OPTIMIZATION OF SMP AND SUGAR PROPORTION FOR WHEY BASED YOGHURT AND ITS QUALITY EVALUATION

by

Dinesh Adhikari

Department of Food Technology

Central Campus of Technology

Institute of Science and Technology

Tribhuvan University, Nepal

2021

Optimization of SMP and Sugar Proportion for Whey Based Yoghurt and its Quality evaluation

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

by

Dinesh Adhikari

Department of Food Technology

Central Campus of Technology, Dharan

Institute of Science and Technology

Tribhuvan University, Nepal

2021

Tribhuvan University

Institute of Science and Technology

Department of Food Technology

Central Campus of Technology, Dharan

Approval Letter

This *dissertation* entitled *Optimization of SMP and Sugar Proportion for Whey Based Yoghurt and its Quality Evaluation* presented by Dinesh Adhikari has been accepted as the partial fulfillment of the requirements for the B. Tech. degree in Food Technology.

Dissertation Committee

1. Head of the Department ______________________________

(Mr. Navin Gautam, Asst. Prof.)

2. External Examiner ____________________________________

(Mr. Anup Halwai, FTQC Division Head)

3. Supervisor _______________________________________

(Mr. Dambar Bahadur Khadka, Asst. Prof.)

4. Internal Examiner _____________________________________

(Mrs. Babita Adhikari Dahal, Assoc. Prof.)

5. RMC Member ______________________________

(Mr. Basanta Kumar Rai, Prof.)

November 22, 2021

Acknowledgements

I express my heartfelt gratitude to my guide Mr. Dambar Bahadur Khadka for his kind support, guidance, encouragement and constructive recommendation on doing this research.

I am also thankful to Asst. Prof. Navin Gautam, Chairperson, Department of Food Technology and Assoc. Prof. Dil Kumar Limbu, Campus Chief, Central Campus of Technology for making available facilities to carry out the best in this dissertation work.

My sincere thanks go to all my friends who assisted me directly and indirectly, throughout the work. Special thanks must be bestowed to my friends, juniors and seniors, Upendra Pokharel, Asish Kshetri, Sanjog Kharel, Ranjan Shrestha, Anup Poudel, Trilochan Pandey, Samyog Sangroula and Nabindra Shrestha for their support during the completion of this work. I salute all those whose perceptions, observation and inputs have helped me directly or indirectly.

Many thanks and gratitude have expressed to all the teachers and staff members, librarian for their support. My special thanks go to Mr. Prajwal Bhandari, Head Laboratory Assistant, CCT, Hattisar, Dharan, Mr. Sachin Basnet, and Mr. Mahesh Shrestha for making my entire study in an enthusiastic and passionate environment.

I would like to express my love and deep regards to my respected parents, whose inspiration and motivation brought me to this stage. Without their support, I would not have been able to make this accomplishment.

Date of Submission:

Mr. Dinesh Adhikari

Abstract

The main aim of this dissertation is to optimization of proportion of SMP and sugar for whey based yoghurt and its quality evaluation. Response surface methodology (RSM) was adopted for optimization of SMP and sugar for the preparation of whey based yoghurt with percentage syneresis, appearance, texture and overall acceptability as response. Paneer whey pH adjusted to 6.8 was used for preparation of yoghurt. Whey based yoghurts prepared from eleven treatment combinations varying SMP (8-14%) and sugar (2-6%) obtained from Central Composite Design using experimental design version 11 was subjected to RSM analysis for optimization. Four optimized formulations solutions having SMP and sugar as coded A (14% SMP and 2% sugar), B (14% SMP and 2.22% sugar), C (14% SMP and 2.65% sugar) and D (14% SMP and 3.46% sugar) obtained after RSM, were prepared for whey based yoghurt and subjected to sensory analysis comparing to control yoghurt from milk as coded E (4% SMP and 3% sugar).

The results revealed that addition of SMP rather but not the sugar had significant linear effect on reducing percentage syneresis of whey based yoghurt. Similarly, the addition of SMP rather but not sugar had significant linear effect on increasing average score of both texture and overall acceptability of whey based yoghurt. But, the addition of SMP had significant linear effect on increasing average score of appearance of whey based yoghurt. While, the addition of sugar have significant linear effect on decreasing average score of appearance of whey based yoghurt. From RSM and sensory evaluation, addition of 14% SMP and 3.46% sugar provided the whey based yoghurt best in terms of sensorial properties, had having minimum syneresis, maximum appearance, maximum texture, maximum overall acceptability and contain protein, fat, acidity, total solid, lactose, ash, moisture and pH were found 5.26%, 0.4%, 1.06%, 24.28%, 6.82%, 1.76%, 75.79% and 4.07 respectively.

Contents

List of Tables

List of Figures

List of Abbreviations

Abbreviations	Full form
SMP	Skim milk powder
NFDM	Non-fat dry milk
UHT	Ultra high temperature
HTST	High temperature short time
LTLT	Low temperature long time
ANOVA	Analysis of variance
LSD	Least significant difference
LAB	Lactic acid bacteria
SNF	Solid not fat
WPC	Whey protein concentrates
WPI	Whey protein isolate
AIDS	Acquired immunodeficiency syndrome
BOD	Biological oxygen demand
RSM	Response surface methodology
DOE	Design of experiments
CLA	Conjugated linoleic acid

Part I

Introduction

1.1 General introduction

Yoghurt is a fermented product obtained through an anaerobic fermentation of lactose in milk by relevant microorganisms most of which are classified as pro-biotic (Tull, 1997). Yoghurt is a cultured dairy product produced by fermenting milk, with or without added non-fat dry milk (NFDM) with *Lactobacillus bulgaricus* and *Streptococcus thermophilus* bacteria. It usually contains 12-14% total milk solids and has soft, friable custard like consistency, and a clear and distinct acid flavor. Yoghurt is usually produced by heating the mix to 80-85°C for 30 min to pasteurize it and to modify the milk proteins so that they will provide the proper viscosity and gelation with a minimum of syneresis in the product (Morr, 1985).

In general, yogurt is made with a variety of ingredients including milk, sugars, stabilizers, fruits and flavors, and a bacterial culture (*Lactobacillus bulgaricus*). During fermentation, these organisms interact with the milk and convert it into a curd. They also change the flavor of the milk giving it the characteristic yogurt flavor of which acetaldehyde is one of the important contributors. The primary byproduct of the fermentation process is lactic acid. The acid level is used to determine when the yogurt fermentation is completed which is usually three to four hours. The suppliers of these yogurt cultures offer various combinations of the two bacterial types to produce yogurts with different flavors and textures (Khadka, 2018). Fermented-milk products such as yoghurt and soured milk contain bacteria from the Lactobacilli group. These bacteria are found in the digestive tract naturally and help to cleanse and repair it. Therefore the introduction of fermented products into the diet can help prevent certain yeasts and bacteria which may cause illness (Fellows and Hampton, 1992).

Whey is the liquid fraction that remains following manufacture of cheese, chhana, paneer and casein. Production of whey in Nepal according to the different projects associated with DDC in the year 2071/2072 was estimated to be more than 3.8 million liters (Bohora, 2018). The world production of whey is estimated at about 165 million tones. Of which cheese whey contributes about 95 %. In India, the major source of whey is from the production of chhana and paneer. In the absence of systematic survey/statistics the estimated production of whey is about 5 million tons per annum (Gupta, 2008).

The utilization was 75% in Europe and probably less than 50% in the rest of the world and as a result a very large amount of material with potential value as a food or feed is wasted. Whey is a rich source of nutritious components and its biological components has proven its effects in treatments of several chronic diseases like cancer, cardiovascular, AIDS etc. As it is nutritionally too rich it can also be used in infant, geriatric and athletic food. Whey protein has potential as a functional food component to contribute to the regulation of body weight by providing satiety signals that affect both short-term and long-term food intake regulation. Whey ingestion activates many components of the food intake regulatory system. Therefore whey protein has potential as physiologically functional food component for persons with obesity (Aneja, 2002).

1.2 Statement of the problem

Modern industrial processing techniques such as ultrafiltration (UF), reverse osmosis (RO), spray drying, hydrolysis, electro-dialysis, ion-exchange, fermentation and protein fractionation, among others, can be applied to transform whey into a major source of ingredients with differing functional and nutritional properties that could be used in food and dairy industry. The predominant driving force behind the development of whey utilization has been stringent regulations imposed by the environmental pollution agencies all over the world. Other aspect relates to economic return from whey, which contains almost half the solids of original milk. Presence of lactose, protein, minerals and water soluble vitamins make the whey a highly nutritious product (Bohora, 2018).

Worldwide whey production is estimated at around 180 to 190million ton/year; of this amount only 50% is processed (Baldasso *et al.*, 2011). Approximately 50% of worldwide cheese-whey (CW) production is treated and transformed into various foods and feed products. About half of this amount is used directly in liquid form, 30% as powdered cheese-whey, 15% as lactose and its byproducts and the rest as cheese whey- protein concentrates (Spalatelu, 2012). Despite significant gains in the amount of whey being processed, a large amount of whey produced still is disposed as raw whey. In small plants, the choice remains some form of disposal, be it municipal treatment, spreading raw whey on local farm lands for its nutrient value or feeding to local livestock. Further, paneer whey, because of high mineral content and low pH pose considerable difficulties in utilization and, therefore, mostly remain unutilized (Bohora, 2018). Paneer whey is produced in large amount in developing country where processing of paneer whey is limited by different factor mainly lack of technology/ machinery. So, direct utilization of such whey into yoghurt production could be economic as well as healthy approach for their best utilization. However, before developing of such paneer whey-based yoghurt, addition and optimization of SMP and sugar to produce good yoghurt gel and evaluation of their quality would be very reliable part of the study.

1.3 Objectives

1.3.1 General objective

The general objective of the study was to determine optimum amount of SMP and sugar addition in whey based yoghurt preparation and to evaluate its quality.

1.3.2 Specific objectives

The specific objectives of this work were to:

a. Study the effect of SMP and sugar addition on syneresis, appearance, texture and overall acceptability of yoghurt.

b. Determine optimum amount of SMP and sugar addition for yoghurt preparation.

c. Determine sensory acceptability of optimized whey based yoghurt.

d. Determine syneresis of optimized whey based yoghurt.

1.4 Significance of the study

Whey is a by-product of the dairy industry, which for years was thought to be insignificant and was either used as an animal feed or it was disposed of as waste. Whey is a serious pollutant as it imposes a very high BOD of 30,000- 50,000 mg/lit and chemical oxygen demand of 60,000-80,000 mg/lit. Discarding of whey also constitutes a significant loss of potential nutrients and energy and has been looked upon seriously by the environmentalists and technologists due to its potent polluting strength (Macwan *et al.*, 2016). In context of

Nepal, whey is most of the time disposed as waste. Over the last years several studies were carried out concerning the importance of whey is nutritional value and the properties of its ingredients. It is now accepted that main content, whey proteins, have antimicrobial, antiviral and anti-oxidant properties. Supplementation of whey helps to improve muscle protein synthesis and promote the growth of lean muscle mass. Whey protein is considered a complete protein as it contains all 9 essential amino acids. It is low in lactose content.

Growing environmental pollution and problems has pressurized the cheese and paneer manufacturers to stop dumping whey into the streams and sewage system. Whey is proved to be serious pollutant with very high BOD when disposed as waste and nutritious when consumed. Therefore, this study is aimed to utilize whey for the preparation of whey based yoghurt. Whey based yoghurt improves the flavors, texture, appearance and shelf life of beverages and in other hand also reduces large part of dairy waste adding environment benefit to the world and economic benefits to the producer and consumer. Thus, preparation of whey based yoghurt may serve as one such effort that can increase the utilization of whey (Spalatelu, 2012).

1.5 Limitations of the study

1. Only the paneer whey was utilized.

2. Shelf life of yoghurt was not carried out.

Part II

Literature review

2.1 A brief history of yoghurt

Yogurt is derived from Turkish word “Jugurt “reserved for any fermented food with acidic test. Currently yogurt of many types including kefir, Greek style yogurt, Swiss and fruit yogurts can be found. Yogurt is one of the most popular fermented dairy product widely consumed all over the world. It’s obtained by lactic acid fermentation of milk by the action of a starter culture containing Streptococcus thermophilus and Lactobacillus delbrueckii ssp. Bulgaricus (Fadela *et al.*, 2009) and Ergo a traditonal femented milk in Ethiopia is semi-solid, smooth and uniform appearance and usually has a white milk color with pleasant odor and taste when prepared carefully. It constitutes primarily sour milk from which other products may be processed (Bereda *et al.*, 2014). Yogurt has assumed different forms like stirred, set and frozen liquid yogurt. The nutritive value of yogurt is attributed to the fat content, sugar and casein (El-Malt *et al.*, 2013). Proteins in yogurt are of excellent biological quality, as are that in milk, because the nutritional value of milk proteins is well preserved during the fermentation process. Therefore, yogurt is recommended for sick and convalescent people.

Yoghurt is one of the most popular fermented milk products worldwide and has gained widespread consumer acceptance as a healthy food. It provides an array of nutrients in significant amounts, in relation to its energy and fat content, making it a nutrient-dense food. In particular, yoghurt can provide the body with significant amounts of calcium in a bioavailable form. Furthermore, yoghurt has many health benefits beyond the basic nutrition it provides, such as improved lactose tolerance, a possible role in body weight and fat loss, and a variety of health attributes associated with probiotic bacteria (Mckinley and Michelle, 2005).

Historically, fermentation was used by humans for preservation of milk. It is thought to have originated in the Middle East area even before the Phoenician era. In Egypt, the consumption of traditional fermented milk such as laban rayeb and laban khad dates back to around 7000BC. The Vedas (Indo-Aryan treatises) also mention dadhi (modern-day yogurt) dating back to 5000 years BC. Dadhi or dahi is still a crucial component of the

South Asian diet. It is produced in most Indian households and consumed daily. The word yogurt is believed to have been first used by the Turks in the 8th century, which appeared as yoghurt. It is thus assumed that the Turkish nomads in Asia made yogurt. Another legend, however, states that yogurt was first prepared or invented by the Balkan people. Sour milk, prokish, was prepared from sheep's milk by the peasants of Thrace. South Asian regions (India, Pakistan, Nepal, and Bangladesh), as well as southwest Asia regions (Iran, Iraq, Balkans, Turkey, Syria) are among the largest producers and consumers of fermented milk products (including yogurt). It is believed that the invasion of Mongols, Tartars, and other Asian rulers to Russia and Europe also contributed to the spread of yogurt and fermented milk to other parts of the world (Chandan *et al.*, 2017).

Yogurt is considered by most regulatory agencies worldwide to be a fermented milk product that provides digested lactose and specifically defined, viable bacterial strains, typically *Streptococcus thermophilus* and *Lactobacillus bulgaricus*. It is a source of several essential nutrients, including protein, calcium, potassium, phosphorus, and vitamins B2 and B12, and serves as a vehicle for fortification. Yogurt is an ancient food that has gone by many names over the millennia: katyk (Armenia), dahi (Nepal), zabadi (Egypt), mast (Iran), leben raib (Saudi Arabia), laban (Iraq and Lebanon), roba (Sudan), iogurte (Brazil), cuajada (Spain), and matsoni (Georgia, Russia, and Japan). It is believed that milk products were incorporated into the human diet around 10 000–5000 BC, with the domestication of milk-producing animals (cows, sheep, and goats, as well as yaks, horses, buffalo, and camels) (Fisberg and Machado, 2015).

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

Yogurt also traces its roots to the Caucasus Mountain region of Russia. The people of this rugged region were commonly nomadic – and as subsistence used both the milk and meat of cows, sheep, goats, and yaks. The fermented milk product traditional to this region, kefir, is a liquid cultured product whose name translates to ‘‘good feeling’’. It also earned the reputation as being a healing drink and was considered a ‘‘gift of the gods’’. Kefir was widely consumed by all families, and the bacteria culture that was used to ferment this product was prized and guarded most closely. The broad popularity of kefir in Russia dates back to the early 1900s.The society was looking to popularize this product for its reputed health and aging benefits. The royal Caucasus family closely guarded the culture used to produce kefir (Tribby, 2008).

