PREPARATION AND QUALITY EVALUATION OF COCONUT MILK INCORPORATED YOGHURT

by

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Preparation and Quality Evaluation of Coconut Milk Incorporated Yoghurt

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

by

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Approval Letter

This dissertation entitled Preparation and Quality Evaluation of Coconut Milk Incorporated Yoghurt presented by Bibek Adhikari has been accepted as the partial fulfillment of the requirements for the B. Tech. in Food Technology

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Bibek Adhikari
Abstract

The current research work was conducted to measure the quality and feasibility of yogurt prepared by using cow milk and different levels of coconut milk with 2% starter culture. The aim of this research is to study the effect of addition of coconut milk in yoghurt. Design expert® version 10 D-optimal design was employed for the formulating the recipe of yoghurt. The obtained five formulations coded A (10%), B (20%), C (30%), D (40%) & E (50%) of coconut milk incorporated yoghurt were prepared in laboratory. The samples were subjected to sensory evaluation by quality scoring method for consumer acceptability. Score was given by individual panelist on the basis of color, taste, aroma and texture. Based on these quality parameter and sensory analysis score, the data were analyzed by two ways ANOVA (no blocking) using Genstat and means were compared using LSD at 5% level of significance.

From sensory evaluation, formulation A (10%) coconut milk incorporated yoghurt was found to be significantly (p<0.05) best using LSD at 5% level of significance quality whose total solid, fat, acidity, protein, carbohydrate, total ash, moisture content, lactose content and pH were found 20%, 5.1%, 0.71%, 3.5%, 5.06%, 0.94%, 82%, 3.8% and 4.4 respectively. Shelf life of the best product was estimated in terms of acidity and total plate count and the shelf life was found to be 2 days at room temperature and 7 days at refrigeration.
Contents

Approval Letter ........................................................................................................ iii

Acknowledgements .................................................................................................... iv

Abstract ...................................................................................................................... v

List of tables ............................................................................................................... xii

List of figures ............................................................................................................. xiii

List of abbreviations ................................................................................................. xiv

1. Introduction .......................................................................................................... 1-4

  1.1 General introduction ......................................................................................... 1

  1.2 Statement of the problem ................................................................................ 2

  1.3 Objectives ......................................................................................................... 3

    1.3.1 General objectives ..................................................................................... 3

    1.3.2 Specific objectives .................................................................................... 3

  1.4 Significance of the study .................................................................................. 3

  1.5 Limitation of the study ..................................................................................... 4

2. Literature review .................................................................................................. 5-27

  2.1 Historical background ..................................................................................... 5

  2.2 Development of dairy industry in Nepal ......................................................... 7

  2.3 Milk .................................................................................................................. 7

  2.4 Milk fermentation ............................................................................................. 8

  2.5 Types of fermented milk ................................................................................ 9

    2.5.1 Traditional fermented milk ....................................................................... 9

    2.5.2 Non-traditional fermented milk ............................................................... 9

  2.6 Advantages of milk fermentation ..................................................................... 9
2.7  The fundamental microbiology of yoghurt .................................................... 10

2.8  Basic stages and CCPs of yoghurt manufacturing ........................................ 11

2.9  Starter culture .................................................................................................. 12

2.10  Types of starter culture .................................................................................... 13

2.10.1 Liquid and freeze-dried culture ................................................................. 13

2.10.2  Pure and mixed culture .............................................................................. 13

2.10.3 Mesophilic and thermophilic culture ......................................................... 13

2.10.4 Physical forms of starter culture ................................................................. 14

2.10.5 Liquid cultures ............................................................................................ 14

2.10.6 Powdered cultures ..................................................................................... 14

2.10.7 Frozen culture ............................................................................................ 14

2.11  Selection of cultures ....................................................................................... 15

2.12  Preparation of starter culture ......................................................................... 15

2.13  Bio-technically important metabolic activities .............................................. 15

2.13.1 Acid production by microorganisms .......................................................... 15

2.13.2 Flavor components in yoghurt ..................................................................... 16

2.13.3 Ropiness and consistency .......................................................................... 16

2.13.4 Proteolytic activity ..................................................................................... 16

2.13.5 Lipolytic activity ....................................................................................... 17

2.13.6 Other metabolic activities .......................................................................... 17

2.14  Role of thermophilic cultures in the intestine ............................................... 17

2.14.1 Production of antibiotic substances ............................................................ 18

2.14.2 Effect on immunoglobulin level, cholesterol, lactose activity and tumor cells .............................................................. 18
2.14.3 Formation of bacteriocins and other inhibitory compounds

2.15 Causes of slow growth

2.15.1 Bacteriophages

2.15.2 Effect of chemical composition of the lactic acid flora

2.16 Coagulum formation in yoghurt

2.17 Syneresis

2.18 Methods for improving the body (viscosity) of yoghurt

2.19 Types of yoghurt

2.19.1 Set type yoghurt

2.19.2 Stirred type yoghurt

2.19.3 Drinking type of yoghurt

2.19.4 Frozen yoghurt

2.19.5 Dried yogurt

2.19.6 Dietetic/therapeutic yoghurt

2.20 Addition of fruit flavoring in yoghurt

2.21 Shelf-life of yoghurt

2.22 Techniques of shelf-life extension

2.23 Coconut

2.23.1 Description and origin

2.23.3 Coconut fat

2.23.4 Coconut meat

2.23.5 Coconut milk

2.23.6 Nutritional and medicinal importance of coconut milk
3. Material and methods ........................................................................................................29-36

3.1 Materials .......................................................................................................................... 29

3.1.1 Milk ................................................................................................................................. 29

3.1.2 Coconut ............................................................................................................................ 29

3.1.3 Milk solid not fat ............................................................................................................. 29

3.1.4 Sweetener ....................................................................................................................... 29

3.1.5 Starter Culture ................................................................................................................. 29

3.1.6 Containers ....................................................................................................................... 29

3.1.7 Equipment and chemicals ............................................................................................. 30

3.2 Method ............................................................................................................................... 30

3.2.1 Preparation of coconut milk ......................................................................................... 30

3.2.2 Preparation of set type coconut milk yoghurt ............................................................... 31

3.2.3 Chemical analysis of coconut milk ............................................................................... 32

3.2.3.1 Acidity ......................................................................................................................... 32

3.2.3.2 Fat .............................................................................................................................. 32

3.2.3.3 Protein ......................................................................................................................... 32

3.2.3.4 Ash ............................................................................................................................. 33

3.2.3.5 pH ............................................................................................................................. 33

3.2.3.6 Total Solid (TS) ......................................................................................................... 33

3.2.3.7 Moisture .................................................................................................................... 33

3.2.3.8 Vitamin C ................................................................................................................... 33

3.2.3.9 Total sugar ................................................................................................................ 33
3.2.4 Chemical analysis of Milk

3.2.4.1 Acidity

3.2.4.2 Fat

3.2.4.3 Protein

3.2.4.4 Ash

3.2.4.5 pH

3.2.4.6 Total Soluble Solid (TSS)

3.2.4.7 Lactose

3.2.5 Design expert

3.2.6 Analysis of yoghurt

3.2.6.1 Sensory evaluation

3.2.6.2 Physical analysis

3.2.6.3 Chemical analysis

3.2.6.4 Microbiological examination

3.2.6.5 Data analysis

4. Results and discussions

4.1 Chemical composition of milk

4.2 Chemical composition of coconut milk

4.3 Sensory evaluation of coconut milk yoghurt

4.3.1 Aroma

4.3.2 Color

4.3.3 Taste

4.3.4 Texture
4.2.5 Overall acceptability.................................................................................. 43
4.4 Chemical analysis of best product .................................................................. 44
4.5 Physical analysis .............................................................................................. 46
4.6 Shelf-life of the product .................................................................................. 46
  4.6.1 Acidity of yoghurt at room temperature .................................................. 47
  4.6.2 Total plate count of yoghurt at room temperature .................................... 47
  4.6.3 Acidity of yoghurt under refrigeration ..................................................... 48
  4.6.4 Total plate count of yoghurt under refrigeration ....................................... 49
4.7 Cost evaluation ............................................................................................... 50

5. Conclusion and recommendations ................................................................... 51
  5.1 Conclusions .................................................................................................. 51
  5.2 Recommendations ....................................................................................... 51

6. Summary .......................................................................................................... 52

References .......................................................................................................... 53-60

Appendix ............................................................................................................. 61-68

Color plates ......................................................................................................... 69
List of Tables

<table>
<thead>
<tr>
<th>Table No.</th>
<th>Title</th>
<th>Page No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.1</td>
<td>Countries where coconut is abundant</td>
<td>24</td>
</tr>
<tr>
<td>2.2</td>
<td>Coconut varieties and characteristics</td>
<td>25</td>
</tr>
<tr>
<td>2.3</td>
<td>Composition of coconut milk</td>
<td>27</td>
</tr>
<tr>
<td>3.1</td>
<td>List of equipments used</td>
<td>30</td>
</tr>
<tr>
<td>3.2</td>
<td>List of chemicals used</td>
<td>30</td>
</tr>
<tr>
<td>3.3</td>
<td>Sample formulation in coded form</td>
<td>34</td>
</tr>
<tr>
<td>4.1</td>
<td>Chemical composition of milk</td>
<td>37</td>
</tr>
<tr>
<td>4.2</td>
<td>Chemical composition of coconut milk</td>
<td>38</td>
</tr>
<tr>
<td>4.3</td>
<td>Chemical analysis of best product</td>
<td>45</td>
</tr>
<tr>
<td>4.4</td>
<td>Syneresis of product</td>
<td>46</td>
</tr>
</tbody>
</table>
## List of Figures

<table>
<thead>
<tr>
<th>S.N.</th>
<th>Title</th>
<th>Page No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.1</td>
<td>Preparation of coconut milk</td>
<td>31</td>
</tr>
<tr>
<td>3.2</td>
<td>Preparation of coconut milk incorporated yoghurt</td>
<td>32</td>
</tr>
<tr>
<td>4.1</td>
<td>Mean sensory score for aroma of coconut milk incorporated yoghurt</td>
<td>39</td>
</tr>
<tr>
<td>4.2</td>
<td>Mean sensory score for color of coconut milk incorporated yoghurt</td>
<td>40</td>
</tr>
<tr>
<td>4.3</td>
<td>Mean sensory score for taste of coconut milk incorporated yoghurt</td>
<td>41</td>
</tr>
<tr>
<td>4.4</td>
<td>Mean sensory score for texture of coconut milk incorporated yoghurt</td>
<td>42</td>
</tr>
<tr>
<td>4.5</td>
<td>Mean sensory score for overall acceptability of coconut milk incorporated yoghurt</td>
<td>43</td>
</tr>
<tr>
<td>4.6</td>
<td>Change in acidity under room temperature with respect to the number of days</td>
<td>47</td>
</tr>
<tr>
<td>4.7</td>
<td>Change in TPC under room temperature with respect to the number of days</td>
<td>48</td>
</tr>
<tr>
<td>4.8</td>
<td>Change in acidity under refrigeration with respect to the number of days</td>
<td>49</td>
</tr>
<tr>
<td>4.9</td>
<td>Change in TPC under refrigeration with respect to the number of days</td>
<td>50</td>
</tr>
</tbody>
</table>
# List of Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Full form</th>
</tr>
</thead>
<tbody>
<tr>
<td>ANOVA</td>
<td>Analysis of variance</td>
</tr>
<tr>
<td>CCP</td>
<td>Critical control point</td>
</tr>
<tr>
<td>LCFA</td>
<td>Long chain fatty acid</td>
</tr>
<tr>
<td>LSD</td>
<td>Least significant difference</td>
</tr>
<tr>
<td>MCT</td>
<td>Medium chain triglycerides</td>
</tr>
<tr>
<td>MSNF</td>
<td>Milk solid not fat</td>
</tr>
<tr>
<td>NFDM</td>
<td>Non-fat dry milk</td>
</tr>
<tr>
<td>SCFA</td>
<td>Short chain fatty acids</td>
</tr>
<tr>
<td>SMP</td>
<td>Skim milk powder</td>
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<tr>
<td>UTH</td>
<td>Ultra high temperature</td>
</tr>
</tbody>
</table>
Part I

