

Pre-heating to 45 °C

↓

Addition of 4 % SMP and 3 % sugar

↓

Heating to 65-70 °C

↓

Cooling until 43-44 °C

↓

Addition of 0, 25, 50, 75, and 100 % almond milk by volume

↓

Inoculation of 2 % culture

↓

Placing mix in several plastic cups

↓

Incubation at 43 °C for 7-8 h (almond yoghurt) and 3.5-4 h for (control)

↓

Set type almond milk yoghurt

↓

Cooling and storage (5-7 °C)

Fig. 3.2 Preparation of almond milk incorporated yoghurt

Source: Biswas (2013)

3.2.2 Analytical procedure

3.2.2.1 Chemical analysis of raw materials and product

3.2.2.1.1 Fat

Fat content of almond, almond milk, milk and yoghurt was determined by Gerber method as described in AOAC (2005).

3.2.2.1.2 Crude fiber

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

3.2.2.1.3 Protein

Protein content of almond, almond milk and yoghurt was determined by kjeldahl method and that of milk was determined by formal titration as given in KC and Rai (2007).

3.2.2.1.4 Ash

Ash content of almond, almond milk, milk and yoghurt was determined as described in Ranganna (1986).

3.2.2.1.5 Moisture

Moisture content of raw material and product was determined as per the methods described by Ranganna (1986).

3.2.2.1.6 Carbohydrate

Total carbohydrate 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)

3.2.2.1.7 Free radical scavenging capacity

The procedure outlined by Shad *et al.* (2012) was modified to test the antioxidant activity of almond oil. 0.1 g of oil sample was dissolved in n-hexane, the volume was made up to 10 ml, and the sample was then mixed with a 40 M DPPH solution produced in n-hexane. At 517 nm, the absorbance was measured after 30 min of dark incubation.

The Yoon *et al.* (2019) approach was used to test the DPPH radical scavenging activities of yoghurt samples with minor modifications. Yoghurt was first diluted in 95 % ethanol for one gram. After fully mixing, the sample was centrifuged at 440×g for 20 min at 4 °C. 100 µL (0.1ml) of samples were added to a 100 µM DPPH solution in methanol at a 1:1 ratio. The mixture was wrapped in aluminium foil and left at room temperature for 30 min in the dark. After that, the activity of yoghurt to scavenge DPPH radicals was measured spectrophotometrically using the DPPH cation de-colorization assay at 517 nm.

Finally, the following equation was used to calculate the percentage of scavenging activity.

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

3.2.2.1.8 Acidity

Acidity of almond milk, milk and yoghurt was determined by titrimetric method as given in KC and Rai (2007).

3.2.2.1.9 pH

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

3.2.2.1.10 Total Solid (TS)

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

3.2.2.1.11 Lactose

Lactose content of milk and yoghurt was determined by Lane and Eynon method as described by Ranganna (1986).

3.2.2.2 Physical analysis

3.2.2.2.1 Syneresis

The degree of syneresis was measured using a technique developed by Lee and Lucey (2004) and expressed as a percentage of free whey. Yoghurt sample weighing 100 g was placed on filter paper lying on top of a funnel. After draining for 10 min under vacuum, the amount of yoghurt that was left was weighed, and syneresis was calculated as follows:

$$\%\ \text{Free whey} = \frac{\text{wt. of initial sample} - \text{wt. of sample after filtration} \times 100}{\text{wt. of initial sample}}$$

3.2.2.3 Microbiological examination

Total Plate Count (TPC) was carried by using plate count agar as described in AOAC (2005).

3.2.3 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. The parameters for sensory evaluation were: texture, appearance, color, flavor and overall acceptability. The specimen of the evaluation of card is shown in Appendix A.

3.2.4 Statistical analysis

ANOVA (Analysis of variance) was used to analyze the data from the sensory evaluation. The Genstat release 12.1 software program developed by VSN International Ltd. was used to analyze the significant differences between them using LSD at the 5 % level of significance, and Microsoft Excel 19 was used to perform a t-Test: Two-Sample Assuming Equal Variances to evaluate the significant differences between the two samples' syneresis. The results of the chemical analysis of the best and control yoghurt were statistically analyzed using the t-Test.

Part IV

Results and discussion

Almond milk incorporated yoghurt was prepared at Central Campus of Technology, Dharan, in a laboratory for the present study. The almond milk incorporated yoghurt samples were prepared by incorporating 0, 25, 50, 75, and 100 % almond 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 43-45 °C. Five formulations of the samples were made by adding 0, 25, 50, 75, and 100 % of almond milk per 100 ml yoghurt mix. Then starter culture was 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 to 4 h until the coagulum was formed.

4.1 Chemical composition of raw almond

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

Table 4.1 Chemical composition of raw almond (dry basis)

Parameters	Values
Crude protein (%, db)	21.7±1.23
Crude fat (%, db)	50.34±0.68
Moisture (%)	4.63±0.61
Ash (%, db)	3.36±0.4
Crude fiber (%, db)	3.1±0.1
Carbohydrates (by difference)	16.87±1.53
Antioxidant activity (% RSA)	72.13±2.55

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

The crude protein content of almond (21.7 %) was similar to that suggested by USDA. (2018) i.e. (21.2 %) and lower than the value obtained by Ibourki *et al.* (2022) i.e. (25.12 %). The decrease in protein content might be due to some loss of nitrogenous material during the digestion of sample. The fat content of almond (50.34 %) was less that the value suggested by DFTQC. (2012) i.e. (58.9 %) and similar to the data obtained by Ibourki *et al.* (2022) i.e. (51.12 %). The moisture content of raw almond was found to be 4.63 % which similar to the value suggested by USDA. (2018) i.e. (4.41 %) but lower than the value obtained by Akpambang *et al.* (2008) i.e. 6.10 %.

The ash content of almond (3.36 %) was similar to the value obtained by Akpambang *et al.* (2008) i.e. (3.34 %) and lower than the value obtained by Ibourki *et al.* (2022) i.e. (5.11 %). The crude fiber of almond was found to be 3.1 % which was similar to the value obtained by Akpakpan and Akpabio (2012) i.e. (3.11 %) and lower than the value obtained by Akpambang *et al.* (2008) i.e. 5.70 %. The carbohydrate content of almond (16.87 %) was less that the value obtained by Akpakpan and Akpabio (2012) i.e. 25.47 % and was similar to the value suggested by USDA. (2018) i.e. (21.6 %). The antioxidant activity of almond was found to be 72.13 % RSA which was similar to the result obtained by Win *et al.* (2019) i.e. 74.19 % RSA of 1000 µg/ml concentration.