2.2 Milk

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

The pH of fresh milk ranges from 6.5 to 6.7 with an initial acidity of 0.14 to 0.16%. pH and acidity measurements are often used as acceptance tests and quality of milk. These tests are used to monitor processes such as cheese-making and yoghurt making. Approximately 80% of the milk proteins are caseins which consist of α-, β-, κ-, and ϒ caseins. The casein micelles and the fat globules give milk most of its physical characteristics, and give taste and flavor to dairy products. Processing of milk by its nature involves the imposition on a changing colloidal system. This is because the colloidal particles in milk alter their nature and behavior. For instance changing pH causes disintegration rearrangement of the micelles and if pH is low enough, new particles of iso-electric casein are formed. Also, heating to high enough temperatures causes the binding of serum proteins to the micelle to breakdown (Mottar *et al*., 1989).

2.3 Milk fermentation and biochemical changes

Food fermentation is one of the oldest known uses of biotechnology. Fermented food and beverages dated back many thousands of years and it continues to provide an important part of human diet supplying about 20-40% of food worldwide (Campbell-Platt, 1994). In recent years this method has been widely used to improve food quality, safety, nutritional values and palatability and to develop new food products. Microbial fermentation in food fermentation involves the breakdown of sugar and protein which results in the production of a large array of organic compounds that contribute to the flavor, preservation and outer appearance of the food product (Hugenholtz *et al.*, 2000). Milk fermentation is initiated by *lactobacilli* and *streptococci* bacteria which use nutrients in milk for their growth and alter the nutritional composition and physical appearance of milk (Urbiene and Leskauskaite, 2006). Milk fermentation can be defined as any modification of the chemical or physical properties of milk or dairy products resulting from the activity of microorganisms or their enzymes. It occurs when bacteria break down milk sugars and other components of milk to give lactic acid, alcohols, carbon dioxide etc. Lactose, fat and citric acid comprise the important fermentable compounds of milk. Lactose a disaccharide, is the chief source of carbon while fat and citric acid provides hydrogen and oxygen source respectively (Davies and Law, 1984).

Lactose is used by lactic acid bacteria (LAB) as the principal source of carbon for growth and energy. It is initially hydrolyzed by lactase into galactose and glucose (Greenberg and Mahoney, 1982) followed by subsequent glucose conversion to D- or L-lactic acid via the glycolytic, Embden-Meyerhof-Parnas pathway (Hemme *et al.*, 1980). The lactic acid fermentation consists of two major pathways that include homolactic fermentation which produces lactic acid and heterolactic fermentation which produce equimolar amount of lactic acid, carbon dioxide and ethanol (Vakil and Shahani, 1970). Protein is degraded by proteolysis and increases the peptide and free amino acid content of fermented milk products (Alm, 1982). Lipids are sparingly hydrolyzed by LAB lipases which are more active towards lower but not higher molecular weight triglycerides (Collins *et al.*, 2003). Although lipases are present in *S. thermophilus* & *L. delbrueckii* subsp. *bulgaricus*, they have little effect on free fatty acid content of fermented milk products (Fernandes *et al.*, 1991). LAB require minerals and vitamins for growth (as mineral catalysis and mediators in the enzymatic reaction respectively) but their requirement is small and would not significantly alter the total content of fermented milk products. The bioavailability of some of the minerals may be changed due to pH changes caused by fermentation (Fernandes and Shahani, 1989).

2.4 Probiotics

Probiotics (derived from the Greek word meaning "for life") are live microbes which influence the well-being of their host through their effect on the intestinal microflora. It was also called "a live microbial food ingredient that is beneficial to health". Probiotic improves intestinal microbial balance and reduction in these bacteria which are naturally found in the human small intestine and large intestine increases the presence of potentially pathogenic microbes (Salminen *et al.,* 1998). Many probiotics are members of the genera of *Lactobacillus* and *Bifidobacteria*. At present approximately ten to fifteen bacterial strains have passed extensive investigations for some of the probiotic criteria. Some probiotic strain with scientific documentation include: *Lactobacillus rhamnosus, Lactobacillus acidophilus, Lactobacillus paracasei, Lactobacillus plantarum, Lactobacillus delbrueckii* subsp. *bulgaricus, Bifidobacterium lactis ,Bifidobacterium longum* and *Lactobacillus gasseri* (Kneifel, 2000). Over the past decade, considerable interest has developed in the use of probiotic organisms in food, pharmaceutical and feed products (Crittenden *et al.,* 2005).

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

2.5 Health benefit of probiotics

Several reported health benefits of probiotic bacteria are reduced duration of diarrhea, antagonistic effects against pathogenic microorganisms, improved lactose digestion regulation of intestinal motility, and reduced activities of cancer-related enzymes, improved calcium resorption and provision of water soluble vitamins (Crittenden *et al.*, 2005). The action of probiotics on intestinal flora results in vital benefits, including protection against pathogens, development of the immune system and positive effects on colonic health and host nutrition. There is also evidence to suggest that certain species/strain of probiotics is anti-carcinogenic. Other important properties that have been attributed to probiotics include prevention and treatment of gastrointestinal disorders, reduction of food intolerance, modulation of the host immune responses, and prevention of cancer and cardiovascular diseases (reduction of serum cholesterol and lipids) (Wollowski *et al.*, 2001). Multiple species or high numbers of probiotic organisms may be required to be administered simultaneously to achieve colonization, as shown in the treatment of pouchitis and in reducing the risk of urogenital infections. It is becoming more apparent that the more these intestinal-friendly bacteria are present in the colon, the lower are the chances of acquiring colon diseases (Reid *et al.*, 2003).

2.6 Yoghurt culture bacteria

The thermophilic LAB, *Streptococcus thermophilus* & *Lactobacillus delbrueckii* subsp. *bulgaricus* are used together as important starter microorganisms in the production of yoghurt and some kind of cheeses. Because both bacteria are able to grow alone in milk, this indirect positive interaction is called proto-cooperation (Fredrickson, 1977). This positive relationship often has a beneficial effect on bacterial growth and on the production of lactic acid and aromatic compounds. Lactic acid production results in the lowering of pH and this makes product unsuitable for growth of spoilage or pathogenic microorganisms (Donkor *et al.*, 2007). *S. thermophilus* grows faster and produces both acid and carbon dioxide. The formate and carbon dioxide produced stimulates *L. bulgaricus* growth. On the other hand, the proteolytic activity of *L. bulgaricus* produces stimulatory peptides and amino acids for use by *S. thermophiles.* These microorganisms are ultimately responsible for the formation of typical yoghurt flavor and texture. The yoghurt mixture coagulates during fermentation due to the drop in pH. The streptococci are responsible for the initial pH drop of the yoghurt mix to approximately 5.0. The lactobacilli are responsible for a further decrease to pH 4.0. Lactic acid, acetaldehyde, acetic acid and diacetyl are the fermentation products that contribute to flavor (Chandan and Shahani, 1995).

2.7 Types of starter culture

2.7.1 Pure and mixed culture

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

2.7.2 Mesophilic and thermophilic culture

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

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

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

2.7.3 Liquid culture

The liquid cultures are generally no longer distributed in commercial practice. To prepare a liquid culture the organisms are propagated in a suitable medium such as milk or whey and maintained in an active condition by periodic transfers. In general, a liquid culture contains about 109 organisms per ml of starter (Neilson and Ullum, 1989).

2.7.4 Powdered culture

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

2.7.5 Frozen culture

Deep frozen cultures are prepared by deep freezing a concentrated, liquid culture at the point of the bacteria growth at which the activity is at its highest. They are preserved by lyophilization in small vials. Super-concentrated, deep frozen cultures are made by adding growth-promoting substances to a milk substrate, continuously neutralizing the lactic acid 14 formed by means of ammonium hydroxide, and finally concentrating the culture in a desludging centrifuge/ bactofuge. The concentrate is pelletized by being frozen as individual drops in liquid nitrogen. The culture is stored at -196°C until it is dispatched to the dairies in foamed plastic boxes containing dry ice (Neilson and Ullum, 1989).

2.8 Preparation of starter culture

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

2.9 Metabolism characteristics of LAB in yoghurt

2.9.1 Carbohydrate metabolism and acid production

LAB needs a sugar for energy production and subsequent growth. Fermentation of lactose is called glycolysis or glycolytic pathway. LAB ferments lactose into pyruvic acid, which is then reduced to lactic acid. Thus, lactic acid is obtained as the sole product and this process is called homo-lactic fermentation (Tamime and Robinson, 1999b). Lactic acid reduces the pH of the milk and leads to a progressive solubilization of micellar calcium phosphate. This causes the demineralization of casein micelles and their destabilization, which generates the complete precipitation of casein in a pH range of 4.6-4.7 (Zourari *et al.*, 1992). The increase in lactic acid decreases pH and causes acid coagulation of milk with a clot formation in the final semi-solid mass (Baglio, 2014).

2.9.2 Protein metabolism

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

2.9.3 Formation of flavor components

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

2.9.4 Synthesis of organic acid and vitamins

Dairy products have generally been considered an excellent source of B vitamins, riboflavin, niacin, vitamin B-6, and vitamin B-12. A greater loss of vitamins may occur during the processing of yogurt because vitamins are more sensitive to changes in environmental factors (Buttriss, 1997). Folate is the best example of a B vitamin that some LAB species synthesize. The folate content of yogurt can vary widely, ranging from 4 to 19 μg/100g (Shahani and Chandan, 1979). In the metabolism of lactic acid bacteria, certain metabolic processes can synthesize a variety of organic acids like fumaric, succinic, benzoic, acetic, butyric, pyruvic, and formic acid. In supplemented yogurts, the content of citric, orotic, and hippuric acids also increases significantly (Venica *et al.*, 2014).

2.9.5 Lipid metabolism

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

2.9.6 Production of exopolysaccharides

Several Gram-negative and Gram-positive bacteria, including lactic acid bacteria, produce exocellular polysaccharides (Macura and Townsley, 1984). Some strains of *S thermophilus* and *L bulgaricus* produce neutral exopolysaccharides. The slime secreted by a strain of *L bulgaricus* and *S thermophilus*, contains galactose, glucose, mannose and small amounts of rhamnose, xylose and arabinose. Production of polysaccharides improves viscosity and texture, increase resistance to mechanical handling and decrease susceptibility to syneresis (Cerning *et al.*, 1990).

2.9.7 Production of antimicrobial compounds

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

2.10 Yoghurt

Yoghurt is a product of the lactic acid fermentation of milk by addition of a starter culture containing *Streptococcus thermophilus* and *Lactobacillus delbrueckii* ssp. bulgaricus. In some countries less traditional microorganisms, such as *Lactobacillus helveticus* and *Lactobacillus delbrueckii* ssp. lactis, are sometimes mixed with the starter culture (Tamime, 2002).

Proteins in yogurt are of excellent biological quality, as are that in milk, because the nutritional value of milk proteins is well preserved during the fermentation process (Gursoy *et al.*, 2010). It has been argued that protein from yogurt is more easily digested than is protein from milk, as bacterial pre-digestion of milk proteins in yogurt may occur

(FAO, 2013). Both the caseins and the whey proteins in yogurt are rich source of Amino acids (93%) and high in nitrogen availability. Amino acids like proline and glycine are present in free form and higher contents in yogurt than in milk (Damian, 2013). Although much of the yogurt sold in industrialized countries is produced from skimmed milk, traditional yogurt has always contained some 3-4 g per100 g milk fat; indeed concentrated yogurt or Greek-style yogurts will contain 9-10 g per100 g fat (Tamine and Robinson, 1999)). Conjugated linoleic acid , a type of essential fatty acid found almost exclusively in the fat of dairy products, can be obtained only through the diet because it is not produced by the human body (Hassan and Amjad, 2010). CLA has been shown to be a powerful natural anti-carcinogen that also can reduce the risk for cardiovascular disease, help fight inflammation, reduce body fat, especially abdominal fat, lower cholesterol and triglycerides, increase metabolism, lower insulin resistance and enhance the immune system (Lee and Lucey, 2010).

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

2.11 Coagulum formation in yoghurt

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

The formation of yoghurt gel is the result of the 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, 1999b).

2.12 Health benefits of yoghurt

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

1. Many people who cannot tolerate milk either because of protein allergy or lactose intolerance can enjoy yoghurt. The culturing process makes yoghurt more digestible than milk.

2. The friendly bacteria in yoghurt reduces the conversion of bile into carcinogenic bile acids and this seems to deactivate harmful substances (such as nitrates and nitrites before they are converted to nitrosamines) before they can become carcinogenic (Commane *et al.*, 2005).

3. Consumption of yoghurt during antibiotic prescription will minimize the effects of the antibiotic removal of friendly bacteria in the intestines. The live bacterial cultures in yoghurt can help replenish the intestines with helpful bacteria before the harmful ones take over.

4. Yoghurt can decrease yeast infection and it has prevention of growth of pathogenic bacteria.

5. Yoghurt is a rich source of calcium. Because of the live active cultures in yoghurt increase the absorption of calcium, serving of yoghurt gets more calcium into the body than same volume of milk. Daily intake of yoghurt reduces the risk of osteoporosis (Smith *et al.*, 1985).

6. Yoghurt is the excellent source of protein. Besides being a rich source of proteins, the limited proteolysis of the milk proteins during fermentation makes these proteins easier to digest. For this reason proteins are often called “pre-digested protein” and have beneficial uses for certain people who lack the digestive enzyme due to disease states(Savaiano and Levitt, 1984).

7. Fermented milk products are excellent sources of dietary minerals particularly calcium, phosphorous, magnesium and zinc.

8. Several LAB are capable of synthesizing B-vitamins and their concentration in fermented milk is generally high.

9. There are few studies that have shown that yoghurt can reduce the blood cholesterol. This is because the live cultures in yoghurt can assimilate the cholesterol or because yoghurt binds bile acids (which has also been shown to lower cholesterol) or both.

10. Yoghurt and various dairy contain LAB are believed to confer a variety of important nutritional and therapeutic benefits to consumers including antimutagenic, anticancer, and anti-carcinogenic activity (Fernandes and Shahani, 1989).

11. Certain whey peptides are known to have biological activity such as opioid and bactericidal activity.

12. Several peptides arising from proteolysis of milk proteins have been cited as exerting biological activity and have pharmalogical effects on the nervous system, cardiovascular system and digestive system including immuno-modulating properties (Meisel and Schlimme, 1990).

2.13 Method for improving body of yoghurt

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

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 simplest and cheapest method is to use a carbohydrate gum (carrageenan, alginate, agar, etc.) at a concentration of about 0.3% (Tamime and Robinson, 1999b).

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

2.14 Yoghurt manufacturing

There are two main types of yoghurt, set and stirred, based on method of production and physical structure of the coagulum. Set yoghurt is the product formed when fermentation/coagulation of milk is carried out in the retail container, and the yoghurt produced is in a continuous semi-solid mass. By contrast, stirred yoghurt results when the coagulum is produced in a bulk and the gel is broken before cooling and packing (Robinson and Tamime, 1993). Manufacturing methods of yoghurt vary considerably depending up on country, raw materials used, size of operation, product formulation and type of product being manufactured, but there are a number of common principles, which determine the nature and quality of the final product (Staff, 1998).

2.14.1 Preparation of base milk

To make a good quality product, raw milk used must be of low bacterial count, free from antibiotics, sanitizing chemicals, mastitis, milk colostrum, and the milk also should be free from contamination by bacteriophages (Haj *et al.*, 2007). The use of lactoperoxidase-thiocyanate - hydrogen peroxide (LP) activated milk and antibiotic (penicillin/

streptomycin) containing milk should be avoided as it retard metabolic activity of yoghurt (Sarkar, 2008).