Introduction

1.1 General introduction

The role of fermented milk in human nutrition is well documented and the virtues of these products were known to man even during the ancient days of civilization. These products have long been an important component of nutritional diet. A fermented milk product has been defined by the International Dairy Federation as the milk product prepared from skimmed milk or not with specific cultures (Gandhi, 2000). Microorganisms employed as starters for production of cultured dairy foods are divided into two types, based on the optimum temperature ranges at which they operate. The lactic acid bacteria incubated at temperatures above 35°C are referred to as thermophilic bacteria and those incubated at 20–30°C are called mesophilic starters. Yogurt is derived by culturing with thermophilic cultures, which act in symbiosis with each other (Chandan and Kilara, 2013).

Yoghurt is a fermented product obtained through an anaerobic fermentation of lactose in milk by relevant microorganisms most of which are classified as pro-biotic (Tull, 1996). Yoghurt is a cultured dairy product produced by fermenting milk, with or without added non-fat dry milk (NFDM) with *Lactobacillus bulgaricus* and *Streptococcus thermophilus* bacteria. It usually contains 12-14% total milk solids and has soft, friable custard like consistency, and a clear and distinct acid flavor. Yoghurt is usually produced by heating the mix to 80-85°C for 30 min to pasteurize it and to modify the milk proteins so that they will provide the proper viscosity and gelation with a minimum of syneresis in the product (Morr, 1985).

Coconut (*Cocos nucifera* L.) is one of the oldest fruits in the world and is confined to seacoast in the humid tropics. It has been estimated that 25% of the world’s output of coconut is consumed as coconut milk (Gwee, 1988). Coconut milk is being used by confectionaries, bakeries, biscuits and ice cream Industries worldwide to enhance flavor and taste of various products (Persley, 1992). Coconut milk was found to be rich in calcium. The milk was reported to be high in minerals and vitamin content (Nieuwentus and Nieuwelink, 2002).
Contrary to widely held opinion, the coconut provides nutritious sources of meat, juice, milk and oil. It is classified as a “functional food” because it provides many health benefits beyond its nutritional content, due to its fiber and oil content (Sanful, 2009). The oil is known to contribute to improved insulin secretion and the utilization of blood glucose; reduce symptoms associated with malabsorption syndrome and cystic fibrosis; help to relieve symptoms associated with crohn’s disease; ulcerative colitis and stomach ulcers; improve the utilization of essential fatty acids and protect them from oxidation (Seow and Gwee, 1997). Current trends and changing consumer needs indicate a great opportunity for innovations and developments in fermented milks (Khurana and Kanawjia, 2007). There is little information about fiber fortification in cultured dairy products however various fibers like psyllium, guar gum, gum acacia, oat fiber, and soy components have potentials to be used (Staffolo et al., 2004).

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

1.2 Statement of the problem

Yoghurt, as a fermented diary product is regarded as a probiotic carrier, is nutritionally rich in available protein, calcium, milk fat, potassium, magnesium. It has nutritional benefits beyond those of milk, because people who are moderately lactose intolerant can enjoy yoghurt without ill effects, as most of the lactose in the milk precursor has been converted to lactic acid by the bacterial culture. Yoghurt also has medical uses because of the probiotic characteristics, in helping out on a variety of gastro intestinal conditions and in preventing antibiotic associated diarrhea (Lourens-Hattingh and Viljoen, 2001).

The addition of fruits and flavors to yoghurt has become very popular in recent years. In context of Nepalese market availability of fruits and flavor yoghurt is rare (Gupta, 2003). Hence, to give a variety of product and also to improve the commercial value of yoghurt, addition of coconut milk can be beneficial to some extent. Yoghurt obtained by using coconut milk has been found to be delicious and a nutritional product (Imele and Atemnkeng, 2001).
Therefore, coconut milk incorporated yoghurt may be a better option to increase its utilization along with the improvement of yoghurt quality.

1.3 Objectives

1.3.1 General objectives

The general objective is to prepare yoghurt by the addition of coconut milk in different proportion.

1.3.2 Specific objectives

To fulfill the general objectives the following specific objectives will be done

i. To study the effect of different levels of coconut milk on the prepared yoghurt and to evaluate its sensory properties.

ii. To analyze the coconut milk yoghurt for its proximate composition.

iii. To study the shelf life of the yoghurt.

1.4 Significance of the study

Yoghurt is the lactic-acid fermented product and has a distinct acidic, sharp flavor. Yoghurt was first produced to preserve the milk. Yoghurt possesses the long shelf life than milk. Different yoghurt based products are being available in the world’s market such as drinking yoghurt, dietetic yoghurt, *shrikhand* etc. With an increase in population in the world the consumption of yoghurt is being increased so the production of yoghurt should also be increased. Considering on the standards of hygiene observed during the manufacture of yoghurt, and the microbiological quality of the ingredients and packaging materials, the shelf life of the yoghurt is around three weeks under refrigerated condition (Tamime and Robinson, 1985).

For many years only natural or plain yogurt was made from whole milk available in the market, but there has been a good demand for fruit yogurt. The scientific name of coconut is *Cocos nucifera*. This coconut tree is called “The Tree of paradise” due to its various uses. On economic point of view, coconut is very important among various fruits. It is one of the oldest fruits cultivated by man from prehistoric times of the world. Coconut is a highly nutrient containing fruit. It is not seasonal in nature like many other fruit crops and in available in large quantity throughout the year. Our children, youth, pregnant women
and poor people are suffering from malnutrition, night blindness, anemia and various kinds of other diseases. In such cases low cost processed supplementary food based on fruits need to be developed (Biswas, 2013). This experiment is very important in Nepal because coconut is available here and price is relatively cheap. Thus incorporating coconut in yogurt could be useful to develop nutritious fermented product.

1.5 Limitation of the study

The limitations of the work are listed below:

1. Best yoghurt could not be compared with commercial yoghurt.
2. Variation of sugar and skim milk could not be carried out.
3. Shelf life of yoghurt could not be compared to control.
Part II

Literature review

2.1 Historical background

Milk fermentation is one of the oldest methods practiced by the human beings to preserve milk with an extended shelf life. The exact origination of milk fermentation is not clear; however, it seems that it is dated back to the dawn of the civilization. It has been reported that the early civilizations such as the Samarians, Babylonians, Pharoes and Indians were well advanced in agricultural and animal husbandry practices (Tamime and Robinson, 1999). This can be supported by the findings of Copley et al., 2003 in which the dairy fat residues were found in pottery fragments from Neolitic Bronze-age and Iron-age settlements, which suggests that the practice of dairying had existed in Britain approximately 6500 years ago (Copley et al., 2003). However, it is questionable that the milk fermentation was practiced during this period. Therefore, the origination of the fermented milk products including yogurt remains unsolved. It has been reported that the Anatolian goatherds conserve their milk by thickening as they used to dry it in the sun and transport in animal stomachs (Anon., 2013a).

The origin of yogurt is dated back to the 6000 B.C. when the Neolitic people in the Central Asia transformed from a status of a food gatherer to a food producer where they began the practice of milking their animals. It is generally accepted that the fermented milk products including yogurt have been discovered accidentally when they used to store milk in sheep-skin bags and has been evolved over centuries into commercial yogurt making which paved the pavement for different commercially available varieties with a range of flavors, forms and textures (Anon., 2013c). With reference to yogurt, it can be suggested that it has been evolved in Turkey as the term “yoghurt” has been derived from a Turkish verb, “jugurt” that means “to be curdled or coagulated” (Mahmood and Gilani, 2008). The earliest writings about yogurt can be found from those of Pliny who lived in the first century A. D. and wrote about ancient barbarous nations that knew how to thicken the milk into a substance with an agreeable acidity. According to the literature, the founder of the Mongol empire, Genghis Khan and his armies was lived on yogurt and spreading of this news among the people had made the yogurt consumption to spread throughout the East (Anon., 2013c). Moreover, according to the Persian tradition, Abraham owed his fecundity
and longevity to the regular ingestion of yogurt, and the emperor Francis I of France was said to be cured of severe diarrhea by consuming yogurt made of goat milk leading to introduce the health benefits of yogurt into the western world in 1542 (Tamime and Robinson, 1999).

The origin of fermented dairy product dates back to the dawn of civilization. The ancient Sanskrit scriptures of India, the Vedas, document the food value of Dahi, a fermented milk product similar to modern yoghurt. The Bible corroborates further evidence for the existence of soured milk as a food in early time (Chandan, 1981). The first industrialized production of yogurt was taken place in 1919, in Barcelona, Spain at a company named Danone (Anon., 2013c).

Yogurt was firstly introduced to the USA in the early 20th century in the form of tablets especially designed for those with digestive intolerance. However, it became popular in the North America when Dannon, a small-scale yogurt factory started manufacture of yogurt in New York in 1940. Even though, yogurt has been evolved for centuries, it was subjected to a significant and dynamic evolution process in the 20th Century to originate a vast array of products. For instance, fruit yogurts, yogurts with fruit on bottom and blended yogurts were introduced in 1937, 1947 and 1963 respectively (Anon., 2013a).

Yoghurt is one of the oldest known cultured milk products, with its origin in the Middle East. It would have been made by Nomadic tribesmen, initially from the milk of goats and sheep (Schmidt, 1992). Although yoghurt has many desirable properties, it is still prone to deterioration, especially at ambient temperature, within a matter of days and one trend in Middle East has been the search for simple techniques to extend keeping quality even further. The first step was the preparation of condensed or concentrated yoghurt by hanging the yoghurt in animal skins. The product had a total solid in the range of 25% and acidity of greater than 2% as lactic acid. Nevertheless, even condensed yoghurt becomes unpalatable within a week or two, and salted yoghurt was prepared which became rapidly popular. An alternative preservation process involved the heating of yoghurt for a few hours over low fires of special type of wood; the end product is referred to as “smoked yoghurt” which was preserved over the winter months. However, as refrigeration become widespread, so interest in these traditional products declined and production of new generation of yoghurt emerged. Initially, production was confined to natural yoghurt, but
gradually production of fruit yoghurt gave the product an entirely fresh image (Tamime and Robinson, 1985).