4.2 Chemical composition of milk and almond milk

The proximate composition of the milk collected from Kamdhenu Dairy Development Cooperative and almond milk is presented in the Table 4.2.

The chemical composition of milk shown in Table 4.2 has little variations over the composition of milk analyzed by Khadka (2018). He had also analyzed the milk of Kamdhenu Dairy Development Co-operative. This variance in milk content may be attributable to the species, nutritional component of the animal, stage of lactation, and animal feeding. The difference could also be related to various dairy processing standards and specification.

The fat content of almond milk (3.43 %) was similar to the result obtained by Miranti and Wati (2021) i.e. 3.4 %. The crude protein content of almond milk (1.71 %) was similar to the data obtained by Miranti and Wati (2021) i.e. 1.7 % and less than the value obtained by Alozie and Udofia (2015) i.e. 2.36 %. The decrease in protein content might be due to some loss of nitrogenous material during the digestion of sample

Table 4.2 Chemical composition of milk and almond milk (dry basis)

Chemical composition	Milk	Almond milk
Crude fat (%, db)	3.1±0.1	3.43±0.12
Crude protein (%, db)	3.6±0.4	1.71±0.11
Lactose (%, db)	4.53±0.03	0
Acidity (%)	0.13±0.01 (lactic acid)	0.036±0.01
Total solid (%, db)	12.9±0.2	12.49±0.56
pH	6.53±0.061	6.70±0.1
Ash (%, db)	0.57±0.046	0.71±0.11
Moisture (%)	87.1±0.2	87.5±0.56

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

The total solid content of almond milk was found to be 12.49 % which was similar to the result obtained by Miranti and Wati (2021) i.e. 12.77 % but is lesser that the value obtained by Kundu *et al.* (2018) i.e. 27.9 %. The pH of almond milk (6.70) is comparable to the result obtained by Ceylan and Ozer (2020) i.e. 6.62. The ash content of almond milk was found to be 0.71 % which was less than the value obtained by Alozie and Udofia (2015) i.e. 0.84 % and was similar to the value obtained by Miranti and Wati (2021) i.e. 0.7 %. The moisture content (87.5 %) was comparable to the value obtained by Alozie and Udofia (2015) i.e. 88.12 % but was higher than that obtained by Kundu *et al.* (2018) i.e. 72.04 %. The acidity of almond milk (0.036 %) was similar to the data obtained by Bernat *et al.* (2015) i.e. 0.039%.

4.3 Sensory evaluation of almond milk yoghurt

Sensory evaluation of five formulation of almond milk yoghurt were carried out by a group of 11 semi-trained panelist. The parameters evaluated were appearance, aroma, taste, color, texture, and overall acceptance. The Analysis of Variance (ANOVA) was carried out using least significant difference (LSD) at 5 % level of significance.

4.3.1 Appearance

Regarding appearance of almond milk incorporated yoghurt, the analysis showed that the mean score for sample A, B, C, D, and E were found to be 8.18, 7.09, 6.00, 4.64, and 8.55 respectively. Statistical analysis showed that the variation in proportion of almond milk had significant effect ($p<0.05$) in the appearance of different yoghurt formulations. LSD at 5 % level of significance indicated that sample A and E were not significantly different to each other but were significantly different from sample B, C and D which is shown graphically in Fig. 4.1. However, the score of sample A was slightly lower than sample E. According to sensory evaluation, following results were obtained.

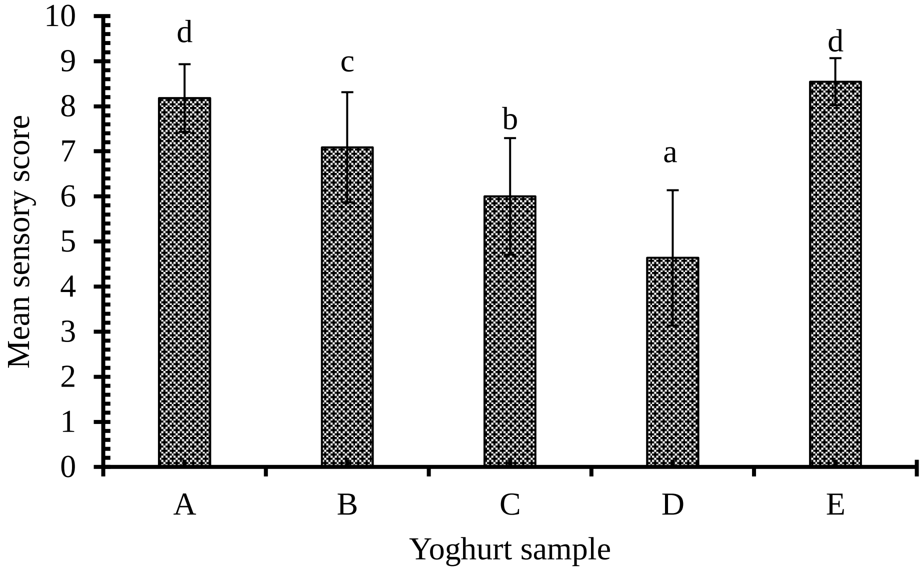

Fig. 4.1 Mean sensory scores for appearance of almond milk incorporated yoghurt

*A, B, C, D, and E denote formulation with 25, 50, 75, 100 and 0 % almond milk respectively. Vertical error bars represent the value of standard deviation. Values of same subscript represents that the samples were similar in terms of appearance.

A similar result was observed by Yilmaz-Ersan and Topcuoglu (2022) in almond milk incorporated yoghurt where control and 25 % almond milk yoghurt had no significant difference in terms of appearance. The score of the appearance decreases as the almond milk ratio of yoghurt sample increases**.**

4.3.2 Aroma

The mean sensory score for aroma were found to be 7.455, 6.636, 5.636, 5.182 and 8 for the almond milk incorporated yoghurt formulation A, B, C, D, and E respectively. Statistical analysis showed that the variation in proportion of almond milk had significant effect ($p<0.05$) in the aroma of different yoghurt formulations. The sample A and E and sample C and D were not significantly different to each other but significantly different to other which is shown graphically in Fig. 4.2. The sample B is significantly different from other.