In order to meet legal requirements and to obtain consistent product quality, standardization of milk total solids content (mostly milk protein content) and milk fat content are important. The level of total solid in the milk is significant for both the consistency and aroma of the manufactured yoghurt (Tamime and HC, 1980). The total solids level in milk for yoghurt manufacture can vary from as low as 9% in skim yoghurt to over 20% in other types of yoghurt. According to Tamime and Robinson (1999a), consistency improves when the content of milk total solids increases from 12–20 % especially protein content. The viscosity of yoghurt is almost wholly dependent on the protein content of the milk. Hence, a high protein concentration is essential for production of viscous yoghurt. Penna *et al.* (2006) concluded that the optimum yoghurt consistency could be achieved from milk containing 14-16% total solids. Although small difference in consistency is achieved when the content of total solids varies from 16–20 %, there is little interest in the use of concentrations above 16 % and, most low-fat yoghurt fall within the range of 14-15%. The protein or SNF content of milk can be increased by concentration of milk or by dry-matter enrichment.

2.14.2 Standardization and mix preparation

In most yoghurt formulation, standardization of milk fat and SNF contents is done to bring uniformity in the product quality. When the milk arrives at the plant, its composition is modified before it is used to make yogurt. This standardization process typically involves reducing the fat content and increasing the total solids. The fat content is reduced by using centrifugation to separate fat from milk. For stirred yogurt manufacture, the solids content of the milk is usually increased to about 16% with 1-5% being fat and 11-14% solids-not-fat (SNF). This is accomplished either by evaporating off some of the water, or adding concentrated milk or milk powder, other ingredients. Increasing the solids content improves the nutritional value of the yogurt, makes it easier to produce a firmer yogurt and improves the stability of the milk substance is fermented until it becomes yogurt. Fruits and flavorings are added to the yogurt before packaging the yogurt by reducing the tendency for it to separate on storage. Yogurt mix should have a minimum SNF of 12% to increase the viscosity and also to increase the resistance to "wheying off". After the solids composition is adjusted, stabilizers are added and the milk is pasteurized (Tribby, 2008).

2.14.3 Homogenization

Homogenization is an integral part of the yoghurt manufacturing process. It is usually carried out before heat treatment. However, in some cases it may take place after heat treatment (Tamime and HC, 1980). It is carried out chiefly to effect a homogeneous dispersion of the milk mix constituents and to increase the viscosity and coagulum stability of the yoghurt. Homogenization of milk for yoghurt manufacture is considered to prevent fat separation (cream layer formation) during storage, improve consistency, increase whiteness and reduce whey separation (Tamime and Robinson, 1999a). It also improves the ‘mouth-feel’ of the product and thus increases the organoleptic quality of yoghurt.

Milk is usually homogenized at pressures in the range 10-20 MPa, at temperatures in the range 55- 65 °C and prior to heat treatment of the mix. The tendency for cream layer formation is reduced in homogenized milk because of the formation of small fat globules (< 2 µm). During homogenization, casein and some whey protein adsorb at the fat globule interface and this effectively increases the number of structure-building components (Walstra, 1999). Since protein was adsorbed onto the surface of homogenised fat globules, higher levels of fat increased the ability of the protein to immobilise water (Keogh and O'kennedy, 1998).

2.14.4 Pasteurization

Pasteurization of yogurt mixes can be accomplished by several different methods. As with any other dairy product, the purpose for pasteurization is to heat treat milk to eliminate pathogenic bacteria. In addition, it is very important to denature the proteins to attain the highest level of functionality from the milk proteins. Pasteurization also aids in the hydration of the stabilizers and dry ingredients that were added during blending, as well as adding a pleasant cooked flavor. The three main types of pasteurization are low temperature long time (LTLT) i.e., 63°C for 30 min, high temperature short time (HTST) i.e., 72-75°C for 15 sec, and ultra-high temperature (UHT) i.e., 125-138°C for 2-4 s (North and Park, 1927).

2.14.5 Cooling

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

2.14.6 Starter addition

The yoghurt starter consists of *Streptococcus thermophilus* and *Lactobacillus bulgaricus* in the ratio of 1:1. The symbiotic relationship between two organisms, at a given ratio is synergistic. Although they can grow independently, the rate of acid production is much higher when used together than either of the two organisms grows individually. The symbiosis is responsible for typical yoghurt flavor and texture (Donkor *et al.*, 2007).

2.14.7 Filling into cups

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

2.14.8 Incubation

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

2.14.9 Cooling and storage

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

2.15 Types of Yoghurt

2.15.1 Set type Yoghurt

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

2.15.2 Stirred type yoghurt

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

2.15.3 Drinking type yoghurt

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

2.15.4 Frozen yoghurt

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

2.15.5 Dried yoghurt

Yogurt powder is produced by fermenting non-fat milk using standard yogurt cultures until attain the desirable pH followed by a step of drying, most probably by freeze-drying. In addition, blended yogurt powder is manufactured by blending cultured non-fat milk, cultured whey, cultured whey protein concentrate, cultured dairy solids, nonfat dry milk and lactic acid which are similar to the flavor and functionality to that of the traditional yogurt powder (Kumar and Mishra, 2004).

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

2.15.6 Therapeutic yoghurt

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

2.16 Quality parameters of yoghurt

2.16.1 Syneresis

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

Syneresis of the acid-induced casein network in yoghurt occurs during storage, and is related to the amount of total solid and casein content in milk, incubation temperature, and rate of acidification and presence of stabilizers that interact with the casein network. The firmness of yoghurt is affected by homogenization, pH, denaturizing of β-lactoglobulin (whey proteins) and adjacency to casein micelles (Lucey *et al.*, 1998). Yoghurt is usually prepared from homogenized milk to improve stability. This process coats the increased surface of fat globules with casein, enabling the fat globules to participate as a copolymer with casein to strengthen the gel network and reduce syneresis (Keogh and O'kennedy, 1998).

2.16.2 Acidity and p^H

Acidity is one of the major indices for consumers' acceptability of plain yogurt since acid and flavor development go hand in hand in this fermented product of bacterial symbiosis. Wide variation in pH ranging between 4.53-3.27 has been reported for commercial yoghurt (Salji and Ismail, 1983). The range of acidity for the best consumption of yoghurt is (0.6-0.9%) as reported by (Ahmed, 2011).

2.16.3 Firmness

Firmness is defined as a force that needs to be applied in order to ensure a certain deformation in the composition of foods. Firmness is a parameter that plays a key role in determining the quality of yoghurt (Yildiz and Ozcan, 2019). Sodini *et al.* (2004) reported that long periods of storage affect some textural properties (firmness, syneresis) and noted that it may result from an increase in acidity and casein hydration. The firmness of yogurts is critical in establishing consumer preference. The most important textural characteristics of yogurt are firmness and the ability to retain water. These two properties are closely related to gel microstructure (Izadi *et al.*, 2015).

2.16.4 Apparent viscosity

Apparent viscosity is a quality parameter of the yoghurt. It is affected by the strength and number of bonds between casein micelles in yogurt, as well as their structure and spatial distribution (Izadi *et al.*, 2015).

2.16.5 Appearance

Appearance Set-style yoghurt gels should have a smooth, custard-like, semi-solid consistency with no surface whey. The appearance of a set gel should be smooth with no cracks, holes or other 'blemishes'(Lucey and Singh, 1997).

2.17 Whey

Whey may be defined, broadly, as the serum or watery part of milk remaining after separation of the curd that's result from the coagulation of milk by acid or proteolytic enzymes. Its composition will vary substantially, depending on the variety of cheese produced or the method of casein manufacture employed. On average, whey contains about 65 g of solids per 1 Kg of whey, comprising about 50 g lactose, 6 g protein, 6 g ash, 2 g non-protein nitrogen and 0.5 g fat. The protein fraction contains about 50% β-lactoglobulin, 25% a-lactalbumin and 25% other protein fractions including immunoglobulins (Zadow, 1994).

Whey is a by-product of the dairy industry, which for years was thought to be insignificant and was either used as an animal feed or it was disposed of as waste. Considering that over 145 million tons of whey is produced worldwide annually, the desire for new methods to utilize whey can be appreciated. Over the last years several studies were carried out concerning the importance of whey is nutritional value and the properties of its ingredients. It is now accepted that main content, whey proteins, have antimicrobial, antiviral and anti-oxidant properties. Due to the substantial difficulties encountered in the treatment of whey as a biological waste and its high potential to be valuable raw material for added value food and bioactive substances production the later tend to be the only accepted and popular trend of dealing with this dairy industry by-product. Productions of whey proteins by ultrafiltration, lactose hydrolysis products, and the use of whole whey or whey permeate as a fermentation feedstock are possible options. A large number of workers have carried out studies on the composition and processing of whey for its use in foods and animal feeds besides studying the nutritive, therapeutic and functional properties of whey (Macwan *et al.*, 2016).

2.17.1 Whey background and production

Whey is the largest byproduct of dairy industry obtained during manufacture of casein, cheese, paneer, chhana etc. Whey had been considered as the milk by products for years and it has been mainly dumped into lands, sewage, waterways, and oceans while partly used as animal feed. Dumping of whey globally pushed the environmental pollution to further increment which leads searching the new possibility for the utilization of whey. Global production of liquid whey from cheese and casein amounted to 192 million MT in 2015 and annual average growth of ~3-4%. The European Union and United state produce ~70% of whey in the world (Affertsholt and Pedersen, 2017).

Lactose is the major product while whey proteins, water soluble vitamins, and minerals are the secondary products. Much of the whey is spray dried to whey powder, whey protein concentrates and isolates which in return is used in food fortification (Smithers *et al.*, 1996).

2.17.2 Nutritional and health effects of whey

Whey protein is a mixture of beta-lactoglobulin, alpha lactalbumin, bovine serum albumin, and immunoglobins. It also contains proteins, albumins and globulins, which have outstanding nutritional qualities. The glutathione in whey protein is an antioxidant reduces the risk of cancer in animals, suggesting an avenue for future medical research. Whey protein is considered a complete protein as it contains all 9 essential amino acids i.e., isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan and valine. It also contains lactose, minerals and biologically active components (Alane *et al.*, 2017).

Whey proteins have antimicrobial, antiviral and anti- oxidant properties can offer a kind of protection against cancer and heart diseases and assist in the enhancement of immune defense (Bohora, 2018). Other benefits include:

1. Aiding weight loss with significantly loss of body fat and greater preservation of lean muscle.

2. Anti-cancer properties.

3. Lowering cholesterol.

4. Improve the immune response in children with asthma.

5. Reduced blood pressure in patients with hypertension and their risk of developing heart disease or stroke.

6. Reducing weight loss in people with HIV.

2.17.3 Net protein utilization of whey protein

The nutritional value of whey protein was found to be higher than that of milk protein by all four indices of nutritional quality. The differences between the two proteins in biological value and net protein utilization were of a high statistical significance. The differences in protein efficiency ratio and in protein retention efficiency were of probable significance ($P = 0.05$ or less considered significant). Net protein utilization, calculated as biological value multiplied by true digestibility, yielded values of 88.5 and 80.0 for whey and milk protein respectively, which agreed well with the values of 85.7 and 78.8 for net protein utilization determined by direct measurement of carcass protein deposition (Tomarelli and Bernhart, 1962).

2.17.4 Composition of whey

The composition of whey is given in Table 2.1

Table 2.1 Composition of whey

Parameter	Acid whey	Rennet whey
Total solids (%)	6.3-7.0	6.3-7.0
pH	3.8-4.6	5.6-6.0
Lactose (%)	5.03	5.01
Protein (%)	0.38	0.98
Fat (%)	0.13	0.34
Ash (%)	0.60	0.54
Lactic acid (%)	0.21	0.14
Calcium(ppm)	710.65	501.50
Phosphorous(ppm)	560.50	441.50

Source: Darade and Ghodake (2012)

2.17.5 Utilization of whey

Worldwide only about 50% of the total whey production is “dried or further processed into whey protein concentrate, lactose and other products for use in formulating human food and animal feed products. The remainder of the whey, especially from small cheese manufacturing plants, is treated by private or municipal waste treatment, fed to livestock or spread on agricultural land as fertilizer. Processing of whey is limited due to less demand for whey powder and further processed whey products, plus the generally unfavorable economics of whey processing, especially for the smaller sized processing operation (Bozanic *et al.*, 2014).

A substantial portion of whey powder, whey concentrate, reduced lactose/ reduced mineral whey, whey protein concentrate and lactose is used in human food product applications, whereas whey blends are used mainly in animal feed products. Whey-based blends are formulated to provide a composition similar to that of non-fat dry milk. Most of the whey concentrate for human food is used in dairy, bakery and confectionery products. Most of the acid whey used in human food product production is produced as dried and modified whey (Darade and Ghodake, 2012).

2.17.6 Therapeutic and functional value of whey

2.17.6.1 Therapeutic value of whey

Whey based beverages target a large scale of consumers - from old people to little children. Because of its health benefits, it was used to treat some illnesses, such as tuberculosis and skin and digestive tract diseases, since the time of Ancient Greece. Whey was also successfully applied for treatments of diarrhea, bile illness, skin problems, scales in the urinary tract and some intoxication. Due to high amount of whey proteins with nutritional value these beverages are ideal source of energy and nutrients for athletes. Whey proteins 11 are a rich source of branched chain amino acids (BCAA) like isoleucine, leucine and valine. BCAAs unlike other essential amino acids are metabolized directly into the muscle tissue and are first amino acids used during periods of exercise and resistance trainings (Shukla *et al.*, 2000).

Whey protein fractions include also lactoferrin, an iron-binding protein, Glycomacropeptide (GMP) which derives after cheese making using rennet and is naturally free of phenylalanine and alpha-lactalbumin which is a calcium binding protein. Due to presence of lactoferrin whey beverages can be used as functional food intended to improve iron absorption from food and/or help to keep pathogens from attaching to the intestinal walls. That is very important for nutrition of little children and babies. Furthermore, these beverages may improve absorption of calcium important for older population which is often suffering from osteoporosis (Russell *et al.*, 2006).

Whey possesses potent antioxidant activity mainly contributed by cysteine-rich proteins that aid in the synthesis of glutathione (GSH), a potent intracellular antioxidant, also investigated as an anti-aging agent. Detoxifying property contributed by Glutathione peroxidase, which is derived from selenium and cysteine, that converts lipid peroxides into less harmful hydroxyl acids and α-lactalbumin, which chelates heavy metals and reduces oxidative stress because of its iron-chelating properties. Immunoglobulin and lysozyme in whey provides immunity enhancing benefits to infants and others. Antihypertensive peptides isolated from bovine β-lactoglubulin, reduces blood pressure (Russell *et al.*, 2006).

Various minerals like, potassium, involved in the transmission of nerve impulses and muscular contractions; magnesium, depolarizes the nerve or muscle causing relaxation and help to lower blood pressure and supports an alkaline tissue pH; Calcium, used by the body to maintain an alkaline tissue pH, maintain bone density, cell wall integrity and nerve impulses. Lactoperoxidase inhibits the growth of iron dependent bacteria. Lactoferrin, inhibits the growth of bacteria (including pathogenic bacteria) and fungi, also regulates iron absorption and bio-availability. Whey also possesses the vitamins necessary for its utilization. It contains vitamins A, B1, B2, B3, B5, B6, C, D, and E (Onwulata and Huth, 2008).

2.17.6.2 Whey proteins as a functional ingredient

Along with physiological benefits, WP possess inherent excellent functional properties and desirable sensory characteristics enabling them to be used in numerous food applications including sport beverages, liquid meat replacements, baked products and processed meats, pasta, salad dressings, spreads and dips, artificial coffee creams, soups, ice cream, confectionary infant foods and various other dairy products. The use of whey proteins as functional ingredient is given in Table 2.2. In order for them to be used in these applications, WP should be extracted from whey using different fractionation methods, mainly membrane processing (Onwulata and Huth, 2008).