2.2 Development of dairy industry in Nepal

Traditionally dairy production was in a back-yard type enterprise in Nepal. Milk and milk products are primarily consumed in home. The surplus milk is converted into ghee and sold in urban areas. This is still followed in most part of the country where market for fresh milk doesn’t exist. This situation is however different in the vicinity in urban areas where farmers primarily sell the fresh milk instead of going for ghee production, since it reduces the profit margin (Dahal, 2009). In Nepal, the history of dairy development is not so long. At first, it was started in Tushal (Kabhre) at 2009 B.S. In 1956, the modern milk processing plant was established with the financial assistance of New Zealand government at Lainchaur, Kathmandu with a capacity of 500 liters. For the implementation of effective dairy development program, DDC (Dairy Development Corporation) was originated in 1969 under the act of Agriculture development, 2001. Similarly in 1974, modern milk processing plant of 2000 liter capacity was established in the eastern region of Nepal, Biratnagar. In 1974, 3000 liter capacity was established in Hetauda. Further in 1977 another plant of 5000 liter capacity was established in Balaju, Kathmandu. In addition Pokhara milk supply scheme, Lumbini milk supply scheme and Kohalpur and Surkhet milk supply scheme was also established (Dahal, 2009). Kathmandu dairy development scheme is also called central dairy because milk is supplied in the dairy from all dairies of Nepal. Beside that, the National Dairy Development Board (NDDB) has been formed as an additional step in the development of dairy in Nepal. This body is responsible for formulating policies, planning and development of dairy profession by being a coordinator between the private and public sectors (Gupta, 2003).

2.3 Milk

Milk is a lacteal secretion of mammary gland of milch animals. It is composed of lipids, carbohydrates, proteins and other many organic compounds and inorganic salts dissolved or dispersed in water. Lipid is composed primarily of fat although there are small amount of phospholipids, sterols, fat soluble vitamins A and D, carotene and xanthophylls. Protein content of milk is classified as a) casein, b) lactalbumin and c) lactoglobulin. Lactose is the carbohydrate in the milk (Meyer, 1960).
Different salt and minerals are found in the milk. Plentiful vitamins are present but vitamin C is limiting. Milk contains a number of enzymes; some of them apparently secreted in the milk and other are formed by microorganism (Meyer, 1960).

2.4 Milk fermentation

A fermented milk product has been defined by the International Dairy Federation as the milk product prepared from skimmed milk or not with specific cultures. The microflora is kept alive until sale to the consumers and may not contain any pathogenic germs (Gandhi, 2000). Milk fermentation can be defined as any modification of the chemical or physical properties of milk or dairy products resulting from the activity of microorganisms or their enzymes. It occurs when bacteria break down milk sugars and other components of milk to give lactic acid, alcohols, carbon dioxide etc. Lactose, fat and citric acid comprise the important fermentable compounds of milk. Lactose a disaccharide, is the chief source of carbon while fat and citric acid provides hydrogen and oxygen source respectively (Davies and Barry, 1984). Fermentation in milk contributes to desirable flavor and texture in product such as cheese and yoghurt or result in spoiled and degraded products. To ensure development of desired fermentation, microbial cultures with known properties are added to milk or dairy product substrate (Yulina, 2010).

Over the period, scientists have tried to isolate and study the characters of such desirable organisms. Among the bacteria, the most important dominant group bringing fermentation is lactic acid bacteria. The lactic acid bacteria are naturally accepted as GRAS (Generally regarded as safe) for human consumption (Aguirre and Collins, 1993). Milk fermentation process has been relied on the activity of lactic acid bacteria, which play a crucial role in converting milk as raw material to fermented milk products. In milk fermentation industry, various industrial strains of LAB are used as starter cultures. Starter cultures of lactic acid bacteria were obtained from sequence activities and passed a process of isolation, selection and confirmation. Several behaviors as the characteristics of each individual selected strains of lactic acid bacteria has been established and used in the production of fermented milk products industrially. The most important properties of LAB are their ability to acidify milk (Mäyrä-Mäkinen and Bigret, 2004) and to generate flavour and texture, by converting milk protein due to their proteolytic activities (Frank and Marth, 1998).
Milk products also serve as the important delivery vehicles for probiotic bacteria. The probiotic bacteria have a long history of association with dairy products. This is because some of the same bacteria that are associated with fermented dairy products also make their homes in different sites on the human body, including the mouth, the gastrointestinal tract etc. Some of these microbes, therefore, can play a dual role in transforming milk into a diverse array of fermented dairy products (yoghurt, cheese, kefir, etc.) and contributing to the important role of colonizing bacteria. Within this sector, probiotic cultures have been incorporated in yoghurts and fermented milk products. The probiotic bacteria used in commercial products today are mainly members of the genera *Lactobacillus* and *Bifidobacterium* (Eshraga *et al.*, 2011).

2.5 Types of fermented milk

2.5.1 Traditional fermented milk

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

2.5.2 Non-traditional fermented milk

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

2.6 Advantages of milk fermentation

The most important advantages of fermented foods are:

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

2.7 The fundamental microbiology of yoghurt

The two organisms are mutually beneficial; *S. thermophilus* by removing oxygen and producing weak acid conditions favoring *L. bulgaricus* and the lactobacillus by hydrolyzing the lactose and the casein. *S. thermophilus* grow best at pH 6.5, growth stopping at pH 4.2-4.4 and the *L. bulgaricus* best at pH 5.5, growth stopping at pH 3.5-3.8 (Rasic and Kurmann, 1978).

The amount of lactic acid production by *L. bulgaricus* is 1.7-1.8% while *S. thermophilus* produces 0.6-0.8%. Lactic acid is very important in yoghurt or yoghurt like fermented milks (Tamime and Robinson, 1985). The lactic acid contributed to the colloidal calcium phosphate complex present in the casein micelle to the soluble calcium phosphate fraction. This leads to gradual loss of calcium from the micelles and the consequent coagulation of casein at pH 4.6-4.7. The lactic produced by the thermophilic cultures gives yoghurt its sharp and acidic taste thus enhancing the flavour of the product. As reported by many workers, *S. thermophilus* produces essentially L (+) lactic acid while *L. bulgaricus* produces mainly D (-) lactic acid. As a consequence the yoghurt usually contains 45-60% L (+) lactic acid and 40-55% D (-) lactic acid (Garvie, 1978).
Yoghurt starter cultures are slightly proteolytic and the peptides and amino acids produced act as precursors for the enzymic and chemical reaction, which produce flavor compounds. Protein degradation is associated mainly with *L. bulgaricus* but peptidase enzymes are produced by *S. thermophilus* and *L. bulgaricus* (Heller, 2001).

### 2.8 Basic stages and CCPs of yoghurt manufacturing

The basic stages of production of yoghurt are:

- Preparation of standard milk with around 12-14% milk solids not fat (MSNF).
- Heating this milk to 85-95°C, preferably in a unit that allows the temperature to be held for some 10-30 min.
- Inoculating the milk with a culture mixture of containing *Lactobacillus bulgaricus* and *Streptococcus thermophilus* as principle organisms.
- Incubating the inoculated milk at 42°C until a smooth coagulum has formed, together with the desired level of acidity and flavor.
- Cooling the finished product and, unless the milk has been incubated in retail cartons (set yoghurt), mixing with fruit or other ingredients (Tamime and Robinson, 1985).

The following critical control points (CCPs) must be followed to produce yoghurt efficiently:

- Milk for yoghurt manufacturing must be free from penicillin, and is checked for penicillin on receipt at the site.
- Milk must be heated to 90-95°C for 15 min before inoculation. It serves the following functions:
  - It destroys pathogens, which is necessary to produce lactic acid at optimum rate.
  - It generates the condition for growth for starter by breakdown of milk protein.
It creates desirable body and texture.

- If the mix is homogenized, it prevents creaming during incubation and storage of cultured dairy products by changing colloidal characteristic of milk. The stabilizer and other component of mix are thoroughly dispersed for optimum textural effect.

- The mix must be cooled to 43°C before starter inoculation.

- The yoghurt starter culture must have the *L. bulgaricus* and *S. thermophilus* in correct proportion (1:1). It must be free from contaminating microorganisms and initiate acid production within thirty minutes of inoculation.

- The inoculation must be done at correct temperature for rapid acid production. The rate of acid production is monitored during 3.5-4 h of incubation (Chandan, 1981).

### 2.9 Starter culture

A starter culture is a product with a high concentration of lactic acid bacteria, which can activate an acidification process in milk. Starter cultures are normally manufactured in special starter culture laboratories but may also be cultured and propagated in dairy (Gandhi, 2006).

The starter culture for most yoghurt production 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 used together than either of the two organisms grown individually. *S. thermophilus* grows faster and produces both acid and carbon dioxide. The formate and carbon dioxide produced stimulates *L. bulgaricus* growth. On the other hand, the proteolytic activity of *L. bulgaricus* produces stimulatory peptides and amino acids for use by *S. thermophilus* (Desai et al., 1994). These microorganisms are ultimately responsible for the formation of typical yoghurt flavor and texture. The yoghurt mixture coagulates during fermentation due to the drop in pH. The streptococci are responsible for the initial pH drop of the yoghurt mix to approximately 5.0. The lactobacilli are responsible for a further decrease to pH 4.0. Lactic acid, acetaldehyde, acetic acid and diacetyl are the fermentation products that contribute to flavor. Modern industrial processes utilize “starters” in the manufacture of modern dairy products. A starter consists of harmless microorganism, which on culturing in milk imparts
desirable and predictable characteristics of flavor and texture. A single strain culture contains an individual strain of bacterial species while a mixed/multi strain culture consists of the mixture of more than one strain or species. Starter cultures are distributed from central laboratory to an operating plant in different forms e.g. liquid culture, frozen concentrate, lyophilized culture etc (Chandan, 1981).

2.10 Types of starter culture

2.10.1 Liquid and freeze-dried culture

The liquid cultures are generally no longer distributed in commercial practice. To prepare a liquid culture the organisms are propagated in a suitable medium such as milk or whey and maintained an active condition by periodic transfers. In general, a liquid culture contains about 10^9 organisms per ml of starter. Freeze-dried cultures are produced by freeze-drying the cultures grown in milk and are preserved by lyophilization in small vials. It can be stored at room temperature for several years but the degree of viability of organism is very low. So reactivation of lyophilized culture is necessary for proper performance (Chandan, 1981).

2.10.2 Pure and mixed culture

A further sub-division is made into either pure cultures or mixed cultures. Pure culture consists of only one species of lactic acid bacteria, whereas mixed cultures consist of several species of lactic acid bacteria. Pure cultures may consist of one or more strains of the same species. Mixed cultures are the most common type in acidification with a mixed culture and on rare occasions on its own. DL cultures used to be cultivated as “dairy cultures” at individual dairies, often the same culture for decades (Kurmann, 1983).