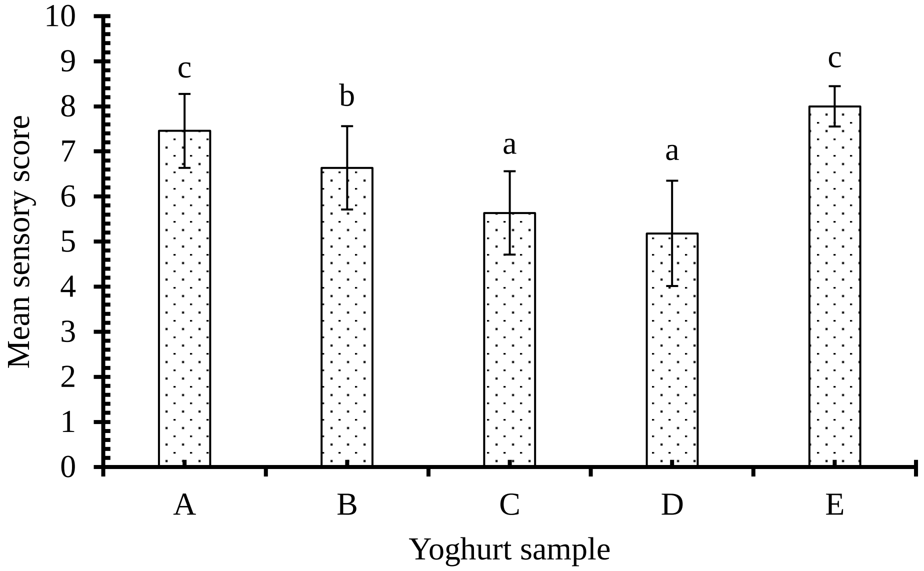

Fig. 4.2 Mean sensory scores for aroma of almond milk incorporated yoghurt

* A, B, C, D, and E denote formulation with 25, 50, 75, 100 and 0 % almond milk respectively. Vertical error bars represent the value of standard deviation. Values of same subscript represents that the samples were similar in terms of aroma.

A similar result was observed by Yilmaz-Ersan and Topcuoglu (2022) in almond milk incorporated yoghurt where control and 25 % almond milk yoghurt had no significant difference in terms of aroma.

4.3.3 Color

The mean sensory score for color were found to be 7.909, 6.545, 6, 5.818, and 8.455 for the almond milk incorporated yoghurt formulation A, B, C, D, and E respectively. Statistical analysis showed that the variation in proportion of almond milk had significant effect

($p<0.05$) in the color of different yoghurt formulations. Sample E had scored higher sensory mean value for color and sample D has the lowest score. Sample A and E were not significantly difference to each other but were significantly different from other samples. Also, sample B, C, and D were not significantly different to each other but were significantly different from A and E. According to sensory evaluation, following results were obtained. The mean sensory score of color for different samples is shown in Fig. 4.3.

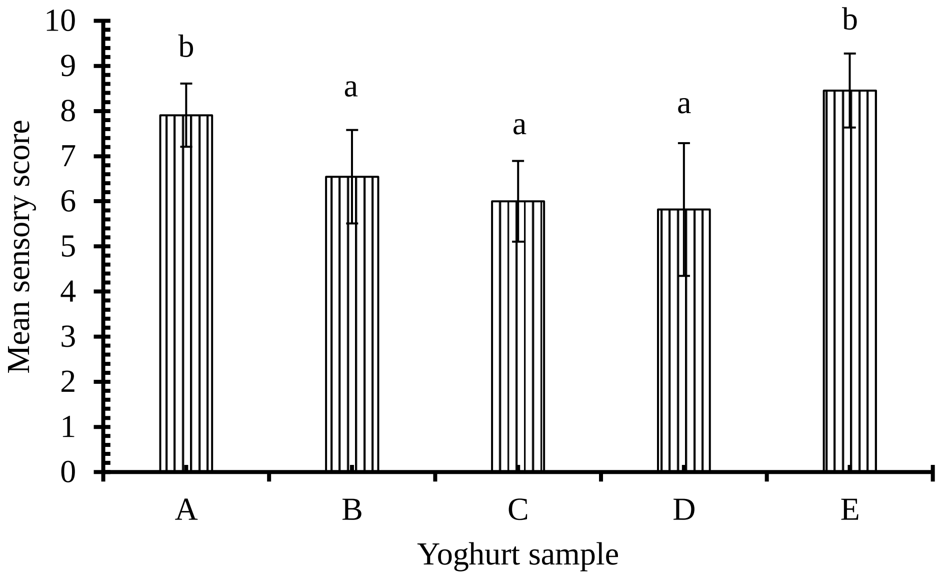

Fig. 4.3 Mean sensory scores for color of almond milk incorporated yoghurt

* A, B, C, D, and E denote formulation with 25, 50, 75, 100 and 0 % almond milk respectively. Vertical error bars represent the value of standard deviation. Values of same subscript represents that the samples were similar in terms of color.

A similar result was observed by Yilmaz-Ersan and Topcuoglu (2022) in almond milk incorporated yoghurt where control has the highest score for color and score of the color decreases as the almond milk rate of yoghurt sample increases. As the storage time increases, the color was less preferred by the panelists. The pigments such as carotenoids and flavonoids, are responsible for the red, yellow, and orange colors in dairy products such as enriched vegetable milks, which explain the difference in color parameters in samples (Yilmaz-Ersan and Topcuoglu, 2022).

4.3.4 Texture

Regarding texture of almond milk incorporated yoghurt, the analysis shows that the mean sensory score for sample A, B, C, D, and E were found to be 7.636, 6.545, 5.364, 4.273 and 8.273 respectively. Statistical analysis showed that the variation in proportion of almond milk had significant effect ($p<0.05$) in the texture of different yoghurt formulations. Sample E had scores higher sensory mean value for texture than other and were significantly different from other samples. Sample D score the lowest score for texture. All the samples were significantly different from each other. According to sensory evaluation, following results were obtained. The mean sensory score of texture for different samples is shown in Fig. 4.4.