Table 2.2 Whey protein as functional ingredient

Function	Benefits	Uses
Emulsification	Creates stable emulsions and prevents fat globules from forming clumps	baked products, beverages, ice-cream, mixes mayonnaise-type dressings
Flavour enhancement	Brings out already present flavour or adds flavour	Baked products , beverages, confectionary, snacks
Gelling and heat setting	Maintain moistness and improves texture and mouth-feel	Baked products, beverages. Dairy
Solubility	Easily dispersed into most system. Prevents sedimentation in beverages ,soups and sauces	Beverages, confectionary ,frozen desserts ,infant foods ,soups and sauces
Water binding and building viscosity	Provides fat-like attributes in products allowing reduction in fat content, improved texture and moistness.	Baked products, beverages, dairy products, coffee creamers, soups and sauces
Whipping, foaming and aeration	Maintains foam properties, enhancing appearance, taste and texture.	Baked products such as meringues and cakes, confectionery, ice-cream, frozen desserts

Source: Abirached *et al.* (2012)

2.17.7 Applications of whey and whey products

In the past whey has been regarded as a cure for many illnesses, and was used in thermal baths or as a medicine in cure centers. The unbalanced composition of whey solids limited the application of whey and whey powder in human food products. In particular the dominant presence of lactose (72%) and minerals (8%) were difficulties which had to be overcome for application of whey in food products. The increasing production of whey and whey powder stimulated their use as nutritional supplements for animal feed, particularly as a cheap replacer for skim milk powder. The introduction of fractionation and isolation techniques for whey components further increased the application possibilities in food products. Nowadays, potential uses for whey components either as functional or as nutritional supplements in food products are numerous (Kelly, 2019).

Applications in confectionery and bakery products are important outlets for whey and whey products in human foods. Lactose, the major component of whey, contributes to color and flavour in these products. Whey and whey-based products have been found to improve the flavour, aroma, color, texture and (in some cases) also the shelf life of bakery products. The use of demineralized whey is preferred, because of its blander taste. The high nutritional quality of whey proteins and the presence of specific growth factors make whey an important source for infant formula and elderly foods. Highly nutritional minor components may be isolated from whey by using column chromatography (Alane *et al.*, 2017).

2.17.7.1 Dairy products

2.17.7.1.1 Yoghurt

A growth in the consumption of fermented milk beverages has been observed in recent years; the most important of these is the consumption of yoghurt. For this reason, the quality characteristics of the finished product are very important. These characteristics can be successfully modified using whey preparations (Liu *et al.*, 2016).

Whey products used in the production of yoghurt includes:

1. Sweet whey powder, which may replace skimmed milk powder at the level 2–5.2%.

2. WPCs, which are most often used by manufacturers of yoghurt. Addition of WPC34 at the level 0.7–2.0% or WPC80 at 0.5–0.8% is sufficient in the case of mixed yoghurt (a greater amount of the additive may adversely affect some quality

characteristics). Replacing skimmed milk powder with WPCs causes, among other effects, increased gel strength in solid yoghurt, increasing the 16 viscosity of mixed yoghurt, and reduces the risk of syneresis in both types of yoghurt.

Furthermore, the addition of whey protein gives yoghurt a smooth and creamy texture; it also increases its nutritional value. The bioactive components present in whey and whey protein can stimulate the growth of probiotic bacterial cultures (both in the finished product, and in the human digestive tract). The addition of *Bifidobacterium bifidum* to standard yoghurt cultures (*Lactobacillus delbrueckii* ssp. bulgaricus, *Streptococcus thermophiles*), increases the number of viable *B. bifidum* in the samples that contain the sweet whey or whey proteins (Kozioł *et al.*, 2014).

2.17.7.1.2 Ice-cream

Whey preparations used in the manufacture of ice-creams and sundaes include whey powder, demineralized whey powder, WPCs and WPI. Along with the most important nutritional, beneficial properties it also includes: water binding capacity, ability to form foam, and high nutritional value (Young, 2007). On the other hand, from the point of view of ice-cream manufacturers, it is important to reduce the costs of production. Rationalization of these costs can be achieved by using cheaper substitutes of certain ingredients of the recipes. The relatively expensive components include milk powder and egg yolk can be replaced with WPCs and with protein-fat preparations (Jasinska *et al.*, 2012).

2.17.7.2 Bakery products

Whey may be widely used in the baking, confectionery, and pastry industries for the production of breads, cakes, cookies, biscuits, crackers, muffins, and icing. Eggs contribute to the development of cakes structure and taste. Replacing eggs with whey proteins is also an effective means of reducing production costs (the obtained product crumbles less during cutting and packaging – which means a lack of additional costs). Furthermore, as a result of the presence of cholesterol in egg yolk, due to dietary reasons, there is a growing interest in the replacement of this component with the addition of WPCs (Stoliar, 2009).

2.17.7.3 Confectionary products

Whey products, including, demineralized whey powders, low-lactose whey powders, WPCs and isolates, and lactose have been used in the following confectionery: chocolates and chocolate chips, candies, jellies and chewing gums. Lactose – milk sugar – can serve as a bulking agent. It is slightly sweet, less soluble than sucrose, and has a low hygroscopicity level; however, it influences the color, the taste, and the texture of the finished product and takes part in the Maillard reaction. For these reasons, use of lactose can be more or less reduced, which depends on many characteristics of the confectionery. Another use of derivatives of WPCs is the production of the so-called aerated confectionery and chocolate. The foaming properties of concentrates are used in this case. In addition, WPI and concentrates of high protein content (WPC80) can be successfully used in the production of protein bars for athletes (Pernot-Barry, 2008).

2.17.7.4 Meat and meat products

Whey processing products used in meat industries especially in the production of comminuted products, such as: frankfurters, sausages, mortadella's, luncheon meat, or surimi. Whey protein partially replaces meat protein, as well as partially or completely substitutes soy protein and other binding agents, fillers, modified starch and hydrocolloids (Prabhu, 2006).

Whey proteins with improved flavour and increased functionality are obtained with new technologies. While choosing a particular whey product, it is essential to match its function to the characteristics we want to achieve. For example, high protein concentrates or isolates are used to modify fat content. A slight increase in sweetness occurs, especially with the addition of sweet whey (which enables reduced addition of sweeteners) (Youssef and Barbut, 2011).

Furthermore, the addition of whey proteins affects the taste and improves the gelation, used in the production of edible sausage casings. They are also used in the finishing of semi-products, as their addition has a positive effect on the adhesion of batter to portions of meat, poultry, or fish. They also exhibit antioxidant activity (this refers to the oxidation of fat in pork meat, in salmon meat, or in products rich in lipids) (Youssef and Barbut, 2011).

2.17.8 Other application of whey

2.17.8.1 Infants formula

Whey preparations, being a source of high-quality protein and of active peptides, are widely used by manufacturers of baby foods. It also increases amount of amino acids in infant formulas especially lysine, methionine and threonine (Murphy *et al.*, 2015).

2.17.8.2 Media preparation

Whey preparations are also used as media in the microencapsulation of sensitive food ingredients which are fragrances, dyes, or various types of probiotic bacteria (e.g. Bifidobacterium-BB-12). The use of whey protein supports protection of active ingredients and prevents the loss of their properties in the long-term. Additionally, after the microencapsulation process, a product is obtained in the form of powder or granules, which allows for controlled release of the component and new uses for food additives (Pinto *et al.*, 2015).

2.17.8.3 Edible coating

Another protective use of whey proteins is as an edible coating for food. Coating based on whey preparations is characterized by good mechanical properties, a good barrier against lipids, aromatics and, especially, oxygen. Fruit and vegetables can be coated successfully with whey protein (Pintado *et al.*, 2009).

Part III

Material and methods

3.1 Materials

The materials collected for the preparation of whey based yoghurt were as follows.

3.1.1 Whey

The paneer whey was collected from Dharan Dairy.

3.1.2 Milk solid not fat / SMP

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

3.1.3 Sweetener

Sugar manufactured by MB food manufacturing company was used as a sweetener. It was bought from the Baraha department store of Dharan.

3.1.4 Starter Culture

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

3.1.5 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.

3.1.6 Milk

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

3.2 Methods

3.2.1 Preparation of paneer whey

Standardized milk was used for the preparation of good quality whey. Milk was heated to 90°C and when temperature decreased to 70°C, 1% citric acid solution gradually added followed by continuous stirring which resulted in complete coagulation of milk protein (casein). The liquid (whey) was filtered using muslin cloth and stored for further use. The process flowchart for the preparation of whey is presented in Fig. 3.1

Standard milk (3 Fat and 8% SNF)

↓

Heating (90ºC)

↓

Cooling (70ºC)

↓

Add 1% citric acid solution gradually followed by slow stirring

↓

Coagulation

↓

Filtering with muslin cloth

↓ → Channa

Whey

Fig. 3.1 Preparation of paneer whey

Source: Ghanshyambhai *et al.* (2015)

3.2.2 Experimental design

Response surface methodology (RSM) by using Design Expert version 11 was used for optimization of level of SMP and sugar addition for the preparation of whey based yoghurt taking % syneresis, appearance, texture and overall acceptability as response variable. A three-level; two factor (central) composite Face Central Design was used for the experimental design (Myers *et al.*, 2016). Two factors were varied as follows; %SMP from 8 to 14% & Sugar from 2 to 6% of paneer whey (pH 6.8 adjusted by sodium bicarbonate). The treatment combinations obtained is presented in the Table 3.1. Whey based yoghurt were prepared by using all these treatment combination, examined and noted for percentage syneresis, appearance, texture and overall acceptability and subjected to further analysis by RSM using Design Expert version 11.

Table 3.1 Experimental combination of un-coded level for SMP and Sugar

S.N.	A (%SMP)	B (% Sugar)
1	11	7
2	11	4
3	7	4
4	14	2
5	15	4
6	8	2
7	14	6
8	11	4
9	11	4
10	11	1
11	8	6

The work was carried out for the preparation of standard quality of different varieties of whey based yoghurt with different proportion of whey, SMP and sugar. The input variation range in design experiment was 8-14% for SMP and 2-6% for sugar. The sugar range made as Dahal *et al.* (2014) used 2% sugar and Muliadi (2011) used 5% sugar. Similarly, the SMP range made. At first, the addition of SMP in the paneer whey was 8% to meet protein content as milk has. Then, the range was made 8-14% as the rate of SMP addition to the yoghurt mix as Tamime and Robinson (2007) used 1-6%.

3.2.3 Preparation of set type control yoghurt

The standardized pasteurized milk was used for the preparation of control yoghurt sample. The milk was mixed with 4% SMP and 3% sugar at 45°C, heated continuously to reach the temperature around 65-70°C for certain period. After it was cooled to 43-45°C and then, the starter culture is added at the rate of 2% to each formulations. The yoghurt mix was incubated at 43°C and was kept for 4 h until the coagulum is formed. The prepared yoghurt was immediately cooled to 5-7°C and stored at 7±1°C in a refrigerator. The flow diagram for preparation of set type of yoghurt is shown in Fig. 3.2

Standardized milk (fat-3%, MSNF-8%)

↓

Pre heating (45°C)

↓

Addition of SMP and sugar

↓

Heating (65-70°C)

↓

Cooling (43-44°C)

↓

Inoculation of culture (2%)

↓

Placing mix in several cups

↓

Incubate at 43°C for 4 hour

↓

Set type yoghurt

↓

Cooling and storing at 7°C

Fig. 3.2 Preparation of set type control yoghurt

Source: Ghanshyambhai *et al.* (2015)

3.2.4 Preparation of set type whey based yoghurt

Paneer whey having pH of 5.36 was first adjusted to pH 6.8 by using sodium bicarbonate, heated around 65-70°C for certain period before used for whey based yoghurt production. Flow chart for set type whey based yoghurt production is shown in Fig 3.3

Paneer whey

↓

pH adjustment (maintained to pH-6.8 by adding sodium bicarbonate)

↓

Addition of 8% SMP

↓

Reconstituted milk

↓

Pre-heating to 45°C

↓

Addition of remaining SMP and sugar according to formulations given by design expert

↓

Heating to 65-70°C

↓

Cooling until 43-44°C

↓

Inoculation of 2% culture

↓

Placing mix in several plastic cups

↓

Incubation at 43°C for 6-7 h

↓

Set type whey based yoghurt

↓

Cooling and storage (5-7°C)

Fig. 3.3 Preparation of set type whey based yoghurt

Source: Biswas (2013)

3.3 Analytical procedure

3.3.1 Chemical analysis of raw materials and final product

The best yoghurt, control yoghurt, SMP and whey was analyzed for its acidity, fat content, protein, total ash, , pH, moisture, total solid and lactose.

3.3.1.1 Acidity

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

3.3.1.2 Fat

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

3.3.1.3 Protein

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

3.3.1.4 Ash

The ash content will be determined as described by AOAC (2005).

3.3.1.5 pH

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

3.3.1.6 Moisture

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

3.3.1.7 Lactose

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

3.3.2 Sensory evaluation

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

3.3.3 Physical analysis

3.3.3.1 Syneresis

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

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

3.3.4 Statistical analysis

The analyses were carried out in triplicate. Statistical calculations were performed in Microsoft office Excel 2013. For optimization of SMP and sugar, design expert 11 RSM was used. Analysis of variance (ANOVA) was carried out for data from sensory evaluation. The significant differences between them were studied by using L.S.D. at 5% level of significance using Genstat release 12.1 software program developed by VSN International Ltd. and t-Test: Two-Sample Assuming Unequal Variances was carried out using Microsoft Excel 10 to evaluate the significant difference between the syneresis of the two samples. The data obtained from chemical analysis of control and best yoghurt were subjected to t-Test for statistical analysis.

Part IV

Results and discussions

Response surface methodology (RSM), two-face Central Composite Design using experimental design version 11 was adopted for optimization of level of SMP and sugar for the preparation of whey based yoghurt with minimum syneresis, maximum appearance, maximum texture and maximum overall acceptability. Paneer whey pH adjusted to 6.8 was used for preparation of yoghurt based on preliminary trial. Eleven treatment combination varying SMP (8-14%) and sugar (2-6%) obtained from experimental design were used for preparation of yoghurt sample, analysed for percentage syneresis, appearance, texture, and overall acceptability and subjected for analysis by RSM for optimization of SMP and sugar. Eight optimum solutions for minimum syneresis and maximum (appearance, texture and overall acceptability) was found having desirability of nearly 1. Among them four solutions having dissimilar combination of SMP and sugar were picked to prepare whey based yoghurt and used for model validation and sensory analysis comparing to control yoghurt. The best formulation of yoghurt (based on the sensory evaluation) was further taken for physicochemical analysis.

4.1 Chemical composition of paneer whey

The chemical composition of the paneer whey used in this study for preparation of whey based yoghurt is presented in the table 4.1.

Table 4.1 Chemical composition of paneer whey

Parameters	Values
Protein (%)	0.61±0.022
Fat (%)	0.37±0.094
Moisture (%)	93.72±0.025
Ash (%)	0.51±0.07
p^H	5.36±0.047
Lactose (%)	4.83±0.025
Acidity (% as lactic acid)	0.22±0.005

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

This chemical composition data for the fresh whey was found to be a little bit different than the data obtained by Rupnar (2006) for the paneer whey. The variation on the whey composition may be due to the variation in the milk constitute obtained and produced in the different parts of the world.