2.10.3 Mesophilic and thermophilic culture

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

1. Acid producing activity

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

Thermophilic cultures have optimum temperature for growth between 37 to 45°C. Thermophilic cultures are generally employed in the production of yoghurt, acidophilus milk, swiss type cheese. Thermophilic cultures include species of *Streptococcus* and *Lactobacillus*. These cultures grow in association with milk and form the typical yoghurt starter culture. This growth is considered symbiotic because the rate of acid development is greater when two bacteria are grown together as compared to single strains (Gandhi, 2006).

### 2.10.4 Physical forms of starter culture

Starter cultures can be manufactured and distributed in three different physical forms: liquid, powder and frozen form (Neilson and Ullum, 1989).

#### 2.10.5 Liquid cultures

The liquid cultures are generally no longer distributed in commercial practice. To prepare a liquid culture the organisms are propagated in a suitable medium such as milk or whey and maintained in an active condition by periodic transfers. In general, a liquid culture contains about 10^9 organisms per ml of starter (Chandan, 1981). The milk used has high total solids (TS) content. Various growth promoting substances may be added (Neilson and Ullum, 1989).

#### 2.10.6 Powdered cultures

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

#### 2.10.7 Frozen culture

Deep frozen cultures are prepared by deep freezing a concentrated, liquid culture at the point of the bacteria growth at which the activity is at its highest. They are preserved by lyophilization in small vials. Super-concentrated, deep frozen cultures are made by adding growth-promoting substances to a milk substrate, continuously neutralizing the lactic acid
formed by means of ammonium hydroxide, and finally concentrating the culture in a desludging centrifuge/ bactofuge. The concentrate is pelletized by being frozen as individual drops in liquid nitrogen. The culture is stored at -196°C until it is dispatched to the dairies in foamed plastic boxes containing dry ice (Neilson and Ullum, 1989). The culture concentrate contains approximate 1011 microorganisms per ml (Chandan, 1981).

2.11 Selection of cultures

The best method is to adopt the procedure used by cheese makers, namely to collect cultures from various sources and access their suitability for particular purpose. Yoghurts are judged on flavour, acidity, body, texture and homogeneity (Kurmann, 1983). It does not follow that the quickest growing are the best for yoghurt. Too rapid growth may lead to instability of coagulum and graininess. A slower culture is easier to control for final acidity and may produce a better flavour (Vanderpoorten and Waes, 1972).

2.12 Preparation of starter culture

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

2.13 Bio-technically important metabolic activities

2.13.1 Acid production by microorganisms

The main role of *S. thermophilus* and *L. bulgaricus* in yogurt manufacture is to acidify milk by producing a large amount of lactic acid from lactose. Lactic acid reduces the pH of the milk and leads to a progressive solubilization of micellar calcium phosphate. This causes the demineralization of casein micelles and their destabilization, which generates the complete precipitation of casein in a pH range of 4.6-4.7 (Fox, 1989). Amount of lactic acid production by *L. bulgaricus* is 1.7-1.8% while *S. thermophilus* is 0.6-0.8%. Acid contributes to the formation of gel by conversion of colloidal calcium phosphate complex present in the casein micelle to soluble calcium phosphate fraction. This leads to loss of
calcium from micelles and coagulation of casein at pH 4.6-4.7. *S. thermophilus* produces L (+) lactic acid while *L. bulgaricus* produces mainly D (-) lactic acid (Garvie, 1978).

### 2.13.2 Flavor components in yoghurt

The typical flavour of yogurt is due to lactic acid and various carbonyl compounds, ie acetaldehyde, acetone and diacetyl, produced by *S. thermophilus* and *L. bulgaricus*. In addition to carbonyl substances, many volatile compounds have also been identified in yogurt, ie volatile fatty acids (Turcic *et al.*, 1969) and several compounds derived from the thermal degradation of lipids, lactose and proteins during the heat treatment of milk before yogurt manufacture eg aldehydes, ketones, alcohols, lactones, sulfur compounds (Tamime and Deeth, 1980).

Both organisms convert nearly all the sugar to lactic acid, producing only trace amounts of byproducts. These are very important for the characteristic yoghurt flavour, *S. thermophilus* by producing diacetyl and *L. bulgaricus* by producing acetaldehyde (Schulz and Hingst, 1954).

### 2.13.3 Ropiness and consistency

Several Gram-negative and Gram-positive bacteria, including lactic acid bacteria, produce exocellular polysaccharides (Macura and Townsley, 1984). Mucous substances are produced by some strains of *S. thermophilus* and *L. bulgaricus* which can have different chemical composition. The thickening ability of the polysaccharides produced by both organisms is different (Galesloot *et al.*, 1968). The quantities of polymer formed by ropy strains of both species vary considerably even under identical experimental conditions (Cerning *et al.*, 1990). It is difficult to establish a good correlation between the quantity of polysaccharide produced and the corresponding viscosity. This difficulty may be due to changes in the 3-dimensional configuration of polymers and to their interactions with some milk compounds, mainly caseins that are precipitated at low pH (Olsen, 1989).

### 2.13.4 Proteolytic activity

Proteolytic activity is greatly involved in both nutrition and interactions of yogurt bacteria, since lactic acid bacteria cannot synthesize essential amino acids. Therefore, they require an exogenous nitrogen source and utilize peptides and proteins in their growth medium by more or less complete enzyme systems. *S. thermophilus* primarily requires glutamic acid,
histidine and methionine, as well as cystine, valine, leucine, isoleucine, tryptophan, arginine and tyrosine for growth (Shankar and Davies, 1977). The uptake of branchedchain amine acids has been studied. It is an active transport which requires an exogenous energy source, depends on temperature and pH and is inhibited by L-cysteine (Akpemado and Bracquart, 1983).

Whey protein hydrolysis is lower when the amount of cocci is higher than rods. Free fatty acid can reduce the proteolytic activity and improve the texture of coagulum. High proteolytic activity is observed during manufacture of lactose hydrolyzed yoghurt (Grous, 1972).

2.13.5 Lipolytic activity

Lipolysis is generally low in yogurt and is therefore not significant in terms of flavor. The free fatty acid content of yogurt differs only slightly from that of milk (Rasic and Kurmann, 1978). Thermophilic starter culture may contain lipolytic enzymes. During the manufacture and storage of yoghurt, hydrolysis of fat usually occurs and the lactic acid bacteria hydrolyze mainly long chain triglycerides (Singh et al., 1980). In general the lipolytic activity of thermophilic starter is weak and most of the volatile acids formed in yoghurt are derived from the hydrolysis of other compounds (Shahini and Reddy, 1979).

2.13.6 Other metabolic activities

Yoghurt starter bacteria utilize or synthesize vitamins during their growth. It has been observed that during yoghurt production niacin, folic acid, biotin, vitamins B6 and B12 are synthesized. It has also been observed that biotin, niacin, folic acid, vitamin B12 is decreased during cold storage of the product or by microbial catabolism (Cerna and Pickova, 1973). Organic acids like fumaric, succinic and benzoic show increased concentrations in yoghurt compared to milk while hipuric acid, orotic acid are reduced by the metabolic activity of the microorganisms (Deeth and Tamime, 1981).

2.14 Role of thermophilic cultures in the intestine

It has been suggested that S. thermophilus and L. bulgaricus can establish themselves in the intestine and generally dominate the natural micro flora. L. bulgaricus is able to resist the high degree of acidity so it is feasible that a percentage of bacteria ingested will reach the intestine in viable state (Lembke, 1963).
Certain strains of *L. bulgaricus* could survive and compete in the human intestine and ensures the absence of putrefactive organisms and hence protect the health of the intestine of the consumer (Acott and Labuza, 1972).

### 2.14 Production of antibiotic substances

There is generally a symbiotic relationship between yogurt bacteria, but growth inhibition is sometimes observed (Moon and Reinbold, 1974). This should be taken into account when selecting starters. Inhibition may be due to competition for one or more nutrients of the growth medium (Moon and Reinbold, 1976) or to inhibitory compounds produced by the strains, such as bacteriocins and inhibitory peptides (Pereira and Luchese, 1988).

#### 2.14.1 Effect on immunoglobulin level, cholesterol, lactose activity and tumor cells

It has been shown that the composition of yoghurt could lead to increase level of immunoglobulin. It has also been shown that the composition of yoghurt could lead to decrease level of cholesterol in the blood (Mann, 1977).

In people who exhibit lactose intolerance upon ingesting milk, the milk can be replaced by yoghurt without any allergic reactions. *L. bulgaricus* has been shown to have a greater inhibitory effect on tumor cells (Mann, 1977).

#### 2.14.2 Formation of bacteriocins and other inhibitory compounds

The study of bacteriocin production has been largely confirmed to *L. acidophilus*. *L. bulgaricus* produces a bacteriocin called “bulgarican” and many other inhibitory compounds (Reddy and Shahini, 1971).

### 2.15 Causes of slow growth

As with cheese starter, partial or complete failure may occur with yoghurt. However, bacteriophages are generally the main causes of failure with starters, antibiotics and preservatives or sterilants are the main causes for failure of yoghurt. *S. thermophilus* and *L. bulgaricus* are the most sensitive of commonly used lactic cultures to penicillin (Tramer, 1973).
2.15.1 Bacteriophages

Knowledge of the specific bacteriophages (phages) of thermophilic lactic acid bacteria has been well documented, primarily on and after 1980. Specified phages may attack *S. thermophilus* and *L. bulgaricus* strains during yogurt manufacture and seriously affect product quality. Moreover, even if phage attacks do not delay acidification during yogurt manufacture, they can lead to an important decrease in the streptococci and to a lower flavor score of the resulting yogurt (Stadhouders *et al.*, 1988).

In order to exert better control against attack of phages it is important to know that the heat treatment of milk at 85°C for 20 min. will ensure the destruction of phage particles. Chemicals such as 0.1% quaternary ammonium, 70 to 90% ethanol, 0.5 to 1% potassium permanganate or 50 to 100 ppm of available chlorine destroy *S. thermophilus* phages (Tamime and Robinson, 1985). The phage problem will never be completely solved; however the frequency of such infection could be reduced (Klaenhammet, 1984).

2.15.2 Effect of chemical composition of the lactic acid flora

Chemical composition can exert an important influence mainly on account of sucrose which may vary from 0-12%. This will not affect the growth of lactic acid flora in fruit yoghurts because the sugar is added after the souring process. Sucrose concentration encourages those types which can ferment sucrose. *L. bulgaricus* is severely inhibited at 24% total solid and over (Carr *et al.*, 1975).

2.16 Coagulum formation in yoghurt

The formation of yoghurt gel is the result of the following biological and physical action of milk. Starter in yoghurt utilizes lactose for its energy and produces lactic acid and other relevant compounds become inevitable. Gradual development of lactic acid destabilizes the calcium caseinate phosphate complex. Aggregates of casein micelles and/or the individual micelles group together and partially coalesce as the pH approaches the isoelectric point (pH 4.6 to 4.7). It is most likely that α-lactalbumin and β-lactoglobulin interaction with the κ-casein (linked by –SH and –SS bridges) partially protects the micelles against complete destabilization or disruption. As a result the gel network or matrix consists of a regular structure, which entraps within all the outer constituents of the basic mix including the water phase (Tamime and Robinson, 1985).
2.17 Syneresis

Syneresis is the oozing out of water on the surface of yoghurt. Syneresis is caused by low SNF or fat content. Insufficient heat treatment and homogenization of milk, too high incubation temperature causes the syneresis problem. These types of problem can solved by standardization of SNF and fat content along with reduction in incubation temperature to 42°C and addition of stabilizers (Rayamajhi, 2011).