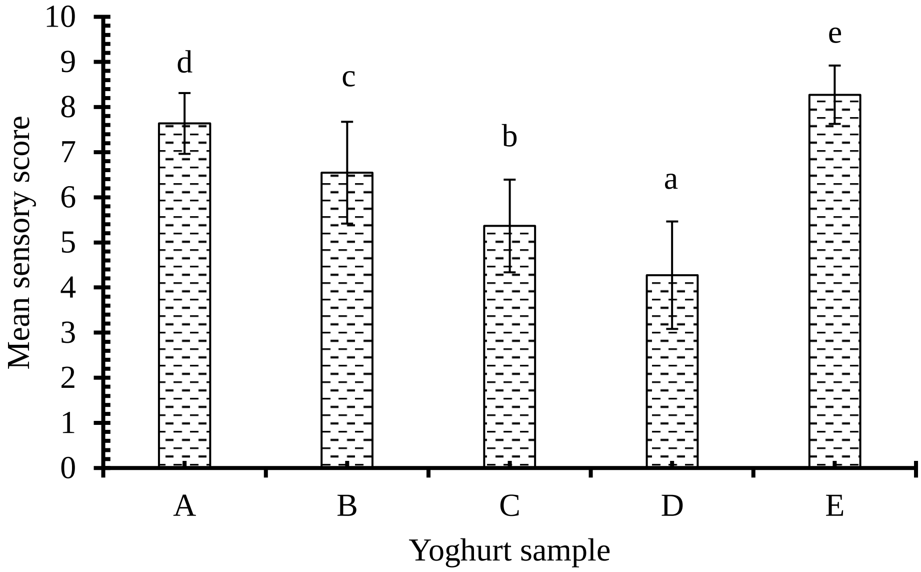

Fig. 4.4 Mean sensory scores for texture of almond milk incorporated yoghurt

* A, B, C, D, and E denote formulation with 25, 50, 75, 100 and 0 % almond milk respectively. Vertical error bars represent the value of standard deviation. Values of same subscript represents that the samples were similar in terms of texture.

A similar result was obtained by Yilmaz-Ersan and Topcuoglu (2022) in almond milk incorporated yoghurt where control has the highest score for texture and score of the texture decreases as the almond milk rate of yoghurt sample increases. The physical states of fats and proteins in milk, milk composition, temperature of heating, solid content and time of heating, homogenization, mechanical handling of coagulum, use of stabilizers, type and quantity of starter culture, acidity, fermentation, and storage conditions all have an impact on the textural properties of yoghurt (Yilmaz-Ersan and Topcuoglu, 2022).

4.3.5 Taste

Regarding taste of almond milk incorporated yoghurt, the analysis showed that the mean sensory score for sample A, B, C, D, and E were found to be 7.818, 6.545, 5.455, 5 and 8.727 respectively. Statistical analysis showed that the variation in proportion of almond milk had significant effect ($p<0.05$) in the taste of different yoghurt formulations. LSD at 5 % level of significance indicated that the sample E was significantly different from rest of the sample and also had the highest score. Sample C and D were not significantly different to each other but were significantly different from other. Sample D scored lowest in terms of texture. According to sensory evaluation, following results were obtained. The mean sensory score of taste for different samples is shown in Fig. 4.5.

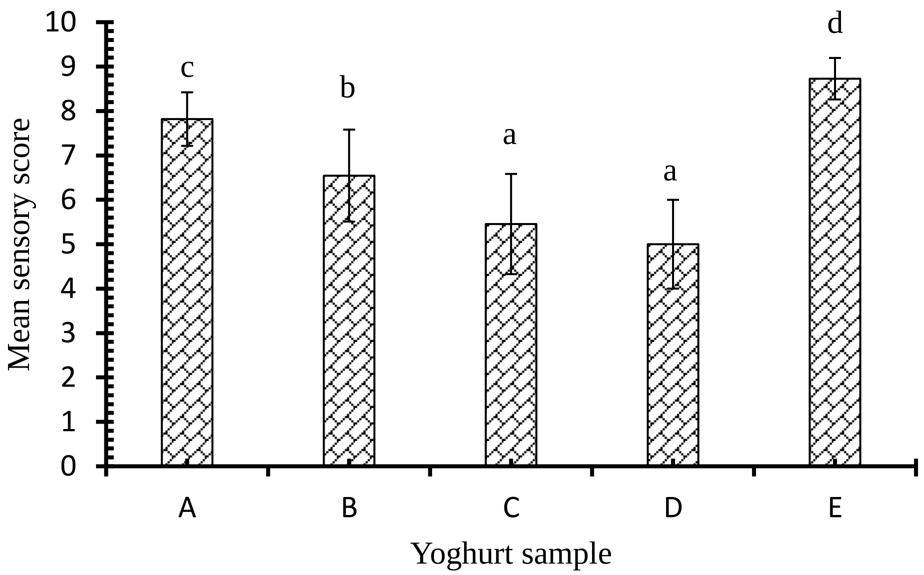

Fig. 4.5 Mean sensory scores for taste of almond milk incorporated yoghurt

* A, B, C, D, and E denote formulation with 25, 50, 75, 100 and 0 % almond milk respectively. Vertical error bars represent the value of standard deviation. Values of same subscript represents that the samples were similar in terms of taste.

A similar result was observed by Yilmaz-Ersan and Topcuoglu (2022) in almond milk incorporated yoghurt where control has the highest score for taste and score of the taste decreases as the almond milk ratio of yoghurt sample increases. Junaid *et al.* (2013) reported that there was significant difference in taste of yoghurt incorporated with different fruit juice in small amount.

4.3.6 Overall Acceptability

Regarding overall acceptance of almond milk incorporated yoghurt, the analysis show that the mean sensory score for sample A, B, C, D, and E were found to be 8.091, 7, 5.909, 5 and 8.636 respectively. Statistical analysis showed that the variation in proportion of almond milk had significant effect ($p<0.05$) in the overall acceptance of different yoghurt formulations. The sample E got highest score due to optimum acceptance of panelists while sample D was least accepted by panelists. A and B scored slightly lower than E. According to sensory evaluation, following results were obtained. The mean sensory score of overall acceptability for different samples is shown in Fig. 4.6.