4.2 Chemical composition of milk solid not fat (SMP)

The analysis of the SMP used in this study for preparation of whey based yoghurt is presented in the table 4.2

Table 4.2 Chemical composition of SMP

Parameters	Values
Protein (%)	33.32±1.28
Fat (%)	1±0.09
Moisture (%)	6.3±0.66
Ash (%)	8.23±0.18
Lactose (%)	50.44±0.5

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

The protein content of the SMP (33.32%) was comparable to the result obtained by Er *et al.* (2019) i.e. 34.8%. The fat content of the SMP (1%) was similar to the data obtained by Murtaza *et al.* (2015) i.e. (0.99%). The moisture content of the SMP (6.3%) was comparable to the data obtained by Pugliese *et al.* (2017) i.e. (4.2%). The ash content of the SMP (8.23%) was similar to the result obtained by Pugliese *et al.* (2017) i.e. (8%). Similarly, the lactose content of the SMP was found to be 50.44% which was similar to the data obtained by Shrestha *et al.* (2008) i.e. 50%.

4.3 Different formulation with responses

First of all, 11 recipe formulation was formulated by using design expert by placing SMP and sugar in range (8-14%) and (2-6%) respectively. The percentage syneresis and average score of appearance, texture and overall acceptability of formulated yoghurt sample is given in Table 4.3.

Table 4.3 Different formulation with responses

Run	Factor 1 A: SMP %	Factor 2 B: Sugar %	Response 1 Syneresis %	Response 2 Appearance Avg. score	Response 3 Texture Avg. score	Response 4 Overall Acceptability Avg. score
1	11	7	39.88	6.2	6.4	6.2
2	11	4	41.89	7.2	7.2	7
3	7	4	59.83	5.4	4.8	5.4
4	14	2	29.17	7.4	7.6	7
5	15	4	22.6	7.4	8.2	7.4
6	8	2	50.07	6.2	5.4	5.6
7	14	6	27.32	6.2	6.4	5.8
8	11	4	40.56	6.4	6	5.8
9	11	4	41.27	6.6	6.4	6.2
10	11	1	38.53	7	6.1	6.2
11	8	6	49.53	5.2	5	5.2

4.4 Effect of addition of SMP and sugar on syneresis of yoghurt

The parameters range was conducted with the help of a design expert, and syneresis was identified. Table 4.5 and 4.6 shows the coefficients of model and other statistical attributes of syneresis. The syneresis of the whey based yoghurt was ranged from 22.6 to 59.83%. The coefficients of the model and other statistical attributes of syneresis are shown in Tables 4.5 and 4.6. The model F-value of 166.03 implies the model is significant. There is only a 0.01% chance that an F-value this large could occur due to noise. P-values less than 0.0500 indicate model terms are significant. In this case A is a significant model term. Values greater than 0.1000 indicate the model terms are not significant. The lack of fit F-value of 10.06 implies there is a 9.32% chance that a lack of fit F-value this large could occur due to noise. Lack of fit is good. So, it can be used to fit model. The predicted R^2 of 0.9506 is in reasonable agreement with the adjusted R^2 of 0.9706; i.e. the difference is less than 0.2. Adequate precision measures the signal to noise ratio. A ratio greater than 4 is desirable. So, the ratio of 34.893 indicates an adequate signal. This model can be used to navigate the design space. Considering all the above criteria, the model (Equation 4.1) was selected for representing the variation of SMP and sugar for syneresis and further analysis.

$$\text{Syneresis} = 40.06 - 11.97 \times A - 0.0601 \times B \qquad \ldots\ldots\ldots\ldots\ldots (4.1)$$

Where, A and B are the coded values of SMP (%) and sugar (%) respectively.

In the above linear equation 4.1, for the whey based yoghurt, syneresis had linear significant ($P>0.05$) negative effect of SMP (A) but, no significant ($P>0.05$) negative effect of sugar (B) at 95% level of confidence.

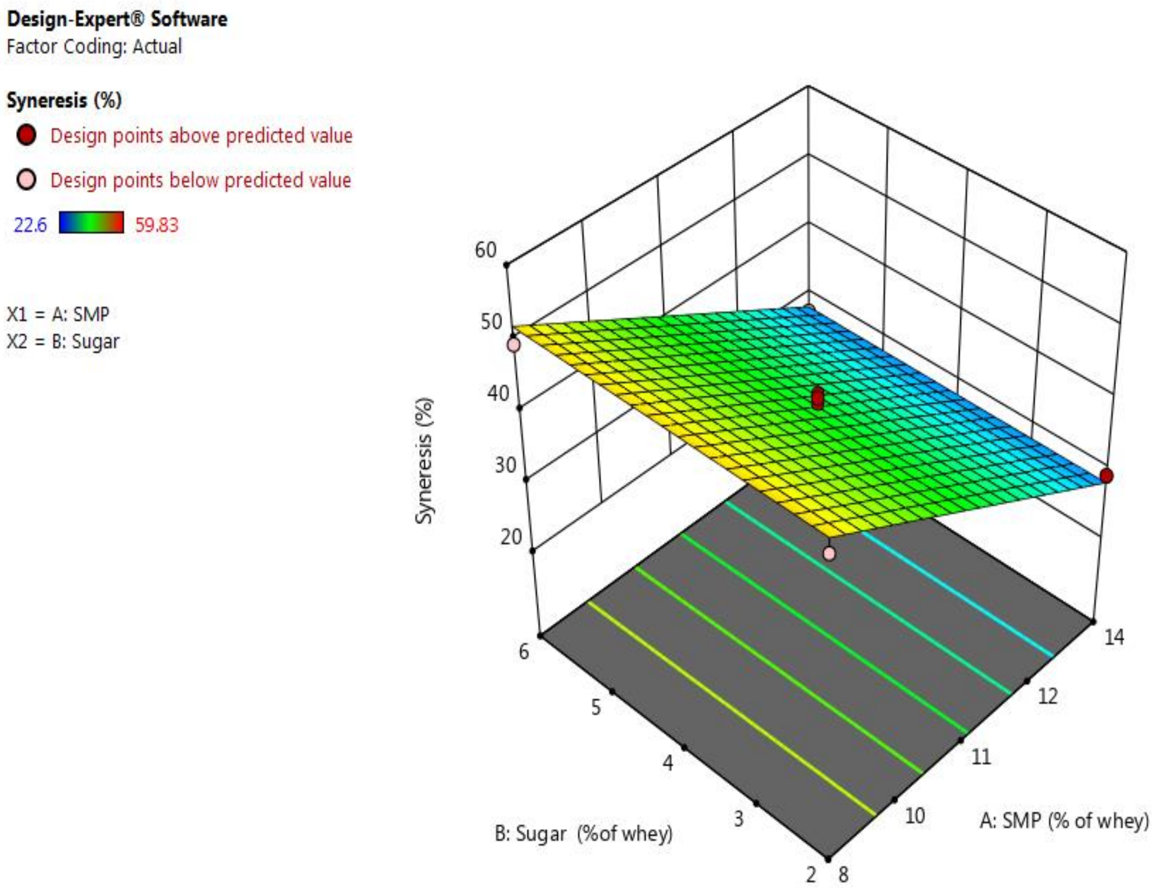

Fig. 4.1 Response surface plot for % syneresis as a function of % SMP and sugar

4.5 Effect of addition of SMP and sugar on appearance of yoghurt

The parameters range was conducted with the help of a design expert, and appearance was identified. Table 4.7 and 4.8 shows the coefficients of model and other statistical attributes of appearance. The appearance of the whey based yoghurt was ranged from 5.2 to 7.4. The coefficients of the model and other statistical attributes of appearance are shown in Tables 4.7 and 4.8. The model F-value of 18.30 implies the model is significant. There is only a 0.10% chance that an F-value this large could occur due to noise. P-values less than 0.0500 indicate model terms are significant. In this case A and B are significant model terms. Values greater than 0.1000 indicate the model terms are not significant. The lack of fit F-value of 0.62 implies the lack of fit is not significant relative to the pure error. There is a 72.38% chance that a lack of fit F-value this large could occur due to noise. Non-significant lack of fit is good. So, it can be used to fit model. The predicted R^2 of 0.6956 is in reasonable agreement with the adjusted R^2 of 0.7758; i.e. the difference is less than 0.2. Adequate precision measures the signal to noise ratio. A ratio greater than 4 is desirable. So, ratio of 11.354 indicates an adequate signal. This model can be used to navigate the design space. Considering all the above criteria, the model (Equation 4.2) was selected for representing the variation of SMP and sugar for appearance and further analysis.

$$\text{Appearance} = 6.47+0.6286\times A-0.4164\times B \quad \text{………..} \ (4.2)$$

Where, A and B are the coded values of SMP (%) and sugar (%) respectively.

In the above linear equation 4.2, for the whey based yoghurt, appearance had significant ($P>0.05$) positive effect of SMP (A) and significant ($P>0.05$) negative effect of sugar (B) at 95% level of confidence.

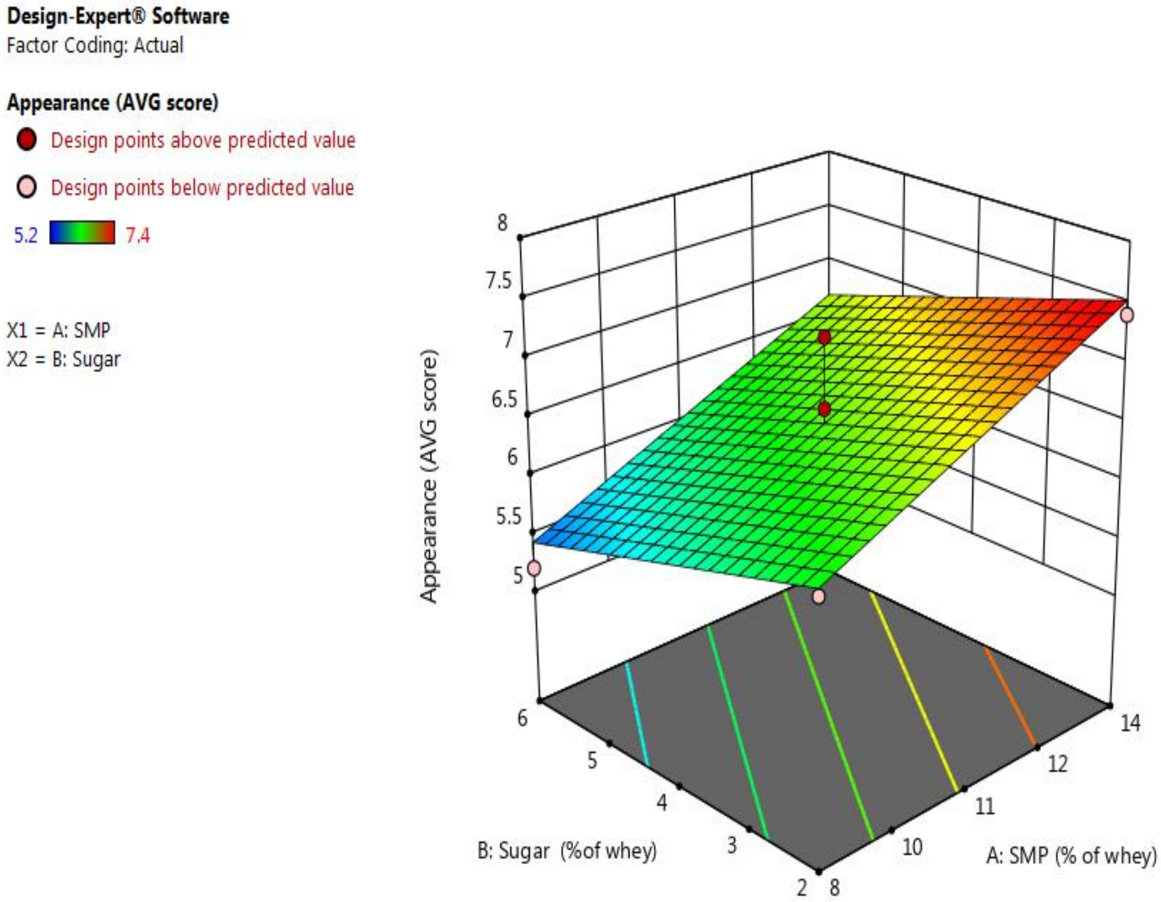

Fig. 4.2 Response surface plot for appearance as function of % SMP and sugar

4.6 Effect of addition of SMP and sugar on texture of yoghurt

The parameters range was conducted with the help of a design expert, and texture was identified. Table 4.9 and 4.10 shows the coefficients of model and other statistical attributes of texture. The texture of the whey based yoghurt was ranged from 4.8 to 8.2. The coefficients of the model and other statistical attributes of texture are shown in Tables 4.9 and 4.10. The model F-value of 17.97 implies the model is significant. There is only a 0.11% chance that an F-value this large could occur due to noise. P-values less than 0.0500 indicate model terms are significant. In this case A is a significant model term. Values greater than 0.1000 indicate the model terms are not significant. The lack of fit F-value of 0.56 implies the lack of fit is not significant relative to the pure error. There is a 75.26% chance that a lack of fit F-value this large could occur due to noise. Non-significant lack of fit is good. So, it can be used to fit model. The predicted R^2 of 0.6686 is in reasonable agreement with the Adjusted R^2 of 0.7724; i.e. the difference is less than 0.2. Adequate precision measures the signal to noise ratio. A ratio greater than 4 is desirable. So, ratio of 11.368 indicates an adequate signal. This model can be used to navigate the design space. Considering all the above criteria, the model (Equation 4.3) was selected for representing the variation of SMP and sugar for texture and further analysis.

$$\text{Texture} = 6.32+1.05\times A-0.1470\times B \quad \ldots\ldots\ldots\ldots (4.3)$$

Where, A and B are the coded values of SMP (%) and sugar (%) respectively.

In the above linear equation 4.3, for the whey based yoghurt, texture had significant ($P>0.05$) positive effect of SMP (A) but, no significant ($P>0.05$) negative effect of sugar (B) at 95% level of confidence.

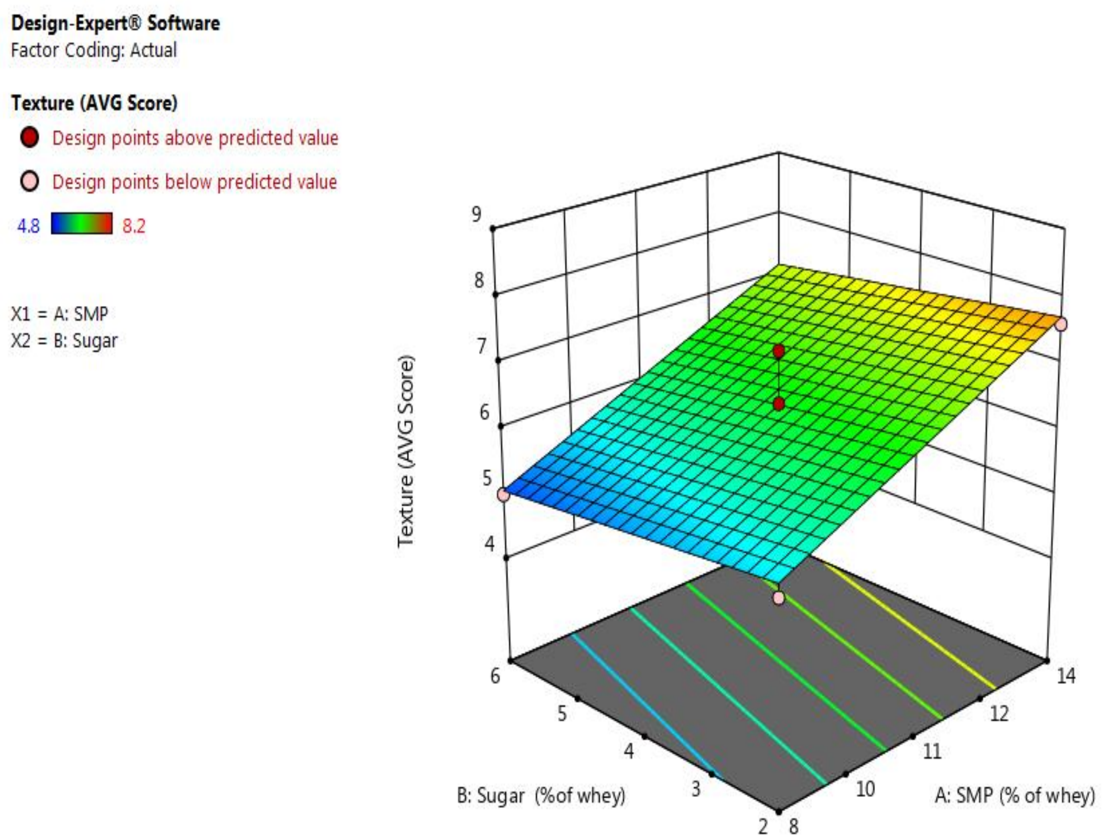

Fig. 4.3 Response surface plot for texture as function of % SMP and sugar

4.7 Effect of addition of SMP and sugar on overall acceptability of yoghurt

The parameters range was conducted with the help of a design expert, and overall acceptability was identified. Table 4.11 and 4.12 shows the coefficients of model and other statistical attributes of overall acceptability. The overall acceptability of the whey based yoghurt was ranged from 5.2 to 7.4. The coefficients of the model and other statistical attributes of overall acceptability are shown in Tables 4.11 and 4.12. The model F-value of 7.22 implies the model is significant. There is only a 1.61% chance that an F-value this large could occur due to noise. P-values less than 0.0500 indicate model terms are significant. In this case A is a significant model term. Values greater than 0.1000 indicate the model terms are not significant. The lack of fit F-value of 0.47 implies the lack of fit is not significant relative to the pure error. There is a 80.17% chance that a lack of fit F-value this large could occur due to noise. Non-significant lack of fit is good. So, it can be used to fit model. The predicted R^2 of 0.3605 is in reasonable agreement with the adjusted R^2 of 0.5545; i.e. the difference is less than 0.2. Adequate precision measures the signal to noise ratio. A ratio greater than 4 is desirable. So, ratio of 6.908 indicates an adequate signal. This model can be used to navigate the design space. Considering all the above criteria, the model (Equation 4.4) was selected for representing the variation of SMP and sugar for overall acceptability and further analysis.