2.18 Methods for improving the body (viscosity) of yoghurt

Traditional yoghurt was made by heating milk in open pans, concentrating it in this way to two-third volume. The higher solids content would also give thicker or more viscous yoghurt. Sheep milk, if used, would also give thicker yoghurt because it is about 50% richer in solids than ordinary cow milk (Tamime and Robinson, 1985).

If yoghurt is made from non-concentrated or unfortified cow milk an attractive gel is obtained, but this is delicate and easily broken by vibration. SMP at 4-5% level is incorporated to overcome this difficulty. The easiest and cheapest way is to incorporate a carbohydrate gum such as carrageenan, alginate, agar, etc. at a level of about 0.3%. This is harmless but does not add to the nutritive value, the milk fat is normally homogenized (Tamime and Robinson, 1985).

Ropy strains of both S. thermophilus and L. bulgaricus can be used at same temperature (43°C) for ordinary yoghurt. However, the lower the temperature and the longer the time of incubation, the higher will be the viscosity. Thus temperature of 30-32°C with an incubation time of 12-15 h may be used (Carr et al., 1975).

2.19 Types of yoghurt

2.19.1 Set type yoghurt

Processed milk is fed directly into the intermediate tank inoculated with starter culture and/or flavor before it is packed in filling machine. The yoghurt cups are filled and transferred to the incubation chamber at 42°C. After 3 hours, the cups are cooled to 15-20°C by means of cold air in the chamber or in the cooling tunnel (Pant, 1992).
2.19.2 Stirred type yoghurt

It is soured in tank after which the product is stirred, cooled and packed. Stirred yoghurt has distinct consistency, thick and smooth, and should make good eating, rather than drinking (Gallesloot and Hassing, 1973). From 0.5-0.7% stabilizer is added in order to impart gel structure, to ensure a smooth body and texture and to prevent wheying off or syneresis after packaging (Chandan, 1981). The stirred type may be plain, fruit and flavored and this form of yoghurt is more popular (Tamime and Robinson, 1985).

2.19.3 Drinking type of yoghurt

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

2.19.4 Frozen yoghurt

The yoghurt base is produced in the conventional way. The milk should be subjected to UHT treatment, before fermentation with starter culture and produced natural stirred yoghurt, then 65-80% yoghurt base, 20-35% fruit syrup base are mixed and 0.85% stabilizer and emulsifier are added. The product is then frozen in an ordinary ice cream freezer (outlet temperature -6°C). Finally, the yoghurt is packed at 0 to -6°C and dispatched (Tamime and Robinson, 1985).

2.19.5 Dried yogurt

Yogurt powder is produced by fermenting non-fat milk using standard yogurt cultures until attain the desirable pH followed by a step of drying, most probably by freeze-drying. In addition, blended yogurt powder is manufactured by blending cultured non-fat milk, cultured whey, cultured whey protein concentrate, cultured dairy solids, nonfat dry milk and lactic acid which are similar to the flavor and functionality to that of the traditional yogurt powder (Childs and Drake, 2008).
The main intention of manufacturing yogurt powder is to store the product in a stable and readily utilizable state, and it can be utilized to replace fresh yogurt for beverage and dip, and in confectionary industry as a coating material for coating of dried fruit, nuts, pretzels, cereal and other snack items (Krasaekoopt and Bhatia, 2012).

2.19.6 Dietetic/therapeutic yoghurt

The fact that most strains of *S. thermophilus* and *L. bulgaricus* do not survive in the intestinal tract, may be limiting factor if yoghurt is used for antibiotic therapy and/or any other medical purpose. However, the inclusion *Lactobacillus acidophilus* and *Bifidobacterium bifidum* as yoghurt starter culture may contravene some existing definitions of yoghurt; the resultant milk product is reported to be of excellent therapeutic value. E.g. lactose-hydrolyzed yoghurt is beneficial for lactose intolerant patient (Tamime and Robinson, 1985).

Apart from these types other varieties of yoghurt are also manufactured. Some important ones are concentrated yoghurt, carbonated yoghurt, yoghurt beverage, soy yoghurt etc.

2.20 Addition of fruit flavoring in yoghurt

In modern technology of yoghurt manufacturing, addition of fruit and flavoring is well popular. In the stirred type, these may be added after fermentation but in the set type, the addition must be done before fermentation. The fruit preparations are especially designed to meet the marketing requirements for different types of yoghurt. They are generally present at the level of 15-20% in the final product (Chandan, 1981). Yogurts are available in a vast array of flavors including fruit (apple, apricot, black cherry, black currant, blue berry, lemon, mandarin, raspberry, strawberry, peach), cereal, vegetables, chocolate, vanilla, caramel, ginger, etc (Anon., 2013b). In general, flavors are added to yogurt during production stage and the addition of flavors not only results a wide array of tastes, but also increases sweetness of the product (Anon., 2013d)

2.21 Shelf-life of yoghurt

Shelf-life of a product may be defined as the number of days after production that can be consumed while still remaining safe, retaining its quality appeal and meeting consumer
expectations. In other words, it should remain microbiologically safe and organoleptically acceptable within its stated shelf-life (Ahmed, 2011).

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

Depending on the standards of hygiene observed during the manufacture of yoghurt, and the microbiological quality of the ingredients and packaging materials, the shelf-life of yoghurt is around 3 weeks under refrigerated condition (Goodluck et al., 2014).

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

### 2.22 Techniques of shelf-life extension

Shelf life can be prolonged by several methods:

- Stopping incubation when the pH has reached 4.6-4.8, rapid cooling and storage at 5°C.
- Aseptic operation on an enclosed production line, including aseptic addition of sterile additives and aseptic packaging.
- Pasteurization of stirred cultured milk products with or without additives in a continuous flow cooler, aseptic cooling, filling and sealing.
- Continuous flow heating, filling while hot, sealing the package, and cooling after a sufficiently long pasteurization time.
- Filling while cold, sealing the package, pasteurization in the package by heating, followed by cooling (Kessler, 2002).
2.23 Coconut

2.23.1 Description and origin

The coconut (Cocos nucifera L.) is a member of the family Arecaceae (palm family). It is the only accepted species in the genus Cocos, and is a large palm, growing up to 30 m tall, with pinnate leaves 4–6 m long and pinnae 60–90 cm long which old leaves break away cleanly, leaving the trunk smooth. The term coconut can refer to the entire coconut palm, the seed, or the fruit, which is not a botanical nut (Afodunrinbi and Onyeukwu, 2000). The coconut palm is found throughout the tropics, where it is interwoven into the lives of the local people. It is particularly important in the low islands of the Pacific where, in the absence of land-based natural resources, it provides almost all the necessities of life-food, drink, oil, medicine, fiber, timber, thatch, mats, fuel, and domestic utensils. For good reason, it has been called the “tree of heaven” and “tree of life.” Today it remains an important economic and subsistence crop in many small Pacific island states (Banzon, 1990).

The coconut's name is a bit of a misnomer, since it is botanically classified as a drupe and not a nut. It is the largest seed known (Banzon, 1990).

Countries where coconut is abundant shown in the Table 2.1

Table 2.1 Countries where coconut is abundant

<table>
<thead>
<tr>
<th>Region</th>
<th>Countries</th>
</tr>
</thead>
<tbody>
<tr>
<td>Southeast Asia</td>
<td>Burma, Indonesia, Malaysia, Philippines, Singapore, Thailand, Vietnam.</td>
</tr>
<tr>
<td>Indian Subcontinent</td>
<td>Bangladesh, South India, Sri Lanka, Nicobar, Seychelles.</td>
</tr>
<tr>
<td>Africa</td>
<td>Cameroon, Ghana, Ivory Coast, Kenya, Mozambique, Nigeria, Tanzania</td>
</tr>
<tr>
<td>Central America</td>
<td>Brazil, Ecuador, Jamaica, Mexico, Trinidad and Tobago, Venezuela.</td>
</tr>
<tr>
<td>Melanesia</td>
<td>Fiji, Papua New Guinea, Solomon Islands, Vanuatu.</td>
</tr>
</tbody>
</table>

Source: Banzon (1990)
2.23.2 Varieties

Varieties and characteristics of coconut shown in the Table 2.2

Table 2.2 Coconut varieties and characteristics

<table>
<thead>
<tr>
<th>Variety</th>
<th>Characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tall</td>
<td>Thick stem with swollen base (bole). Late flowering (5–6 yr from out planting). Little or no overlapping of male and female phases of an inflorescence encouraging out-crossing.</td>
</tr>
<tr>
<td>Dwarf</td>
<td>Slender stem with short internodes. Bole slight or absent. Early flowering (3 yr from out planting). Considerable overlapping of male and female phases of an inflorescence resulting in self-pollination</td>
</tr>
</tbody>
</table>

Source: Romney (1997)

2.23.3 Coconut fat

All fats and oils are composed of molecules called fatty acids. There are two methods of classifying fatty acids. The first is based on saturation; there are saturated fats, monounsaturated fats, and polyunsaturated fats. The other system of classification is based on molecular size or length of the carbon chain within each fatty acid. In this system there are short-chain fatty acids (SCFA), medium-chain fatty acids (MCFA), and long-chain fatty acids (LCFA) (Thompson et al., 1961).

Coconut oil is composed predominately of medium-chain fatty acids (MCFA), also known as medium-chain triglycerides (MCT). The size of the fatty acid is important because the human body responds to and metabolizes each fatty acid differently depending on its size. So the physiological effects of MCFA in coconut oil are distinctly different from those of LCFA more commonly found in our foods. The saturated fatty acids in coconut oil are predominately medium-chain fatty acids. Both the saturated and unsaturated fat found in meat, milk, eggs, and plants (including almost all vegetable oils)
are composed of LCFA (Pamplona-Roger, 2007). MCFA are very different from LCFA. They do not have a negative effect on cholesterol and help to protect against heart disease. MCFA help to lower the risk of both atherosclerosis and heart disease. It is primarily due to the MCFA in coconut oil that makes it so special and so beneficial. There are only a very few good dietary sources of MCFA. The best sources of MCFA are coconut and palm kernel oils (Pamplona-Roger, 2007).

2.23.4 Coconut meat

Coconut meat is the edible white meat of a coconut; often shredded for use in cakes and curries. It contains essential mineral salts particularly magnesium, calcium and phosphorus which are of great importance to the musculoskeletal system. Though present in small amounts (32 mg/100 g of magnesium) in coconut meat, the Magnesium content surpasses that of all animal-based foods including meat, fish, milk and eggs (Pamplona-Roger, 2007).