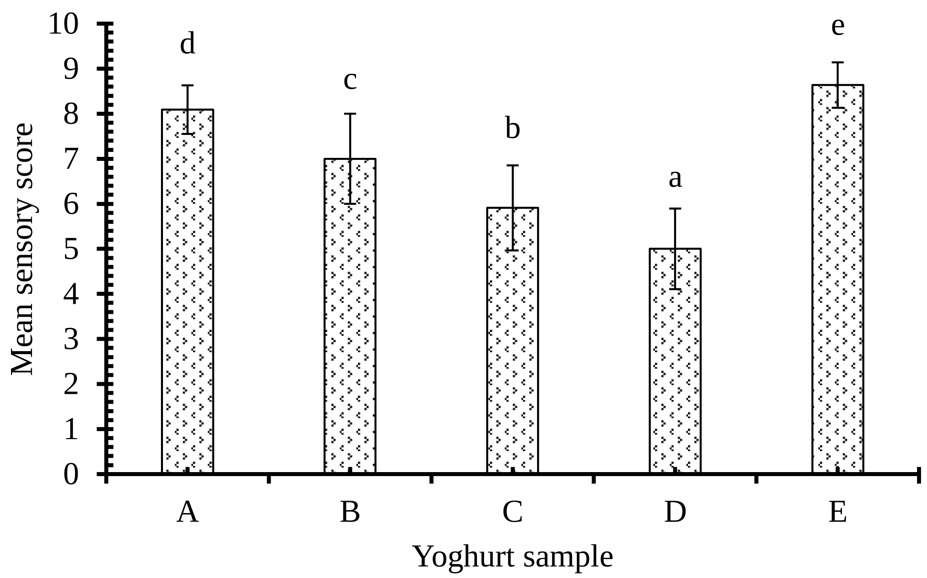

Fig. 4.6 Mean sensory scores for overall acceptance of almond milk incorporated yoghurt

* A, B, C, D, and E denote formulation with 25, 50, 75, 100 and 0 % almond milk respectively. Vertical error bars represent the value of standard deviation. Values of same subscript represents that the samples were similar in terms of overall acceptance.

The overall acceptability of sample A and E was higher due to the improvement in color, taste and texture with respect to other samples. Sample C and D got lowest score may be due to the incorporation of high volume of almond milk to yoghurt. With respect to the control (Sample E), sample A got high score in terms of overall acceptability as shown in Fig. 4.6.

Yilmaz-Ersan and Topcuoglu (2022) concluded that the addition of 25 % of almond milk to yogurt was most appreciated by the panelists. The score of the overall acceptance decreases as the almond milk ratio of yoghurt sample increases and not preferred by panelists. This was similar to our study.

Therefore, from the sensory evaluation of the product conducted on the attributes like appearance, aroma, color, texture, taste and overall acceptability, the product containing 25 % almond milk and 75 % cow milk by volume was rated as best in all attributes.

4.4 Chemical analysis of best product

Thus, from statistical sensory analysis, the best product was found to be sample A yoghurt containing 25 % almond milk and 75 % cow milk. Chemical analysis of sample A and sample E i.e., control yoghurt (100 % cow milk) was done. The value of chemical analysis is shown in Table 4.3.

Table 4.3 Chemical analysis of product

Parameters	Sample A (Best)	Sample E (Control)
Moisture	81.45^{a}±0.38	79.13^{b}±0.77
Crude protein (%, db)	3.74^{a}±0.14	2.88^{b}±0.12
Crude fat (%, db)	3.34^{a}±0.06	2.93^{b}±0.15
Ash (%, db)	0.79^{a}±0.01	0.83^{a}±0.1
Acidity (% lactic acid)	0.64^{a}±0.01	0.62^{a}±0.07
pH	4.57^{a}±0.01	4.62^{a}±0.06
Lactose	2.76^{a}±0.23	3.84^{b}±0.06
Total solid (%, db)	18.54^{a}±0.38	20.87^{b}±0.78
Antioxidant activity (% RSA)	70.56^{a}±1.3	56.7^{b}±2.06

*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 moisture content, fat and total solid of yoghurt from whole milk (control) found in our study was comparable to the data reported by Matela *et al.* (2019) i.e. 79.16 %, 2.24 %, and 20.84 % respectively. Protein content of the control yoghurt was similar to the result obtained by Farinde *et al.* (2009) i.e. 3 %. Ash content was similar to the data reported by Chhetri (2021) i.e. 0.96 %. Acidity and pH of control sample in our study was similar to the result obtained by Salji and Ismail (1983). The lactose content of the control was similar to that reported by Gaglio *et al.* (2019) i.e. 3.37 %. The antioxidant activity of control was found to be 56.7 % RSA. The result was comparable to the data obtained by Nguyen and Hwang (2016) i.e. 59.47 % RSA.

The moisture content of the best yoghurt was found to be 81.45 % which was similar to the yoghurt prepared form camel milk by Eshraga *et al.* (2011). The protein and fat content of the best sample was found to be higher than that of control sample. This may be due to high protein and fat content in the almond milk compared to dairy milk. However, the lactose content of control sample was higher than the best sample because almond milk does not contain lactose. Similar result was obtained by Chhetri (2021). Acidity of best sample was found to be increased slightly than control sample but was not significant. Desai *et al.* (1994) reported that addition of fruit juice/pulp increases percent acidity. The radical scavenging capacity was found to be increased in the best sample due to incorporation of almond 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
Best yoghurt	$21.3^{a}\pm0.75$
Control yoghurt	$20.36^{a}\pm0.8$

t-Test was carried out to evaluate the significant difference between two samples. There was 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 Chhetri (2021).

4.6 Shelf-life of the product

The best product (Sample A) of almond milk incorporated yoghurt was found best with respect to appearance, color, aroma, texture, taste and overall acceptance. Hence it was subjected to further study for the 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 0.64 % to 1.029 % within 2 days of storage under room temperature. Acid production by culture bacteria could be the cause of increase in acidity of the yoghurt during storage period. The optimum temperature for the growth of bacterial culture *Lactobacillus bulgaricus* and *Streptococcus thermophilus* ranges between 37 °C to 45 °C hence they may have the high rate of metabolic activity at room temperature (Dave and Shah, 1997). Moreover, the rate of acidity was due to the addition of almond milk which increases availability of higher nutrients for the production of lactic acid. The acidity of yoghurt at 2 days of room temperature was found to be 1.029 %. Therefore, yoghurt sample remain suitable for consumption up to 2 days. The results are in agreement with Ahmed (2011) within the range of (0.6-0.9 %) acidity in yoghurt.

4.6.2 Acidity of yoghurt under refrigeration

The acidity of chilled yoghurt increased very slowly from 0.64 % to 0.97 % within 6 days of storage under refrigeration. The acidity of yoghurt might have increased due to acid generation by culture bacteria during storage due to their activity even at low temperatures (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 yoghurt samples also increased in refrigeration temperature but not as rapid as at room temperature due to temperature effect. Yoghurt samples were suitable for consumption up to 6 days which is shown in Fig. 4.7.