Overall acceptability = $6.16+0.6036\times A-0.2000\times B$ ……….. (4.4)

Where, A and B are the coded values of SMP (%) and sugar (%) respectively.

In the above linear equation 4.4, for the whey based yoghurt, overall acceptability had significant (P>0.05) positive effect of SMP (A) but, no significant (P>0.05) negative effect of sugar (B) at 95% level of confidence.

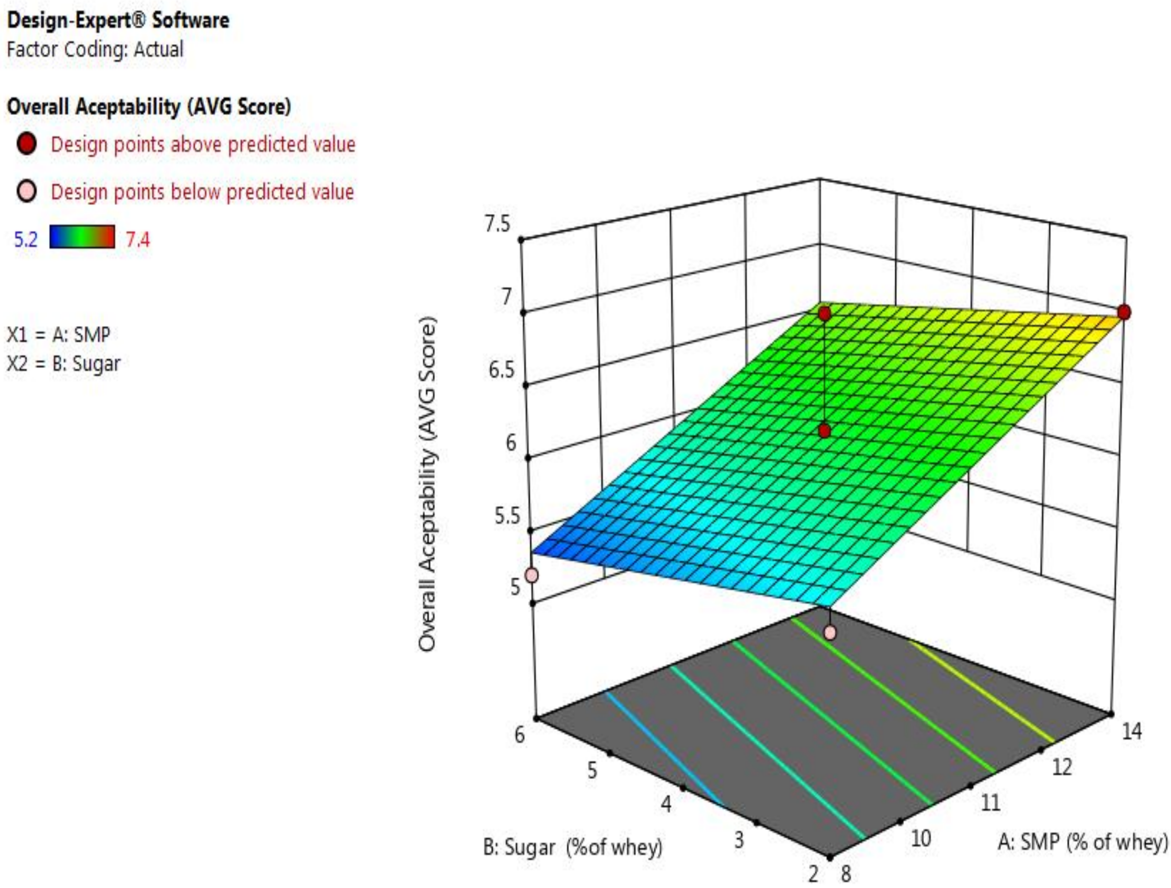

Fig. 4.4 Response surface plot for overall acceptability as function of % SMP and sugar

4.8 Optimized solution

The model output (RSM) provided altogether 8 solutions for % SMP and % sugar level addition for whey based yoghurt preparation with minimum syneresis and maximum appearance, maximum texture and maximum overall acceptability (Appendix E). All 8 solutions had desirability of nearly 1. But only four solutions having slightly different combination level of SMP and sugar were chosen for post analysis (% Variability and model validation) of model output and for further sensory evaluation. The combination levels of %SMP and sugar taken for evaluation of model output and sensory evaluation are presented in the Table 4.4.

Table 4.4 Comparison of % syneresis of selected formulation for model validation

Samples	% SMP	% Sugar	% Syneresis from lab test (Actual)	% Syneresis from model prediction	% Variation
A	14.0	2.000	28.85	28.03	2.93
B	14.0	2.224	29.75	28.27	5.24
C	14.0	2.653	29.89	28.63	4.40
D	14.0	3.461	29.35	28.97	1.31

In order to evaluate model performance, all the four levels of SMP and sugar (Table 4.13) were used for preparation of whey based yoghurt, analyzed for the % syneresis and compared with the model prediction. The percentage variation between model output and real response found to be ranged from 1.31 to 5.24% showing 94.76-98.69% of model validity.

4.9 Effect of optimized formulations on sensory characteristics

The four optimized levels of SMP and sugar were used for preparation of whey based yoghurt (Table 4.4) was subjected to 9 point hedonic test compared with control sample coded as E (milk yoghurt without whey). The four optimized samples were coded as A, B, C and D respectively. The result of sensory analysis for appearance, color, texture, taste and overall acceptability is presented and discussed on the following headings.

4.9.1 Aroma

The average sensory score for aroma obtained for all the yoghurt samples are presented in the fig. 4.5. Regarding aroma of whey based yoghurt, the analysis shows that the mean sensory score for sample A, B, C, D and E were found to be 6.1, 6.5, 7, 8.7 and 7.4 respectively. Statistical analysis showed that the variation in proportion of SMP and sugar had significant effect in aroma of yoghurt ($p<0.05$). LSD at 5% level of significance indicated that the sample D was significantly different from rest of the sample and also had the highest score.

According to Isleten and Karagul-Yuceer (2006), slight acidic or bitter aroma in the yoghurt is due to the addition of skim milk powder. This aroma was attributed to the interaction among whey proteins, calcium phosphate, and caseins in whey based yoghurt (Lemieux and Simard, 1994).

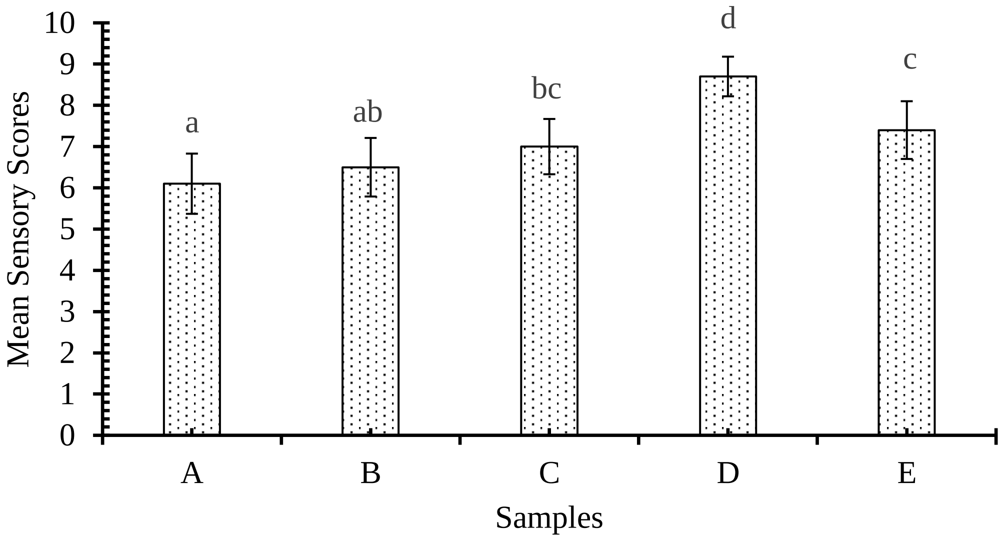

Fig. 4.5 Effect of SMP and sugar on aroma of yoghurt

Fig. 4.5 represents the mean sensory scores for aroma of whey based 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.

4.9.2 Color

The average sensory score for color obtained for all the yoghurt samples are presented in the fig. 4.6 The mean color score for samples A, B, C, D and E were found to be 6.4, 6.6, 7.2, 8.5 and 7.4 respectively. Statistical analysis showed that the variation in proportion of SMP and sugar had significant effect in color of yoghurt ($p<0.05$). LSD at 5% level of significance indicated that sample A, B, C and E were not significantly different from each other but were significantly different from sample D.

According to Gonzalez-Martınez *et al.* (2002), the use of whey implies a slower acidification rate in yoghurts which becomes a little yellowish. However, the color of whey based yoghurt is as same as the color of SMP used for the yoghurt production.

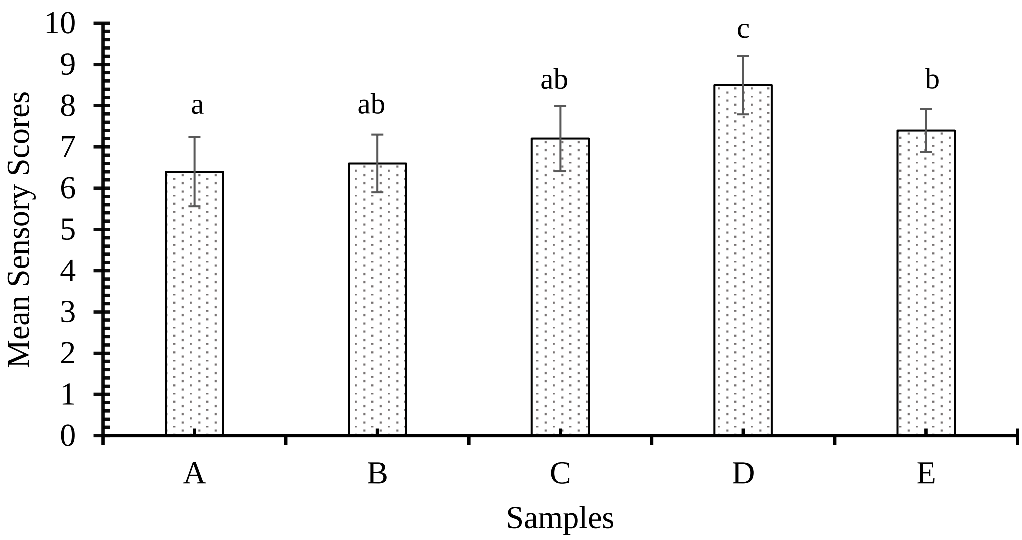

Fig 4.6 Effect of SMP and sugar on color of yoghurt

Fig. 4.6 represents the mean sensory scores for color of whey based 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.

4.9.3 Taste

The average sensory score for taste obtained for all the yoghurt samples are presented in the fig. 4.7. Regarding taste of whey based yoghurt; the analysis showed that the mean sensory score of sample A, B, C, D and E were found to be 6.3, 6.5, 6.6, 8.3, and 7.3.

Statistical analysis showed that the variation in proportion of SMP and sugar had significant effect in taste of yoghurt ($p<0.05$). LSD at 5% level of significance indicated that the sample D was significantly different from rest of the sample and also had the highest score. The sample A, B, C, and E were not significantly different to each other but were significantly different with D.

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

The addition of sugar in the mix significantly increases the sensory score in terms of taste of the product (Dahal *et al.*, 2014). According to Isleten and Karagul-Yuceer (2006), Slight acidic or bitter taste in the yoghurt is due to the addition of skim milk powder.

In yogurt products, adding significant amounts of SMP (more than 6% w/w) results in a powdery taste (Karam *et al.*, 2013).

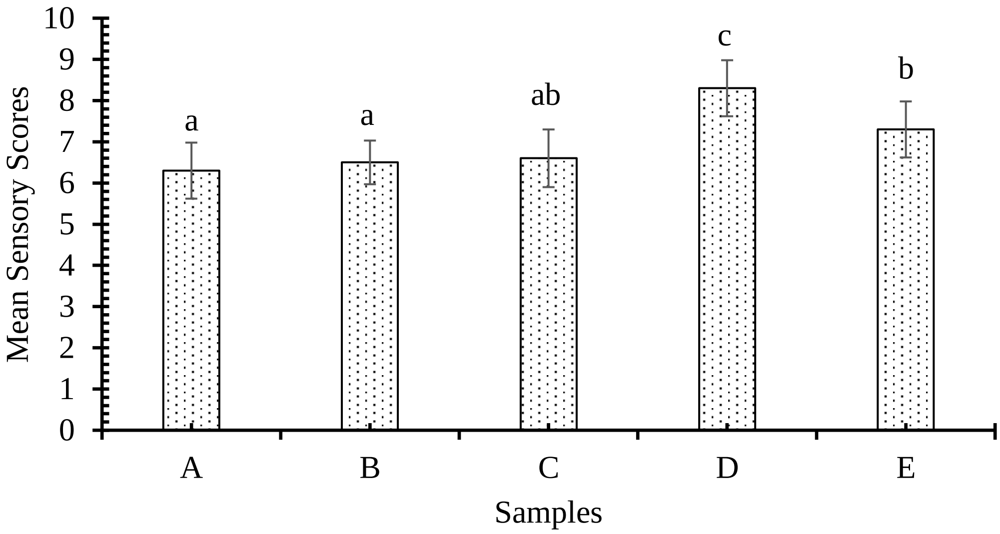

Fig 4.7 Effect of SMP and sugar on taste of yoghurt

Fig. 4.7 represents the mean sensory scores for taste of whey based 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.

4.9.4 Texture

The average sensory score for aroma obtained for all the yoghurt samples are presented in the fig. 4.8. The average sensory of texture was found to be 6.2, 6.3, 6.9, 8.3 and 7.4 for samples A, B, C, D and E respectively. Statistically there was significant effect on texture of yoghurt due to variation of proportion of SMP and sugar. LSD between the samples indicated that sample A, B, C and E were not significantly different from each other but were significantly different from sample D. Sample D had highest score due to the optimum acceptance of panelist.