2.23.5 Coconut milk

Coconut milk should not be confused with coconut water, although some studies have used the two terms interchangeably. The aqueous part of the coconut endosperm is termed coconut water, whereas coconut milk, also known as “santan” in Malaysia and Indonesia, and “gata” in the Philippines, refers to the liquid products obtained by grating the solid endosperm, with or without addition of water. Coconut milk is usually used as a food ingredient in various traditional cooking recipes, while coconut water is served directly as a beverage to quench thirst (Banzon, 1990).

Coconut milk is the term used to designate the liquid obtained by the manual or mechanical extraction of grated coconut meat with or without added water. The term coconut milk and coconut cream are used interchangeably. But coconut milk refers to the milky fluid, freshly extracted from the coconut kernel with or without added water, and coconut cream refer to the high fat cream like material obtained from the coconut milk by either gravity separation or centrifugation (Banzon, 1990).

Maturity of the coconut greatly affects the yield of coconut milk. Mature brown husked coconuts with no protruding sprouts produce higher yields of milk. Coconut milk is generally produced from mature nuts of 12 months in age. At this stage, the meat is hard
and thick, with a typical composition of as follows: 50% moisture, 34% oil, 3.5% protein, 3% fiber, 2.2% ash and 7.3% carbohydrates (Banzon, 1990).

Composition of coconut milk is shown in the Table 2.3

**Table 2.3 Composition of coconut milk**

<table>
<thead>
<tr>
<th>Components</th>
<th>Quantity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture</td>
<td>72.88</td>
</tr>
<tr>
<td>Ash</td>
<td>1.7</td>
</tr>
<tr>
<td>Protein</td>
<td>2.02</td>
</tr>
<tr>
<td>Fat</td>
<td>5</td>
</tr>
<tr>
<td>Acidity (as %citric acid)</td>
<td>0.13</td>
</tr>
<tr>
<td>Carbohydrate</td>
<td>2.81</td>
</tr>
</tbody>
</table>

Source: USDA (2012)

### 2.23.6 Nutritional and medicinal importance of coconut milk

Some of the most important benefits of coconut milk are:

- A major part of the fats found in coconut milk is lauric acid, which has been found to exhibit antibacterial, antifungal and antiviral properties. This fatty acid can boost the immune system and its disease fighting ability.
- Lauric acid can also be helpful in maintaining the elasticity of the blood vessels and in keeping them clean, which can lower the risk for conditions like, atherosclerosis and heart disease.
- Coconut milk also contains several antioxidant compounds, which can provide protection against the harmful free radicals and their damaging effects on the body cells and tissues.
- Coconut milk can improve the health of the digestive system and promote digestion. It can relieve the symptoms of stomach ulcers and acid reflux disease as well.
- Coconut milk can give about 22% of the recommended daily allowance of iron. With such a high level of iron, it can help to treat anemia caused by iron deficiency.
• Coconut milk health benefits are mentioned in Traditional Medicine for the human body. It is also used for the treatment of mouth ulcers.
• Coconut is a dairy free alternative to those who are lactose intolerant and are also allergic to animal milk. This milk is also nut free, soy free and gluten free.
• It is known to relieve the symptoms of sore throat.
• It is good for the health of your skin and hair. Many cosmetic giants use it as a base in products for skin and hair.
• Apply coconut milk to the scalp to have dandruff free hair and condition your hair naturally.
• Coconut milk is a reservoir of antioxidants. Antioxidants help the body fight aging, low vision and low bone density.
• It also aids in digestion and is also used as a laxative. It can also be a remedy for urinary and kidney problems.
• Coconut milk is an excellent source of Vitamin E. It helps in the nourishment of the skin.
• The saturated fat content in coconut is made up of short and medium chain fatty acids. These fatty acids are quickly converted into energy instead of storing as fat in the body.
• The medium chain fatty acids present in coconut milk are full of lauric acid. Lauric acid is anti fungal, anti viral and anti microbial. Lauric acid present in coconut milk helps to keep the arteries of the heart clean and healthy (Banzon, 1990).
Part III

Material and methods

3.1 Materials

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

3.1.1 Milk

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

3.1.2 Coconut

Coconut was collected from local market of Amarhatt Dharan.

3.1.3 Milk solid not fat

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

3.1.4 Sweetener

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

3.1.5 Starter Culture

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

3.1.6 Containers

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

**Table 3.1** List of equipment used

<table>
<thead>
<tr>
<th>Physical apparatus</th>
<th>Physical apparatus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heating arrangement</td>
<td>Refrigerator</td>
</tr>
<tr>
<td>Electric balance (Phoenix)</td>
<td>Daily routine glassware</td>
</tr>
<tr>
<td>Gerber centrifuge</td>
<td>Muffle furnace (Accuma, India)</td>
</tr>
<tr>
<td>Thermometer</td>
<td>Hot air oven (Vitco, India)</td>
</tr>
<tr>
<td>Refractrometer (Hand refractometer model WYT-32, Zhongyou Optical Instruments)</td>
<td>Stainless steel vessels</td>
</tr>
<tr>
<td>Titration apparatus</td>
<td>Incubator (Vitco, India)</td>
</tr>
<tr>
<td>Desiccators</td>
<td>Gerber butyrometer</td>
</tr>
<tr>
<td>Kjeldahal digestion and distillation set</td>
<td></td>
</tr>
</tbody>
</table>

**Table 3.2** List of chemicals used

<table>
<thead>
<tr>
<th>Chemicals</th>
</tr>
</thead>
<tbody>
<tr>
<td>40 % Formaldehyde</td>
</tr>
<tr>
<td>Culture medium (plate count agar)</td>
</tr>
<tr>
<td>Starter culture</td>
</tr>
<tr>
<td>Saturated potassium oxalate</td>
</tr>
<tr>
<td>0.0005% Fuchsin solution</td>
</tr>
</tbody>
</table>

3.2 Method

3.2.1 Preparation of coconut milk

The coconut was de-husked. The de-husked nut was cracked open into halves. The split nuts were de-shelled to separate the coconut meat (kernel). Coconut meat was washed and comminuted using an electric blender with water. This was then pressed through a muslin cloth and strained to obtain coconut milk.
Flow chart for the preparation of coconut milk is shown in Fig. 3.1

```
De-husked whole coconut → Drilling → Coconut water

De-watered, de-husked whole coconut

De-shelling, grinding

Splitting

Comminuted coconut meat

Milk extract

Coconut milk

Coconut residue
```

Fig. 3.1 Preparation of coconut milk

Source: (Tamrakar, 2017)

3.2.2 Preparation of set type coconut milk yoghurt

The standardized and pasteurized milk from DDC (Dairy Development Corporation) was taken for the preparation of yoghurt. The milk was mixed with 4% SMP (Skim milk powder) and 3% sugar at 45°C. Heating of milk was further continued till the temperature reached to around 65-70°C for certain period. After that the heated milk was cooled to around 43-44°C. After cooling, five formulations of the samples were made by adding 10, 20, 30, 40, and 50% of coconut milk per 100 ml yoghurt mix. Then the starter culture is added at the rate of 2% to each formulations. Then the mix is placed in plastic cups. The yoghurt mix was then kept in an incubator which was maintained at a temperature of about 43°C and was kept for 3.5-4 h until the coagulum is formed. Now the prepared yoghurt was immediately cooled to 5-7°C and stored at that temperature in a refrigerator.
Flow chart for the preparation of coconut milk incorporated is shown in Fig. 3.2

![Flow chart for the preparation of coconut milk incorporated](image)

**Fig. 3.2** Preparation of coconut milk incorporated yoghurt

Source: Biswas (2013)

### 3.2.3 Chemical analysis of coconut milk

#### 3.2.3.1 Acidity

Acidity was determined by titrimetric method as Pearson (1981).

#### 3.2.3.2 Fat

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

#### 3.2.3.3 Protein

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

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

3.2.3.5  **pH**

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

3.2.3.6  **Total Solid (TS)**

Total solid was determined by subtracting the moisture from the 100 according to Ranganna (2000).

3.2.3.7  **Moisture**

Moisture content was determined as per the methods described by Ranganna (2000).

3.2.3.8  **Vitamin C**

Vitamin C was determined following the method of KC and Rai (2007).

3.2.3.9  **Total sugar**

The total sugar was determined following the method of Ranganna (2000).

3.2.4  **Chemical analysis of Milk**

3.2.4.1  **Acidity**

Acidity and was determined by titrimetric method as per Pearson (1981).

3.2.4.2  **Fat**

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

3.2.4.3  **Protein**

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

3.2.4.4  **Ash**

The ash content was determined as described by Ranganna (2000).
3.2.4.5 pH

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

3.2.4.6 Total Soluble Solid (TSS)

The total soluble solid of milk was determined by using Hand refractometer (Model WYT-32, Zhongyou Optical Instruments).

3.2.4.7 Lactose

Lactose content was determined by Lane and Eynon method as per Pearson (1981).

3.2.5 Design expert

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

Table 3.3 Sample formulation in coded form

<table>
<thead>
<tr>
<th>Sample</th>
<th>Milk</th>
<th>Coconut milk</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>90%</td>
<td>10%</td>
</tr>
<tr>
<td>B</td>
<td>80%</td>
<td>20%</td>
</tr>
<tr>
<td>C</td>
<td>70%</td>
<td>30%</td>
</tr>
<tr>
<td>D</td>
<td>60%</td>
<td>40%</td>
</tr>
<tr>
<td>E</td>
<td>50%</td>
<td>50%</td>
</tr>
</tbody>
</table>

3.2.6 Analysis of yoghurt

3.2.6.1 Sensory evaluation

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

3.2.6.2.1 Syneresis

Degree of syneresis, expressed as proportion of free whey was measured by a method used by Lee and Lucey (2004). A 100 g sample of yoghurt was placed on a filter paper resting on the top of a funnel. After 10 min of drainage in vacuum condition, the quantity of the remained yoghurt was weighed and syneresis was calculated as follows:

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

3.2.6.3 Chemical analysis

3.2.6.3.1 Fat

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

3.2.6.3.2 Lactose

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

3.2.6.3.3 pH

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

3.2.6.3.4 Titrable acidity

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

3.2.6.3.5 Protein

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

3.2.6.3.6 Ash

Ash content was determined as described in Ranganna (2000).
3.2.6.3.7 **Total carbohydrate**

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

3.2.6.4 **Microbiological examination**

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

3.2.6.5 **Data analysis**

Analysis of variance (ANOVA) was carried out for data from sensory evaluation. The significant differences between them were studied by using L.S.D. at 5% level of significance using Genstat release 12.1 software program developed by VSN International Ltd. (VSNi) in the collaboration with practicing statisticians at Rothamsted Research and the organization in Australia, New Zealand, Switzerland and the UK. t-Test: Two-Sample Assuming Equal Variances was carried out using Microsoft Excel 10 to evaluate the significant difference between the syneresis of the two samples.
Part IV

Results and discussions

Coconut milk yoghurt was prepared at CCT, Dharan, in a laboratory for the present study. The coconut milk incorporated yoghurt samples were prepared by incorporating 10, 20, 30, 40, and 50% coconut milk. The milk was mixed with 4% SMP (Skim milk powder) and 3% sugar at 45°C. Heating of milk was further continued till the temperature reached to around 65-70°C for certain period. After that the heated milk was cooled to around 43-44°C. After cooling, five formulations of the samples were made by adding 10, 20, 30, 40, and 50% of coconut milk per 100 ml yoghurt mix. Then the starter culture is added at the rate of 2% to each formulations. Then the mix is placed in plastic cups. The yoghurt mix was then kept in an incubator which was maintained at a temperature of about 43°C and was kept for 3.5-4 h until the coagulum is formed. Coconut milk yoghurt with the different levels of coconut milk was subjected to sensory evaluation and compared with each other to assess its acceptable level.