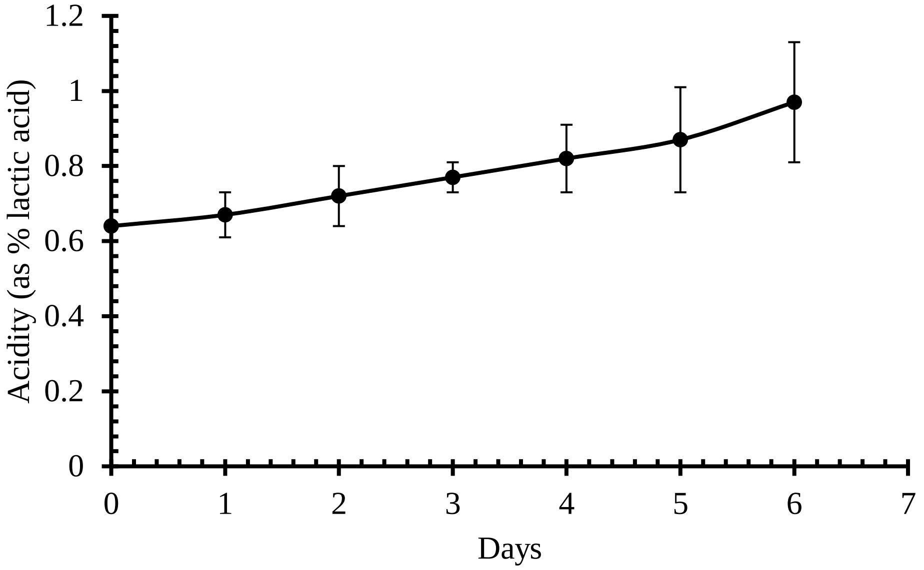

Fig. 4.7 Change in acidity under refrigeration with respect to the number of days

*Vertical error bars represent standard deviation

4.6.3 Total plate count of yoghurt at room temperature

The total plate count (TPC) of yoghurt increased from 4.1×10^4 to 16×10^4 CFU/ml within 6 days of storage under room temperature as shown in fig. 4.8. The increase in TPC of yoghurt was due to the production of lactic acid bacteria which increases with the addition of almond milk. Similar results was obtained by Goodluck *et al.* (2014) for the consumable range of total bacterial count as in the range of (3.0×10^4 – 10.5×10^4 CFU/ml). The optimum temperature for the growth of bacterial culture *Lactobacillus bulgaricus* and *Streptococcus thermophilus* ranges between 37 °C to 45 °C hence they may have the high rate of metabolic activity at room temperature due to temperature effect (Dave and Shah, 1997). Yoghurt sample was suitable for consumption up to 2 days at room temperature. The results are in agreement with Goodluck *et al.* (2014).

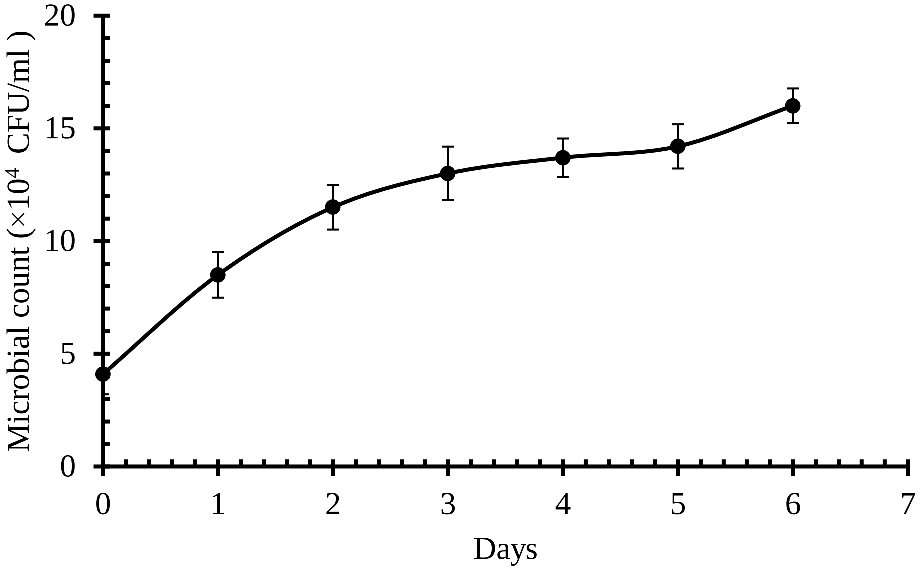

Fig. 4.8 Change in TPC under room temperature with respect to the number of days

*Vertical error bars represent standard deviation

4.6.4 Total plate count of yoghurt under refrigeration

The total plate count (TPC) of yoghurt increased slowly from 4.1×10^4 to 11.7×10^4 CFU/ml within 6 days of storage under refrigeration. The increase in TPC of yoghurt was 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). Similar results were obtained by Goodluck *et al.* (2014) for the consumable range of total bacterial count as in the range of (3.0×10^4 – 10.5×10^4 CFU/ml). The optimum temperature for the growth of bacterial culture *Lactobacillus bulgaricus* and *Streptococcus thermophilus* ranges between 37 °C to 45 °C hence they may have the high rate of metabolic activity at room temperature due to temperature effect (Dave and Shah, 1997). Yoghurt sample was suitable for consumption up to 6 days at refrigerated temperature as shown in Fig. 4.9.

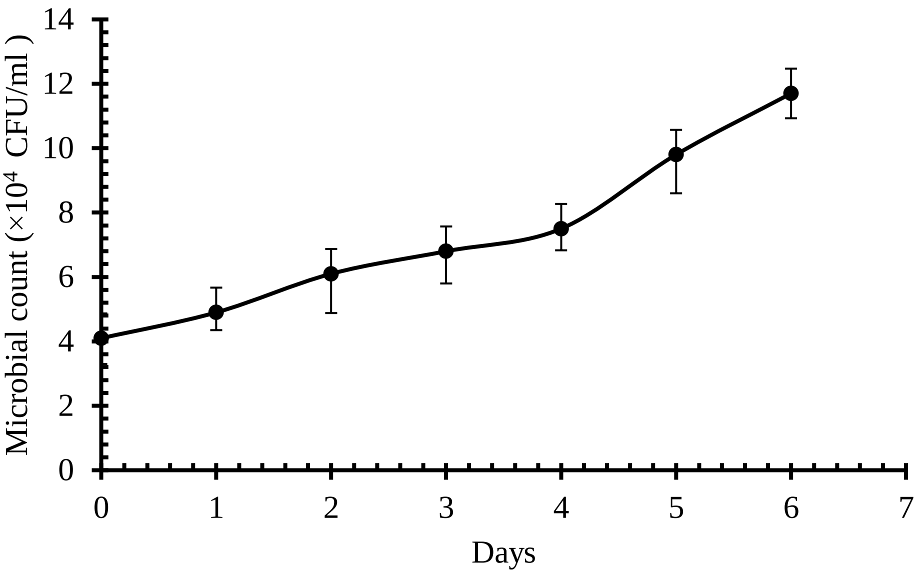

Fig. 4.9 Change in TPC under refrigeration with respect to the number of days

*Vertical error bars represent standard deviation

4.7 Cost evaluation

The total cost of the best sample of almond milk incorporated yoghurt and the control sample was calculated. It is shown in Appendix E in Tables E.1 and E.2. The price for 100 ml of almond milk incorporated yoghurt was found to be NRs. 24.02, and that of the control sample was found to be NRs. 18.08 (as of 2023). From the cost evaluation, it can be seen that the price of almond milk yoghurt was only Rs 6 higher than the control yoghurt given that almonds are much more expensive.