The positive effect of sugars on yogurt texture can be explained by their water-binding capacity, which increases the viscosity of the serum phase and, therefore, the serum-holding capacity of yogurt (Jelen, 1997).

In general, addition of 2% of SMP is considered appropriate for improving yogurt textural quality. When compared to yogurt made without fortification, the addition of SMP generated good quality yogurt and helped to increase yogurt viscosity and gel strength (Karam *et al.*, 2013).

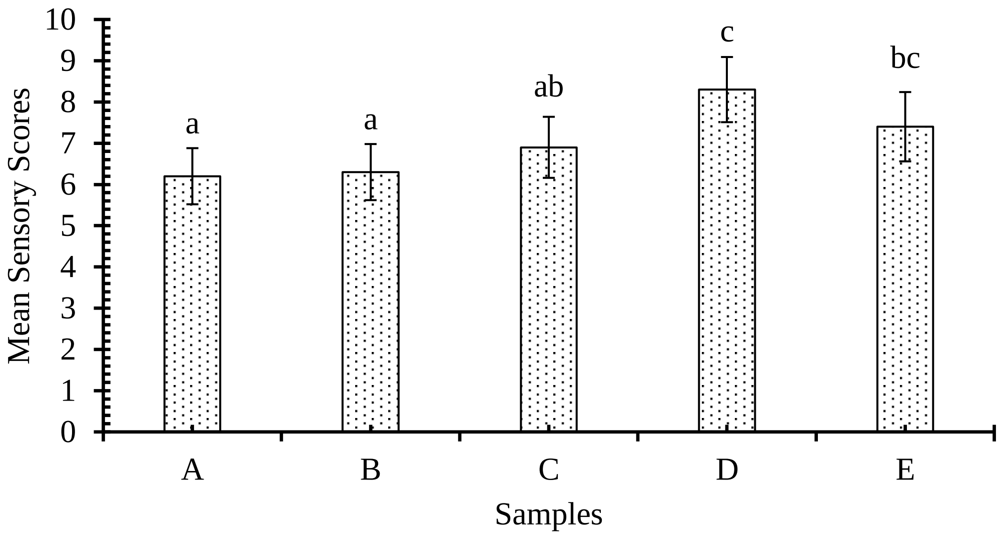

Fig 4.8 Effect of SMP and sugar on texture of yoghurt

Fig. 4.8 represents the mean sensory scores for texture of whey based 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.

4.9.5 Overall acceptability

The average sensory score for aroma obtained for all the yoghurt samples are presented in the fig. 4.9. Regarding overall acceptability of whey based yoghurt; the analysis showed that the mean sensory score for sample A, B, C, D and E were found to be 6.3, 6.7, 6.9, 8.2 and 7.4. Statistical analysis showed that effect of different proportion of SMP and sugar on overall acceptability of the product was significant ($p<0.05$). LSD showed that sample A, B, C and E were not significantly different from each other but were significantly different from sample D. Sample D got the high mean score due to optimum acceptance of panelist.

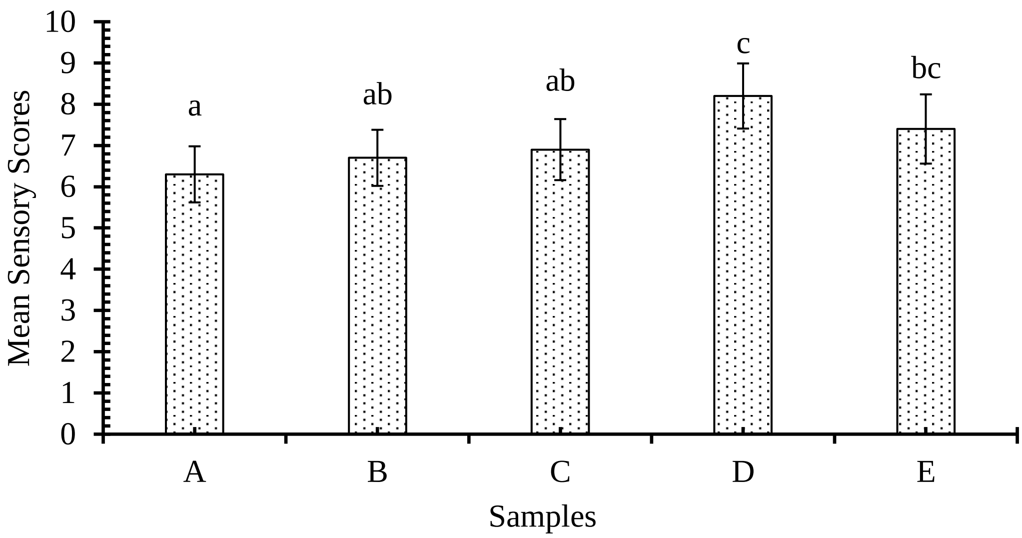

Fig. 4.9 Effect of SMP and sugar on overall acceptability of yoghurt

Fig. 4.9 represents the mean sensory scores for overall acceptability of whey based yoghurt. Values on the top of the bars bearing similar superscript are not significantly different at 5% level of significance. Vertical error bar represent ±standard deviation of scores given by panelist.

There was significant difference between the samples D with respect to other samples but there was no significant different between other samples except sample E. The overall acceptability of Sample D was higher due to the improvement in taste and texture with respect to other samples. Sample A, B and C got lowest score may be due to incorporation of comparatively low SMP and sugar.

From the sensory evaluation of the product conducted on the attributes like aroma, color, taste, texture and overall acceptability, the product containing 14% SMP and 3.46% sugar was rated as best in all attributes.

4.10 Chemical composition of milk yoghurt and whey based yoghurt

From the statistical analysis, the best product was found to be sample D yoghurt containing 14% SMP and 3.46% sugar. Chemical analysis of sample D and control yoghurt from whole milk (containing 4% SMP and 3% sugar) was done. The values of the chemical analysis are shown in Table 4.5.

Table 4.5 Chemical analysis of product

Parameters	Product E (Control)	Product D (Best)
Acidity (% Lactic acid)	0.77^{a}±0.02	1.06^{b}±0.01
Ash (%)	1.02^{a}±0.13	1.75^{b}±0.2
Fat (%)	2.77^{a}±0.2	0.47^{b}±0.1
Moisture (%)	82.78^{a}±0.54	75.57^{b}±0.4
Protein (%)	4.72^{a}±0.21	5.39^{a}±0.46
pH	4.33^{a}±0.05	4.07^{b}±0.05
Lactose (%,)	3.77^{a}±0.21	6.60^{b}±0.44

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

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

The lactose and ash content of sample D (best) was found to be higher than that of control sample. This may be due to high lactose and ash content in the SMP compared to dairy milk. However, the fat content of control sample was higher than best sample. This is because SMP and whey contain very low fat Murtaza *et al.* (2015), Rupnar (2006). Acidity of best sample was found to be increased slightly and was significantly different. This is due to long incubation time (6-7 h) for best product than control product (3-4 h).

4.11 Physical analysis

The physical analysis of the control yoghurt and best product was performed. The values obtained for the syneresis are shown in Table 4.6.

Table 4.6 Syneresis of yoghurt

Samples	Syneresis (%)
Control yoghurt	$24.42^{a}\pm0.81$
Best yoghurt	$29.35^{b}\pm0.82$

t-Test was carry out to evaluate the significant different between the two samples. There were significant difference between control and best product ($P<0.05$) as shown in Appendix D.9. The results are similar to the work done by Younus *et al.* (2002) i.e. 22.8-29.64%.

4.12 Cost evaluation

The total cost of the best whey based yoghurt was calculated. It is shown in the appendix B. The price for 100 ml whey based yoghurt was found to be NRs. 10.87 (as of 2021) which was cheaper than commercial yoghurt.

Part V

Conclusion and recommendations

5.1 Conclusion

Whey based yoghurt was prepared and the physicochemical properties of the prepared yoghurt was studied. On the basis of result obtained from physicochemical analysis of yoghurt following conclusions were drawn:

- Percentage syneresis in yoghurt was decreased when % SMP was increased. Where percentage sugar don't have significant effect on % syneresis. Similarly, the addition of SMP rather but not sugar had significant linear effect on increasing average score of both texture and overall acceptability of whey based yoghurt. But, the addition of both SMP and sugar had significant linear effect on increasing and decreasing average score of appearance of whey based yoghurt respectively.
- Whey based yoghurt can be prepared by adding 14% SMP & 3.46% sugar from acid whey with adjusted to pH 6.8 with accepted sensory quality.
- The protein, fat, acidity, total solid, lactose, ash, moisture and pH of best product were found 5.26%, 0.4%, 1.06%, 24.28%, 6.82%, 1.76%, 75.79% and 4.07 whereas milk yoghurt contain less amount of ash i.e. 1.02% and lactose 3.77%. So that it could be more important from the nutritional point of view. Whey based yoghurt have low fat content compared to milk yoghurt which may be useful for heart patients.

5.2 Recommendations

- Preparation and quality evaluation of cheese whey based yoghurt can be carried out.
- Flavored whey based yoghurt can be prepared by incorporating different type of flavor.

Part VI

Summary

Whey both paneer and cheese whey, although being nutritionally important, huge amount is still discarded as byproduct of dairy industries. It not only increases the environmental burden but also account greater loss of valuable components present in it. Transforming whey into various products is an important strategy to add value and economic benefit. Hence this study is carried out to optimize the proportion of SMP and sugar for the development of whey based yoghurt, and evaluates its quality.

Response surface methodology was adopted for optimization of level of SMP and sugar for the preparation of whey based yoghurt with percentage syneresis, appearance, texture, and overall acceptability as response. Paneer whey pH adjusted to 6.8 was used for preparation of yoghurt based on preliminary trial. Whey based yoghurts prepared from eleven treatment combinations varying SMP (8-14%) and sugar (2-6%) obtained from two faced Central Composite Design using experimental design version 11were subjected to RSM analysis for optimization. From response analysis, four optimized formulations solutions having SMP and sugar as coded A (14% SMP and 2% sugar), B (14% SMP and 2.22% sugar), C (14% SMP and 2.65% sugar) and D (14% SMP and 3.46% sugar) were prepared for whey based yoghurt and subjected to sensory analysis comparing to control yoghurt. The best formulation of yoghurt was further taken for physicochemical analysis.

The results revealed that addition of SMP rather but not the sugar had significant linear effect on reducing percentage syneresis of whey based yoghurt. Similarly, the addition of SMP rather but not sugar had significant linear effect on increasing average score of both texture and overall acceptability of whey based yoghurt. But, the addition of SMP had significant linear effect on increasing average score of appearance of whey based yoghurt. While, the addition of sugar have significant linear effect on decreasing average score of appearance of whey based yoghurt. From RSM and sensory evaluation, addition of 14% SMP and 3.46% sugar provided the whey based yoghurt best in terms of sensorial properties, had having minimum syneresis, maximum appearance, maximum texture, maximum overall acceptability and contain protein, fat, acidity, total solid, lactose, ash, moisture and pH were found 5.26%, 0.4%, 1.06%, 24.28%, 6.82%, 1.76%, 75.79% and 4.07 respectively.

References

Abirached, C., Medrano, C. A., Araujo, A. C., Moyna, P., Anon, M. C. and Panizzolo, L. A. (2012). Comparison of interfacial and foaming properties of soy and whey protein isolates. *J. Food Eng.* **2** (7), 376-381. [doi:10.17265/2159-5828/2012.07.003].

Abu-Tarboush, H. (1996). Comparison of associative growth and proteolytic activity of yogurt starters in whole milk from camels and cows. *J. Dairy Sci.* **79** (3), 366-371. [doi:10.3168/jds.S0022-0302(96)76373-7].

Affertsholt, T. and Pedersen, D. (2017). Whey & Lactose: Market Fueled by High Protein. *Mark. TI (ed.)*. **1** (3), 26-28.

Ahmed, F. (2011). Study on the quality and storage stability of laboratory made and local market yogurt. Master of Science in Food Engineering Thesis. Bangladesh Agricultural Univ., Bangladesh.

Alane, D., Raut, N., Kamble, D. B. and Bhotmange, M. (2017). Studies on preparation and storage stability of whey based mango herbal beverage. *Int. J. Chem. Stud.* **5** (3), 237-241.

Alm, L. (1982). Effect of fermentation on proteins of Swedish fermented milk products. *J. dairy sci.* **65** (9), 1696-1704.

Amatayakul, T., Halmos, A., Sherkat, F. and Shah, N. (2006). Physical characteristics of yoghurts made using exopolysaccharide-producing starter cultures and varying casein to whey protein ratios. *Int. dairy j.* **16** (1), 40-51.

Andrews, T. (2000). Nectar and Ambrosia: An Encyclopedia of Food in World Mythology. **8** (21), 2006.

Aneja, R. (2002). "Technology of Indian milk products". Dairy India Yearbook. New Delhi. [ISBN 8190160303].

AOAC. (2005). "Official Methods of Analysis" (18th ed.). Association of Official Analytical Chemists Washington, DC. USA. [ISBN 0935584676].

Baglio, E. (2014). "Chemistry and technology of yoghurt fermentation". Springer International Publishing. Switzerland. [ISBN 978-3-319-07376-7].

Baldasso, C., Barros, T. and Tessaro, I. (2011). Concentration and purification of whey proteins by ultrafiltration. *J. Desalination*. **278** (3), 381-386.

Bereda, A., Eshetu, M. and Yilma, Z. (2014). Microbial properties of Ethiopian dairy products: A review. *Afr. J. Microbiol. Res.* **8** (23), 2264-2271.

Bintsis, T. (2018). Lactic acid bacteria as starter cultures: An update in their metabolism and genetics. *AIMS microbiol.* **4** (4), 665-684.

Biswas, A. R. (2013). Yogurt from cow milk and coconut milk-a kinetic approach Master of Science In Food Engineering Thesis. Department of Food Technology and Rural Industries Bangladesh Agricultural Univ., Bangladesh.

Bohora, G. (2018). Preparation of whey based pineapple beverage and its storage quality evaluation. B. Tech. Dissertation. Tribhuvan Univ., Nepal.

Bozanic, R., Barukcic, I. and Lisak, K. (2014). Possibilities of whey utilisation. *J. Nutr. Food Sci.* **2** (7), 7.

Buttriss, J. (1997). Nutritional properties of fermented milk products. *Int. J. Dairy Technol.* **50** (1), 21-27.

Bylund, G. (1995). "Dairy processing handbook". Tetra Pak Processing Systems AB. Sweden.

Campbell-Platt, G. (1994). Fermented foods—a world perspective. *Food Res. Int.* **27** (3), 253-257.

Cerning, J., Bouillanne, C., Landon, M. and Desmazeaud, M. (1990). Comparison of exocellular polysaccharide production by thermophilic lactic acid bacteria. *Sci. Des Aliments*. **10** (2), 443-451.

Chandan, R. C., Gandhi, A. and Shah, N. P. (2017). Yogurt: Historical background, health benefits, and global trade. *In:* "Yogurt in Health and Disease Prevention".). pp. 3-29. Elsevier. [ISBN 978-0-12-805134-4].

Chandan, R. C. and Shahani, K. M. (1995). Other fermented dairy products. 385-418.

Chandan, R. C., White, C. H., Kilara, A. and Hui, Y. H. (2008). "Manufacturing yogurt and fermented milks". John Wiley & Sons. [ISBN 0470276533].

Collins, Y. F., McSweeney, P. L. and Wilkinson, M. G. (2003). Lipolysis and free fatty acid catabolism in cheese: a review of current knowledge. **13** (11), 841-866. [doi:10.1016/S0958-6946(03)00109-2].