4.1 Chemical composition of milk

The proximate composition of the milk collected from DDC (Dairy Development Corporation) is presented in the Table 4.1. The collected milk was standardized and pasteurized.

Table 4.1 Chemical composition of milk

<table>
<thead>
<tr>
<th>Parameters</th>
<th>*Values (% dry basis)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total soluble solid</td>
<td>8.4 (0.057)</td>
</tr>
<tr>
<td>Acidity as lactic acid (%)</td>
<td>0.14 (0.005)</td>
</tr>
<tr>
<td>pH</td>
<td>6.5 (0.057)</td>
</tr>
<tr>
<td>Lactose (%)</td>
<td>4.5 (0.057)</td>
</tr>
<tr>
<td>Protein (%)</td>
<td>3.4 (0.057)</td>
</tr>
<tr>
<td>Fat (%)</td>
<td>3.0 (0.057)</td>
</tr>
<tr>
<td>Ash (%)</td>
<td>0.72 (0.005)</td>
</tr>
</tbody>
</table>

*Values in the table are arithmetic mean of triplicate samples. Figure in the parentheses indicates standard deviation.
The composition of DDC milk in Table 4.1 has little variation over the composition of milk analyzed by Dahal, (2009). The milk analyzed by Dahal is produced from Kamdhenu Dairy Co-operative (KDDC). This variation in composition of milk may be due to the species, nutritional aspect of animal, stage of lactation and feeding of animals. The variation may also be due to different processing standard and specification of dairies.

4.2 Chemical composition of coconut milk

Coconut milk was analyzed. The results of analysis of coconut milk in dry basis are tabulated in the Table 4.2.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acidity (% as citric acid)</td>
<td>0.13 (0.005)</td>
</tr>
<tr>
<td>Ash (% dry basis)</td>
<td>0.7 (0.057)</td>
</tr>
<tr>
<td>Fat (% dry basis)</td>
<td>4.2 (0.057)</td>
</tr>
<tr>
<td>Moisture (%)</td>
<td>91.2 (0.75)</td>
</tr>
<tr>
<td>pH</td>
<td>6.4 (0.057)</td>
</tr>
<tr>
<td>Protein (% dry basis)</td>
<td>2.8 (0.057)</td>
</tr>
<tr>
<td>Total solid (% dry basis)</td>
<td>8.2 (0.11)</td>
</tr>
<tr>
<td>Vitamin C (mg/100g)</td>
<td>3 (0.057)</td>
</tr>
<tr>
<td>Total sugar (% Dextrose)</td>
<td>3.1 (0.057)</td>
</tr>
</tbody>
</table>

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

There was little variation in the value obtained during this work from the value given by Tamrakar (2017) and Biswas (2013). This may be due to difference in variety, climatic conditions of cultivation, fruit maturity.

4.3 Sensory evaluation of coconut milk yoghurt

Sensory evaluation of all five formulation of the product which were carried out by a group of seven semi-trained panelists evaluating aroma, color, texture and overall acceptance of prepared coconut milk yoghurt. The Analysis of Variance (ANOVA) was carried out using least significant difference (LSD) at 5% level of significance.
4.3.1 Aroma

Regarding aroma of coconut milk incorporated yoghurt, the analysis shows that the mean sensory score for sample A, B, C, D, and E were found to be 7.57, 7.57, 7.14, 6.71 and 6.71 respectively. Statistical analysis shows that effect of different coconut milk portion on aroma of the product was not significantly different at 5% level of significance.

![Bar graph showing mean sensory scores for aroma of coconut milk yoghurt. Values on the top of the bars bearing similar superscript are not significantly different at 5% level of significance. Vertical error bar represent ±standard deviation of scores given by panelist.](image)

**Fig. 4.1** Effect of coconut milk on aroma of yoghurt

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

4.3.2 Color

Regarding color of cococnut milk incorporated yoghurt, the analysis shows that the mean sensory score for sample A, B, C, D, and E were found to be 8.143, 7.571, 6.571, 6.429 and 6.286 respectively. Statistical analysis shows that effect of different coconut milk portion on color of the product was significant (p<0.05). LSD shows that sample A and B, A and C, B and C, A and D, B and D, A and E, B and E were significantly different but
there was no significant difference between samples C, D and E. Among five samples, sample A got the high mean score, due to optimum acceptance of panelist.

As the proportion of cow milk was decreased the color preference became decrease. It may be due to addition of coconut milk. Similar results were reported by Biswas (2013).

**Fig. 4.2** Effect of coconut milk on color of yoghurt.

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

Regarding taste of coconut milk incorporated yoghurt, the analysis shows that the mean sensory score for sample A, B, C, D, and E were found to be 8, 7.571, 6.857, 6.143, and 5.857 respectively. Statistical analysis shows that effect of different coconut milk portion on taste of the product was significant (p<0.05). LSD shows that sample A and C, A and D, A and E, B and C, B and D, B and E were significantly different but there was no significant difference between samples A and B and C and D.

Among five samples, sample A and B got the high mean score, due to optimum acceptance of panelist.

As the proportion of cow milk was decreased the taste preference became decrease. It may be due to addition of coconut milk. Similar results were reported by Biswas (2013).

![Fig. 4.3 Effect of coconut milk on taste of yoghurt](image)

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

Regarding texture of coconut milk incorporated yoghurt, the analysis shows that the mean sensory score for sample A, B, C, D, and E were found to be 8.29, 7.57, 6.42, 5.71, and 5.14 respectively. Statistical analysis shows that effect of different coconut milk portion on texture of the product was significant (p<0.05). LSD shows that sample A and B, A and C, A and D, A and E, B and C, B and D, B and E were significantly different but there was no significant difference between samples D and E.

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

As the proportion of cow milk was decreased the texture preference became decrease. It may be due to addition of coconut milk. Similar results were reported by Biswas (2013).

FIG. 4.4 Effect of coconut milk on texture of yoghurt

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

Regarding overall acceptability of coconut milk incorporated yoghurt, the analysis shows that the mean sensory score for sample A, B, C, D, and E were found to be 8, 7.71, 6.85, 6.14 and 5.71 respectively. Statistical analysis shows that effect of different coconut milk portion on overall acceptability of the product was significant (p<0.05). LSD shows that sample A and C, A and D, A and E, B and C, B and D, B and E, C and D, C and E were significantly different but there was no significant difference between samples A and B and D and E.

Among five samples, sample A and B got the high mean score, due to optimum acceptance of panelist.

As the proportion of cow milk was decreased the overall acceptability preference became decrease. It may be due to addition of coconut milk. Similar results were reported by Biswas (2013).

![Fig. 4.5 Effect of coconut milk on overall acceptability of yoghurt](image-url)
Fig. 4.5 represents the mean sensory scores for overall acceptability of coconut milk yoghurt. Values on the top of the bars bearing similar superscript are not significantly different at 5% level of significance. Vertical error bar represent ±standard deviation of scores given by panelist.

There was significant difference between the samples A with respect to other samples but there was no significant difference between other samples. The overall acceptability of sample A was higher due to improvement in color, taste and texture with respect to other samples. From the sensory evaluation of the product conducted on the attributes like aroma, color, taste, texture and overall acceptability, the product containing 10% coconut milk and 90% cow milk by volume was rated as best in all attributes.

4.4 Chemical analysis of best product

The standardized and pasteurized milk from DDC (Dairy Development Corporation) was taken for the preparation of yoghurt. The milk was mixed with 4% SMP (Skim milk powder) and 3% sugar at 45°C. Heating of milk was further continued till the temperature reached to around 65-70°C for certain period. After that the heated milk was cooled to around 43-44°C. After cooling, five formulations of the samples were made by adding 10, 20, 30, 40, and 50% of coconut milk per 100 ml yoghurt mix. Then the starter culture is added at the rate of 2% to each formulations. Then the mix is placed in plastic cups. The yoghurt mix was then kept in an incubator which was maintained at a temperature of about 43°C and was kept for 3.5-4 h until the coagulum is formed.
Chemical analysis of best product was done. The values of the chemical analysis are shown in Table 4.4

**Table 4.4** Chemical analysis of coconut milk incorporated yoghurt.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Yoghurt</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acidity (% lactic acid)</td>
<td>0.71 (0.01)</td>
</tr>
<tr>
<td>Ash (% dry basis)</td>
<td>0.94 (0.04)</td>
</tr>
<tr>
<td>Total Carbohydrate (% dry basis)</td>
<td>5.06 (0.08)</td>
</tr>
<tr>
<td>Fat (% dry basis)</td>
<td>5.1 (0.1)</td>
</tr>
<tr>
<td>Moisture</td>
<td>82 (1.5)</td>
</tr>
<tr>
<td>pH</td>
<td>4.4 (0.1)</td>
</tr>
<tr>
<td>Protein (% dry basis)</td>
<td>3.5 (0.1)</td>
</tr>
<tr>
<td>Total solid (% dry basis)</td>
<td>20 (1)</td>
</tr>
<tr>
<td>Lactose (% dry basis)</td>
<td>3.8 (0.2)</td>
</tr>
</tbody>
</table>

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

The chemical analysis of the best product was done. Percent acidity was to be 0.71%. It is within the range (0.6-0.9%) reported by Yulina (2010). Desai *et al.* (1994) reported that addition of fruit juice/pulp increases percent acidity. Ash content of the product was found to be 0.94%. The result is within the range of value (0.8-1.5%) showed by Ahmed (2011). Mahmood and Gilani (2008) reported that addition of fruit juice/pulp increases ash content which may be due to insoluble solids and fiber content which may contribute in increasing the ash content. Carbohydrate content was found to be 5.06% which was similar to the result obtained by Afodunrinbi and Onyeukwu (2000). Fat content was found to be 5.1%
which is nearly same to the result obtained by Biswas (2013). Percentage fat is higher than normal dairy yoghurt which is nearly (3-4%), this may be due to higher percent of fat content in coconut milk than cow milk (Biswas, 2013).

Moisture content in the yoghurt was found to be 82% that is approximately similar to the yogurts prepared from camel milk by Eshraga et al. (2011). PH was found to be 4.4 which agrees quite well with the results of Akpan et al. (2007). Protein content was found to be 3.5% which was approximately similar to the result obtained by Afodunrinbi and Onyeukwu (2000). Total solid was found to be 20%, which is nearly similar to the result obtained by Biswas (2013). Lactose content was found to be 3.8% which was similar to the result obtained by Mahmood and Gilani (2008) during the preparation of yoghurt blended with apple and banana.