Part V

Conclusion and recommendations

5.1 Conclusion

Yoghurt was prepared by incorporating almond milk and storage stability of the prepared yoghurt was studied. On the basis of result obtained from physiochemical analysis of yoghurt following conclusions were drawn;

1. From sensory analysis of the product conducted on the attributes like appearance, aroma, color, taste, texture and overall acceptability, the product containing 25 % almond milk and 75 % cow milk by volume i.e., Sample A was rated as best in all attributes.
2. The moisture, protein, fat, ash, acidity, pH, lactose, total solid and antioxidant activity of best product were found to be in acceptable range. Antioxidant activity also seemed to be increased compared to control yoghurt
3. The shelf life of the best product was increased to 6 days in refrigerated condition with respect to TPC and acidity.
4. The syneresis of best product was 21.3 % and that of control was 20.036 %. There was only 1 % increment in the syneresis of the best product.
5. The cost of 100 ml of almond milk incorporated yoghurt was found to be NRs. 24.02 was only Rs 6 higher than the control yoghurt given that almonds are much more expensive.

5.2 Recommendations

The experiment can be further continued with the following recommendations:

1. 100 % almond milk yoghurt can be prepared by blending different proportions of sugar, stabilizer and MSNF.
2. Almond milk-based ice cream or energy drink can be prepared and quality evaluation
3. Roasted almonds can be used for the yoghurt preparation.

Part VI

Summary

Yoghurt includes a high quantity of milk solids in addition to nutrients produced during the fermentation process, making it more nutrient-dense. Almond (*Prunus amygdalus*) is a deciduous tree native to west Asia. However, it has not been commercialized in the context of Nepal. Almond milk incorporated yoghurt is a cultured dairy product produced by fermenting milk and almond milk, with or without added non-fat dry milk (NFDM) with *Lactobacillus bulgaricus* and *Streptococcus thermophilus* bacteria.

To make yoghurt, standardized and pasteurized milk of 3 % fat and 8 % SNF from Kamdhenu Dairy Development Limited was taken. At 45 °C, 4 % SMP (Skim milk powder) and 3 % sugar were added to the milk. The milk was heated for a little longer until it reached a temperature of about 65 to 70 °C. The heated milk was then cooled to a temperature of 43–44 °C. Five formulations of the samples were created after cooling, using 0, 25, 50, 75and 100 % of almond milk per 100 ml of yoghurt mix. The starter culture was then added to each formulation at a rate of 2 %. The mixture was then put in plastic cups. For each formulation, 1 liter of yoghurt was made. After that, the yoghurt mix was stored in an incubator that was kept at a temperature of roughly 43 °C for 3.5 to 4 h till the coagulum formed.

Sensory evaluation of five formulations of almond milk yoghurt was carried out. According to the sensory evaluation, it was concluded that the yoghurt containing 25 % almond milk and 75 % milk by volume was best whose moisture, protein, fat, ash, acidity, pH, lactose, total solid, and antioxidant activity were found to be 81.45 %, 3.74 %, 3.43 %, 0.79 %, 0.64 %, 4.57, 2.76 %, 18.54 % and 70.56 % respectively. Shelf life of the best product was found to be 2 days at room temperature and 6 days at refrigeration which was estimated in terms of acidity and total plate count.

According to the overall analysis of the result, it is clear that good quality yoghurt could be prepared by adding almond milk with cow milk. Almond milk has the benefit that it improves gut health, lowers cholesterol and blood pressure, and its consumption should be increased. This new product will help to foster the growth and commercialization of almond in Nepal.

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Appendices

Appendix-A

SENSORY ANALYSIS SCORE CARD

Name: …………………………   Date: ……………

Name of the product: Almond milk incorporated yoghurt

Dear panelist, you are provided with five samples of yoghurt prepared from almond milk. Please test the following samples of yoghurt and check how much you prefer for each of the samples. Give the points for your degree of preferences for each parameter for each sample as shown below:

Judge the characteristics on the 1-9 scale as below:

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

Parameters	**Sample**				
	A	**B**	**C**	**D**	**E**
Appearance					
Aroma					
Taste					
Color					
Texture					
Overall acceptability					

Comments if any:

Signature: …………

Appendix B

Table B.1 List of equipment used

Physical apparatus	
Heating arrangement	Electric balance
Refrigeration	Gerber centrifuge
Incubator	Gerber butyrometer
Thermometer	Muffle furnance
Refractometer	Hot air oven
Kjeldahl digestion and distillation set	Desiccators
Titration apparatus	Daily routine glassware

Table B.2 List of chemicals used

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

Appendix C

ANOVA results of sensory analysis

Table C.1 ANOVA (no interaction) for appearance of almond milk incorporated yoghurt

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.	l.s.d
Yoghurt type	4	113.5273	28.3818	45.64	<.001	0.680
Panelist	10	28.9455	2.8945	4.65	<.001	1.008
Residual	40	24.8727	0.6218			
Total	54	167.3455				

Table C.2 ANOVA (no interaction) for aroma of almond milk incorporated yoghurt

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.	l.s.d
Yoghurt type	4	61.9273	15.4818	38.53	<.001	0.5463
Panelist	10	23.3818	2.3382	5.82	<.001	0.8103
Residual	40	16.0727	0.4018			
Total	54	101.3818				

Table C.3 ANOVA (no interaction) for taste of almond milk incorporated yoghurt

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.	l.s.d
Yoghurt type	4	108.0727	27.0182	42.34	<.001	0.688
Panelist	10	13.7455	1.3745	2.15	0.042	1.021
Residual	40	25.5273	0.6382			
Total	54	147.3455				

Table C.4 ANOVA (no interaction) for color of almond milk incorporated yoghurt

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.	l.s.d
Yoghurt type	4	60.8364	15.2091	19.27	<.001	0.766
Panelist	10	20.4364	2.0436	2.59	0.016	1.135
Residual	40	31.5636	0.7891			
Total	54	112.8364				

Table C.5 ANOVA (no interaction) for texture of almond milk incorporated yoghurt

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.	l.s.d
Yoghurt type	4	117.2000	29.3000	63.70	<.001	0.5845
Panelist	10	27.7818	2.7782	6.04	<.001	0.8669
Residual	40	18.4000	0.4600			
Total	54	163.3818				

Table C.6 ANOVA (no interaction) for overall acceptability of almond milk incorporated yoghurt

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.	l.s.d
Yoghurt type	4	99.3455	24.8364	89.87	<.001	0.4530
Panelist	10	21.3091	2.1309	7.71	<.001	0.6720
Residual	40	11.0545	0.2764			
Total	54	131.7091				

Appendix D

Table D.1 t-test two-sample assuming unequal variance) for moisture of the best sample (sample A) with control (sample E).

	Sample A (Best)	Sample E (Control)
Mean	81.45333	79.13
Variance	0.143433	0.6057
Observations	3	3
Hypothesized Mean Difference	0	
df	3	
t Stat	4.649354	
P(T<=t) one-tail	0.009382	
t Critical one-tail	2.353363	
P(T<=t) two-tail	0.018763	
t Critical two-tail	3.182446	

Table D.2 t-test two-sample assuming unequal variance) for protein of the best sample (sample A) with control (sample E).

	Sample A (Best)	Sample E (Control)
Mean	3.743333333	2.88
Variance	0.018233333	0.0144
Observations	3	3
Hypothesized Mean Difference	0	
df	4	
t Stat	8.277675899	
P(T<=t) one-tail	0.000581261	
t Critical one-tail	2.131846786	
P(T<=t) two-tail	0.001162523	
t Critical two-tail	2.776445105	

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

	Sample A (Best)	Sample E (Control)
Mean	3.426667	2.933333
Variance	0.004133	0.023333
Observations	3	3
Hypothesized Mean Difference	0	
df	3	
t Stat	5.155824	
P(T<=t) one-tail	0.007074	
t Critical one-tail	2.353363	
P(T<=t) two-tail	0.014147	
t Critical two-tail	3.182446	

Table D.4 t-test two-sample assuming unequal variance) for ash of the best sample (sample A) with control (sample E).

	Sample A (Best)	Sample E (Control)
Mean	0.79	0.836667
Variance	0.0001	0.012033
Observations	3	3
Hypothesized Mean Difference	0	
df	2	
t Stat	-0.7338	
P(T<=t) one-tail	0.269717	
t Critical one-tail	2.919986	
P(T<=t) two-tail	0.539434	
t Critical two-tail	4.302653	

Table D.5 t-test two-sample assuming unequal variance) for acidity of the best sample (sample A) with control (sample E).

	Sample A (Best)	Sample E (Control)
Mean	0.641	0.623333
Variance	0.000103	0.005633
Observations	3	3
Hypothesized Mean Difference	0	
df	2	
t Stat	0.404016	
P(T<=t) one-tail	0.363654	
t Critical one-tail	2.919986	
P(T<=t) two-tail	0.725307	
t Critical two-tail	4.302653	

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

	Sample A (Best)	Sample E (Control)
Mean	4.576667	4.626667
Variance	0.000133	0.004133
Observations	3	3
Hypothesized Mean Difference	0	
df	2	
t Stat	-1.32583	
P(T<=t) one-tail	0.158029	
t Critical one-tail	2.919986	
P(T<=t) two-tail	0.316059	
t Critical two-tail	4.302653	

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

	Sample A (Best)	Sample E (Control)
Mean	2.76	3.843333
Variance	0.0508	0.004433
Observations	3	3
Hypothesized Mean Difference	0	
df	2	
t Stat	-7.98403	
P(T<=t) one-tail	0.007664	
t Critical one-tail	2.919986	
P(T<=t) two-tail	0.015328	
t Critical two-tail	4.302653	

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

	Sample A (Best)	Sample E (Control)
Mean	18.54666667	20.87
Variance	0.143433333	0.6057
Observations	3	3
Hypothesized Mean Difference	0	
df	3	
t Stat	-4.649353736	
P(T<=t) one-tail	0.009381703	
t Critical one-tail	2.353363435	
P(T<=t) two-tail	0.018763406	
t Critical two-tail	3.182446305	

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

	Sample A (Best)	Sample E (Control)
Mean	70.56667	56.70667
Variance	1.703333	4.258133
Observations	3	3
Hypothesized Mean Difference	0	
df	3	
t Stat	9.832123	
P(T<=t) one-tail	0.001118	
t Critical one-tail	2.353363	
P(T<=t) two-tail	0.002237	
t Critical two-tail	3.182446	

Table D.10 t-test two-sample assuming unequal variance) for syneresis of the best sample (sample A) with control (sample E).

	Sample A (Best)	Sample E (Control)
Mean	21.3	20.36667
Variance	0.57	0.643333
Observations	3	3
Hypothesized Mean Difference	0	
df	4	
t Stat	1.467599	
P(T<=t) one-tail	0.10806	
t Critical one-tail	2.131847	
P(T<=t) two-tail	0.216119	
t Critical two-tail	2.776445	

Appendix E

Table E.1 Cost evaluation of 100 ml of 25 % almond milk incorporated yoghurt

Particulars	Quantity (g)	Rate (Rs.)	Amount (Rs.)
Milk	75 (ml)	110/liter	8.25
Almond	6.7	1150/kg	7.7
SMP	4	950/kg	3.8
Sugar	3	90/kg	0.27
Raw material cost			20.02
Overhead cost (20 %)			4.004
Total cost			24.02

Table E.2 Cost evaluation of 100 ml of Control

Particulars	Quantity (g)	Rate (Rs.)	Amount (Rs.)
Milk	100 (ml)	110/liter	11
SMP	4	950/kg	3.8
Sugar	3	90/kg	0.27
Raw material cost			15.07
Overhead cost (20 %)			3.014
Total cost			18.08

Color Plates

P1: Grinding almond

P2: Incubation

P3: Sensory evaluation

P4: Kjeldahl method of protein determination