4.5 **Physical analysis**

The physical analysis of the commercial yoghurt and the best product was performed. The values obtained for the syneresis are shown in Table 4.5

<table>
<thead>
<tr>
<th>Samples</th>
<th>Syneresis (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Commercial yoghurt</td>
<td>19.26&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Best product</td>
<td>20.28&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup> T-Test was carry out to evaluate the significant difference between the two samples. There were no significant difference between commercial and best product (P<0.05) as shown in Appendix C. The results are similar to the work done by Rayamajhi (2011).

4.6 **Shelf-life of the product**

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

The acidity of yoghurt increased from 0.71% to 1.19% within 7 days of storage under room temperature. The increase in acidity of the yoghurt could be due to the acid production by culture bacteria during storage period. Moreover increase in value of acidity is due to addition of coconut milk which increases availability of higher nutrients for the production of lactic acid. Yoghurt sample remain suitable for consumption up to 2 days. The results are in agreement with Ahmed (2011).

![Graph showing change in acidity under room temperature with respect to the number of days](image_url)

**Fig. 4.6** Change in acidity under room temperature with respect to the number of days

In fig. 4.6 vertical error bars represents standard deviation

4.6.2  Total plate count of yoghurt at room temperature

TPC of yoghurt increases from $4.2 \times 10^4$ to $18.2 \times 10^4$ CFU/ml within 7 days of storage under room temperature. The increase in TPC of yoghurt is due to the production of lactic acid bacteria which increases with the addition of coconut milk. The results are in agreement with Ahmed (2011). Similar results was obtained by Goodluck et al. (2014) for the consumable range of total bacterial count as in the range of ($3.0 \times 10^3$-$10.5 \times 10^3$ cfu/ml). Yogurt samples were suitable for consumption up to 2 days.
Fig. 4.7 Change in TPC under room temperature with respect to the number of days

In fig. 4.7 vertical error bars represents the standard deviation

4.6.3 Acidity of yoghurt under refrigeration

The acidity of chilled yoghurt increased very slowly from 0.71 to 0.91 % within 7 days of storage under refrigeration. The increase in acidity of yoghurt could be due to the acid production by culture bacteria during storage period owing to their activity even at low temperature. Acidity in yogurt 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 7 days. The results are in agreement with Ahmed (2011)
In fig. 4.8 vertical error bars represents the standard deviation.

4.6.4 Total plate count of yoghurt under refrigeration

TPC of yoghurt increased slowly from $4.4 \times 10^4$ to $7.6 \times 10^4$ CFU/ml within 7 days of storage under refrigeration. The increase in TPC of yoghurt is due to the production of lactic acid even at low temperature. Though 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. The results are in agreement with Ahmed (2011). Similar results was obtained by Goodluck et al. (2014) for the consumable range of total bacterial count as in the range of $(3.0 \times 10^3-10.5 \times 10^4 \text{ cfu/ml})$. Yoghurt samples were suitable for consumption up to 7 days.
In fig. 4.9 vertical error bars represents the standard deviation

4.7 Cost evaluation
The total cost of the best coconut milk incorporated yoghurt was calculated. It is shown in the Appendix B. The price for 100 ml coconut milk incorporated yoghurt was found to be Rs.10.47 which was cheaper than commercial yoghurt.
Part V

Conclusion and recommendations

5.1 Conclusions

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

- From the sensory evaluation of the product conducted on the attributes like aroma, color, taste, texture and overall acceptability, the product containing 10% coconut milk and 90% cow milk by volume was rated as best in all attributes.
- From the storage analysis the product at refrigerated condition had increased self life.
- The coconut milk incorporated yoghurt thus prepared was found to be cheaper and more nutritional than the commercial yoghurt.
- The syneresis was not affected by the incorporation of coconut milk in yoghurt.

5.2 Recommendations

- Yoghurt can be prepared by blending different proportion of sugar and MSNF.
- Thermal treatment of coconut milk yoghurt can be studied to extend the shelf life of the product.
- Coconut milk based ice cream could be prepared and their quality studied.
Part VI

Summary

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

The standardized and pasteurized milk from DDC (Dairy Development Corporation) was taken for the preparation of yoghurt. The milk was mixed with 4% SMP (Skim milk powder) and 3% sugar at 45°C. Heating of milk was further continued till the temperature reached to around 65-70°C for certain period. After that the heated milk was cooled to around 43-44°C. After cooling, five formulations of the samples were made by adding 10, 20, 30, 40, and 50% of coconut milk per 100 ml yoghurt mix. Then the starter culture is added at the rate of 2% to each formulations. Then the mix is placed in plastic cups. The yoghurt mix was then kept in an incubator which was maintained at a temperature of about 43°C and was kept for 3.5-4 h until the coagulum is formed.

Sensory evaluation of five products was carried out. The sensory evaluation revealed that the product containing 10% coconut milk and 90% milk by volume was found to be best whose total solid, fat, acidity, protein, carbohydrate, total ash, moisture content, lactose and pH were found to 20%, 5.1%, 0.71%, 3.5%, 5.06%, 0.98%, 82%, 3.8% and 4.4 respectively. Shelf life of the best product was estimated in terms of acidity and total plate count and the shelf life was found to be 2 days at room temperature and 7 days at refrigeration.

From the overall analysis of the result it is clear that good quality yogurt could be prepared by adding coconut milk with cow milk. This new product will help to utilize coconut to some extent. Coconut is available through the year for yogurt preparation. It is expected that consumer will be interested to consume this type of yogurt day by day. Considering commercial point of view, production of maximum volume yogurt from minimum volume of cow milk by incorporating coconut milk make this business profitable.
References


Appendix

Appendix-A

Sensory evaluation Card (specimen)

(Hedonic Rating test)

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

Product: Coconut milk incorporated yoghurt

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

Quality Description

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

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

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Sample A</th>
<th>Sample B</th>
<th>Sample C</th>
<th>Sample D</th>
<th>Sample E</th>
</tr>
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<tbody>
<tr>
<td>Aroma</td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Color</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Taste</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Texture</td>
<td></td>
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<tr>
<td>OA</td>
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<td></td>
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</tbody>
</table>

Comments if any: .........................................................................................

Signature ______________
### Appendix B

**Table B.1** Cost evaluation of 100ml of 10% coconut milk incorporated yoghurt

<table>
<thead>
<tr>
<th>Particulars</th>
<th>Quantity (g)</th>
<th>Rate (Rs.)</th>
<th>Amount (Rs.)</th>
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<tbody>
<tr>
<td>Milk</td>
<td>90 (ml)</td>
<td>80/liter</td>
<td>7.2</td>
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<tr>
<td>Whole Coconut</td>
<td>15</td>
<td>50/kg</td>
<td>0.75</td>
</tr>
<tr>
<td>SMP</td>
<td>40</td>
<td>300/kg</td>
<td>1.2</td>
</tr>
<tr>
<td>Sugar</td>
<td>30</td>
<td>80/kg</td>
<td>0.24</td>
</tr>
<tr>
<td>Overhead cost (20%)</td>
<td></td>
<td></td>
<td>1.08</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td></td>
<td></td>
<td><strong>10.47</strong></td>
</tr>
</tbody>
</table>
### Appendix C

**Table C.1 t-Test: Two-Sample Assuming Equal Variances**

<table>
<thead>
<tr>
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<th>Commercial</th>
<th>Best</th>
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<tr>
<td>Mean</td>
<td>19.48666667</td>
<td>20.42</td>
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<tr>
<td>Variance</td>
<td>0.0434333333</td>
<td>0.0436</td>
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<tr>
<td>Observations</td>
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<td>3</td>
</tr>
<tr>
<td>Pooled Variance</td>
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</tr>
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<td>Hypothesized Mean Difference</td>
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<tr>
<td>Df</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>t Stat</td>
<td>-5.479672404</td>
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</tr>
<tr>
<td>P(T&lt;=t) one-tail</td>
<td>0.002699888</td>
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<td>t Critical one-tail</td>
<td>2.131846786</td>
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<tr>
<td>P(T&lt;=t) two-tail</td>
<td>0.005399775</td>
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<tr>
<td>t Critical two-tail</td>
<td>2.776445105</td>
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</table>
## Appendix D

**Table D.1** Two way ANOVA no blocking for Aroma

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>d.f</th>
<th>s.s</th>
<th>m.s</th>
<th>V.r</th>
<th>F pr.</th>
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<tbody>
<tr>
<td>Formulation</td>
<td>4</td>
<td>5.143</td>
<td>1.286</td>
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<td>Panelist</td>
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<td>24.057</td>
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<td>Total</td>
<td>34</td>
<td>58.286</td>
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**Table D.2** LSD for Aroma

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<th>Panelist</th>
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<td>5</td>
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<tr>
<td>d.f</td>
<td>24</td>
<td>24</td>
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<td>l.s.d</td>
<td>1.105</td>
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Table D.3 Two way ANOVA no blocking for Color

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<th>s.s</th>
<th>m.s</th>
<th>V.r</th>
<th>F pr.</th>
</tr>
</thead>
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<td>Formulation</td>
<td>4</td>
<td>18.5714</td>
<td>4.6429</td>
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<td>Panelist</td>
<td>6</td>
<td>22.4000</td>
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<td>Residual</td>
<td>24</td>
<td>5.0286</td>
<td>0.2095</td>
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<td>Total</td>
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Table D.4 LSD for Color

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<tr>
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<tr>
<td>d.f</td>
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<td>l.s.d</td>
<td>0.5050</td>
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### Table D.5 Two way ANOVA no blocking for Taste

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<th>s.s</th>
<th>m.s</th>
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<th>F pr</th>
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<tr>
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<td>23.2571</td>
<td>5.8143</td>
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<td>Panelist</td>
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<td>24.3429</td>
<td>4.0571</td>
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<td>Residual</td>
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<td>5.9429</td>
<td>0.2476</td>
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<td>Total</td>
<td>34</td>
<td>53.5429</td>
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### Table D.6 LSD for Taste

<table>
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<tr>
<td>d.f</td>
<td>24</td>
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<tr>
<td>l.s.d</td>
<td>0.5490</td>
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**Table D.7** Two way ANOVA no blocking for Texture

<table>
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<tr>
<th>Source of variation</th>
<th>d.f</th>
<th>s.s</th>
<th>m.s</th>
<th>V.r</th>
<th>F pr.</th>
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</thead>
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<tr>
<td>Formulation</td>
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<td>47.0286</td>
<td>11.7571</td>
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<td>16.5714</td>
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<td>Residual</td>
<td>24</td>
<td>8.5714</td>
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<td>72.1714</td>
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**Table D.8** LSD for Texture

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<tr>
<td>d.f</td>
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<td>l.s.d</td>
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### Table D.9 Two way ANOVA no blocking for OA

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### Table D.10 LSD for OA

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</tr>
<tr>
<td>d.f</td>
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<td>24</td>
</tr>
<tr>
<td>l.s.d</td>
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<td>0.783</td>
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Color plates

Plate no 1. Sensory evaluation of yoghurt

Plate no 2. Lab analysis

Plate no 3. Lab analysis

69