Commane, D., Hughes, R., Shortt, C. and Rowland, I. (2005). The potential mechanisms involved in the anti-carcinogenic action of probiotics. *Mut. Res.* **591** (1-2), 276-289. [doi:10.1016/j.mrfmmm.2005.02.027].

Crittenden, R., Bird, A. R., Gopal, P., Henriksson, A., Lee, Y. and Playne, M. (2005). "Probiotic research in Australia, New Zealand and the Asia-Pacific region". Vol. 11. Bentham Science Publishers. Australia. [ISBN 1381-6128].

Dahal, S., Suman, A. B. and Acharya, P. P. (2014). Effect of sucrose, SMP addition and duration of storage on the quality parameters of probiotic yoghurt. B. Tech. Dissertation. Tribhuvan Univ., Nepal.

Damian, C. (2013). Influence of dietary fiber addition on some properties of yoghurt. *Ovidius Univ. Ann. Chem.* **24** (1), 17-20.

Darade, R. and Ghodake, S. (2012). An overview of whey beverages. *Res. J. Anim. Husb. Dairy Sci.* **3** (1), 41-44.

Dave, R. I. and Shah, N. P. (1997). Viability of yoghurt and probiotic bacteria in yoghurts made from commercial starter cultures. *Int. Dairy J.* **7** (1), 31-41. [doi:10.1016/S0958-6946(96)00046-5].

Davies, F. L. and Law, B. A. (1984). "Advances in the microbiology and biochemistry of cheese and fermented milk". Sole distributor in the USA and Canada, Elsevier Science. USA. [ISBN 0853342873].

Davoodabadi, A., Dallal, M. M. S., Lashani, E. and Ebrahimi, M. T. (2015). Antimicrobial activity of *Lactobacillus spp.* isolated from fecal flora of healthy breast-fed infants against diarrheagenic *Escherichia coli. Jundishapur J. Microbiol.* **8** (12). [doi:10.5812/jjm.27852].

Donkor, O., Henriksson, A., Singh, T., Vasiljevic, T. and Shah, N. P. (2007). ACE-inhibitory activity of probiotic yoghurt. *Int. Dairy J.* **17** (11), 1321-1331.

El-Malt, L. M., Abdel Hameed, K. and Mohammed, A. (2013). Microbiological evaluation of yoghurt products in Qena city, Egypt. *Vet. World*. (7), 400-404.

Er, B., Sert, D. and Mercan, E. (2019). Production of skim milk powder by spray-drying from transglutaminase treated milk concentrates: Effects on physicochemical, powder flow, thermal and microstructural characteristics. *Int. Dairy J.* **99**, 104-544. [doi:10.1016/j.idairyj.2019.104544].

Fadela, C., Abderrahim, C. and Ahmed, B. (2009). Physico-chemical and rheological properties of yoghurt manufactured with ewes milk and skim milk. *Afr. J. Biotechnol.* **8** (9), 1938-1942.

Farinde, E. O., Adesetan, T. O., Obatolu, V. A. and Oladapo, M. O. (2009). Chemical and microbial properties of yogurt processed from cow's milk and soymilk. *J. Food Process. preserv.* **33** (2), 245-254.

Fellows, P. and Hampton, A. (1992). "Small-scale food processing: a guide to appropriate equipment". Intermediate Technology Publications. London, UK. [ISBN 1853391085].

Fernandes, C. and Shahani, K. (1989). Lactose intolerance and its modulation with lactobacilli and other microbial supplements. *J Appl. Nutr*. **41** (2), 50-64.

Fernandes, C. F., Shahani, K. M., Staudinger, W. L. and Amer, M. (1991). Effect of *Lactobacillus acidophilus* on growth of Ehrlich ascites tumor in Swiss mice. *J. Nutr. Med.* **2** (1), 25-34. [doi:10.3109/13590849109084097].

Fisberg, M. and Machado, R. (2015). History of yogurt and current patterns of consumption. *Nutr. Rev.* **73**, 4-7. [doi:10.1093/nutrit/nuv020].

Fox, P. and Wallace, J. (1997). Formation of flavor compounds in cheese. *Adv. Appl. Microbiol.* . **45**, 17-86.

Fredrickson, A. (1977). Behavior of mixed cultures of microorganisms. *Annu. Rev. Microbiol.* **31** (1), 63-88.

Gaglio, R., Gentile, C., Bonanno, A., Vintaloro, L., Perrone, A., Mazza, F., Barbaccia, P., Settanni, L. and Di Grigoli, A. (2019). Effect of saffron addition on the microbiological, physicochemical, antioxidant and sensory characteristics of yoghurt. *Int. J. Dairy Technol.* **72** (2), 208-217.

Ghanshyambhai, M. R., Balakrishnan, S. and Aparnathi, K. (2015). Standardization of the method for utilization of paneer whey in cultured buttermilk. *J. Food Sci. Technol.* **52** (5), 2788-2796.

Gonzalez-Martınez, C., Becerra, M., Chafer, M., Albors, A., Carot, J. and Chiralt, A. (2002). Influence of substituting milk powder for whey powder on yoghurt quality. *Trends Food Sci. Technol.* **13** (9-10), 334-340.

Greenberg, N. and Mahoney, R. (1982). Production and characterization of β-galactosidase from *Streptococcus thermophilus*. *J. Food Sci.* **47** (6), 1824-1835. [doi:10.1111/j.1365-2621.1982.tb12891.x].

Guarner, F. and Malagelada, J.-R. (2003). Gut flora in health and disease. *Appl. Environ. Microbiol.* **361** (9356), 512-519. [doi:10.1016/S0140-6736(03)12489-0].

Gupta, V. (2008). "Overview of production, processing and utilization of dairy by-products in compendium on technological advances in the utilization of dairy by-products". Dairy Technology division. Karnal, India.

Gursoy, A., Durlu-Ozkaya, F., Yildiz, F. and Aslim, B. (2010). Set type yoghurt production by exopolysaccharide producing turkish origin domestic strains of *Streptococcus thermophilus* (W22) and *Lactobacillus delbrueckii ssp. bulgaricus* (B3). *J. Dairy Technol.* **16**, 81-90. [doi:10.9775/kvfd.2009.1520]

Haj, M. H., El Owni, O. A. and El Zubeir, I. E. (2007). Assessment of chemical and microbiological quality of stirred yoghurt in Khartoum State, Sudan. *Res. J. Vet. Anim. Sci*. **2**, 56-60.

Hassan, A. and Amjad, I. (2010). Nutritional evaluation of yoghurt prepared by different starter cultures and their physiochemical analysis during storage. *Afr. J. Biotechnol.* **9** (20).

Hemme, D., Nardi, M. and Jette, D. (1980). Beta-galactosidases et phospho-beta-galactosidases de *Streptococcus thermophilus. Dairy Sci. Technol.* **60** (599-600), 595-618. [doi:10.1051/lait:1980599-60032].

Huang, Y. and Adams, M. C. (2004). In vitro assessment of the upper gastrointestinal tolerance of potential probiotic dairy propionibacteria. *Int. J. Food Microbiol.* **91** (3), 253-260. [doi:10.1016/j.ijfoodmicro.2003.07.001].

Hugenholtz, J., Starrenburg, M., Boels, I., Sybesma, W., Chaves, A., Mertens, A. and Kleerebezem, M. (2000). Metabolic engineering of lactic acid bacteria for the improvement of fermented dairy products. *Int. J. Dairy Sci.,* 3009-3013.

Isleten, M. and Karagul-Yuceer, Y. (2006). Effects of dried dairy ingredients on physical and sensory properties of nonfat yogurt. *J. Dairy Sci.* **89** (8), 2865-2872.

Izadi, Z., Nasirpour, A., Garoosi, G. A. and Tamjidi, F. (2015). Rheological and physical properties of yogurt enriched with phytosterol during storage. *J. Food Sci. Technol.* **52** (8), 5341-5346.

Jasinska, M., Trzcinski, J., Dmytrow, I. and Mituniewicz-Małek, A. (2012). Whey protein concentrates and whey-fat preparations as powdered milk substitutes in soft serve ice cream. *J. Dairy Sci.* **19** (1), 37-50.

Jelen, P. (1997). Texture of fermented milk products and dairy desserts. *Trends Food Sci. Technol.* **10** (8), 345-347.

Karam, M. C., Gaiani, C., Hosri, C., Burgain, J. and Scher, J. (2013). Effect of dairy powders fortification on yogurt textural and sensorial properties: a review. *J. Dairy Res.* **80** (4), 400-409.

KC, J. and Rai, B. (2007). "Basic Food Analysis Handbook" (1st ed.). Prompt Printers Pvt. Ltd. Kathmandu, Nepal.

Kelly, P. (2019). Whey protein ingredient applications. *In:* "Whey Proteins".). pp. 335-375. Elsevier.

Keogh, M. and O'kennedy, B. (1998). Rheology of stirred yogurt as affected by added milk fat, protein and hydrocolloids. *J. Food Sci.* **63** (1), 108-112.

Khadka, G. (2018). Preparation and shelf life study of cinnamon oleoresin incorporated yoghurt. B. Tech. Dissertation. Tribhuvan Univ., Nepal.

Kneifel, W. (2000). In vitro growth behaviour of probiotic bacteria in culture media with carbohydrates of prebiotic importance. *Microb. Eco. H. Dis.* **12** (1), 27-34. [doi:10.1080/089106000435563].

Kozioł, J., Gustaw, W., Wasko, A., Skrzypczak, K., Sławinska, A. and Sołowiej, B. (2014). Effect of selected milk protein preparations on growth and survival of *Lactobacillus acidophilus* as well as on rheological properties of fermented milk beverages. *Food Sci. Technol.* **3** (94), 41-55.

Krasaekoopt, W. and Bhatia, S. (2012). Production of yogurt powder using foam-mat drying. *AU J. Technol.* **15** (3), 166-171.

Kumar, P. and Mishra, H. (2004). Yoghurt powder—a review of process technology, storage and utilization. *Food Bioprod. Process.* **82** (2), 133-142.

Lee, W.-J. and Lucey, J. (2010). Formation and physical properties of yogurt. *Asian-australas. J. Anim. Sci.* **23** (9), 1127-1136.

Lee, W. and Lucey, J. A. (2004). Structure and physical properties of yogurt gels: Effect of inoculation rate and incubation temperature. *J. Dairy Sci.* **87** (10), 3153-3164. [doi:10.3168/jds.S0022-0302(04)73450-5].

Lemieux, L. and Simard, R. (1994). Astringency, a textural defect in dairy products. *J. Dairy Sci.* **74** (3), 217-240.

Liu, K., Tian, Y., Stieger, M., van der Linden, E. and van de Velde, F. (2016). Evidence for ball-bearing mechanism of microparticulated whey protein as fat replacer in liquid and semi-solid multi-component model foods. *Food Hydrocoll.* **52**, 403-414.

Lock, A. L., Destaillats, F., Kraft, J. and German, J. B. (2008). Introduction to the proceedings of the symposium “Scientific Update on Dairy Fats and Cardiovascular Diseases”. *J. Am. Coll. Nutr.* **27** (6), 720S-722S. [doi:10.1080/07315724.2008.10719749].

Lucey, J. and Singh, H. (1997). Formation and physical properties of acid milk gels: a review. *Food Res. Int.* **30** (7), 529-542.

Lucey, J., Tamehana, M., Singh, H. and Munro, P. (1998). A comparison of the formation, rheological properties and microstructure of acid skim milk gels made with a bacterial culture or glucono-δ-lactone. *Food Res. Int.* **31** (2), 147-155.

Lucey, J. A. (2016). Acid coagulation of milk. *In:* "Advanced dairy chemistry" (4th ed.). (E. L. H. Paul and O. A. James, Ed.). pp. 309-328. New York. Springer. [ISBN 978-1-4939-7974-5].

Macura, D. and Townsley, P. (1984). Scandinavian ropy milk—identification and characterization of endogenous ropy lactic streptococci and their extracellular excretion. *J. Dairy Sci.* **67** (4), 735-744. [doi:10.3168/jds.S0022-0302(84)81363-6].

Macwan, S. R., Dabhi, B. K., Parmar, S. and Aparnathi, K. (2016). Whey and its utilization. *Int. J. Curr. Microbiol. Appl. Sci.* . **5** (8), 134-155.

Matela, K. S., Pillai, M. K., Matebesi-Ranthimo, P. and Ntakatsane, M. (2019). Analysis of proximate compositions and physiochemical properties of some yoghurt samples from maseru, lesotho. *J. Food Sci. Nutr. Res.* **2** (3), 245-252.

Mckinley and Michelle. (2005). The nutrition and health benefits of yoghurt. *Int. J. Dairy Technol.* **58** (1), 1-12.

Meisel, H. and Schlimme, E. (1990). Milk proteins: precursors of bioactive peptides. *Trends Food Sci. Technol.* **1**, 41-43.

Morr, C. (1985). Functionality of heated milk proteins in dairy and related foods. *J. Dairy Sci.* **68** (10), 2773-2781.

Mottar, J., Bassier, A., Joniau, M. and Baert, J. (1989). Effect of heat-induced association of whey proteins and casein micelles on yogurt texture. *J. dairy Sci.* **72** (9), 2247-2256. [doi:10.3168/jds.S0022-0302(89)79355-3].

Muliadi, J. (2011). Effect of milk ratio and skim milk powder concentration on the characteristics of milk-soy yoghurt. B. Tech. Thesis. Pelita Harapan Univ., Indonesia.

Murphy, E. G., Roos, Y. H., Hogan, S. A., Maher, P. G., Flynn, C. G. and Fenelon, M. A. (2015). Physical stability of infant milk formula made with selectively hydrolysed whey proteins. *Int. Dairy J.* **40**, 39-46.

Murtaza, M. A., Huma, N., Mustafa, I., Nawaz, H., Murtaza, M. S. and Ullah, M. I. (2015). Composition and microstructure of buffalo skim milk powder as influenced by concentration and drying temperatures. *Pak. J. Zool.* **47** (4), 1157-1161.

Myers, R. H., Montgomery, D. C. and Anderson-Cook, C. M. (2016). "Response surface methodology: process and product optimization using designed experiments". John Wiley & Sons. New York, USA. [1118916034].

Neilson, E. and Ullum, J. A. (1989). "Dairy Technology". Vol. 1. Danish Turnkey Dairies Ltd. Technical food and Dairy publishing house. Denmark.

North, C. E. and Park, W. H. (1927). Standards for Milk Pasteurization. *Am. J. Hyg.* **7**, 147-173.

Onwulata, C. and Huth, P. (2008). "Whey Processing" (1st ed.). Blackwell. USA. [ISBN 978-0-813-80903-8].

Pant, G. (1992). Formulation, process optimization and quality evaluation of soya yoghurt. B. Tech Dissertation. Tribhuvan Univ., Central Campus of Technology, Nepal.

Papadimitriou, K., Zoumpopoulou, G., Foligne, B., Alexandraki, V., Kazou, M., Pot, B. and Tsakalidou, E. (2015). Discovering probiotic microorganisms: in vitro, in vivo, genetic and omics approaches. *Food Microbiol.* **6**, 58. [doi:10.3389/fmicb.2015.00058].

Pearson, D. (1976). "The Chemical Analysis of Foods" (7 ed.). Longman Group Ltd. New York. [ISBN 0443014116].

Penna, A. L. B., Converti, A. and De Oliveira, M. N. (2006). Simultaneous effects of total solids content, milk base, heat treatment temperature and sample temperature on the rheological properties of plain stirred yogurt. *Food Technol. Biotechnol.* . **44** (4), 515.

Pernot-Barry, A. (2008). Importance of whey ingredients in confectionery products. *Polish J. Food Nutr. Sci.* **66** (3), 157-165. [doi:10.1515/pjfns-2015-0052].

Pintado, C. M., Ferreira, M. A. and Sousa, I. (2009). Properties of whey protein–based films containing organic acids and nisin to control *Listeria monocytogenes*. *J. Food Prot.* **72** (9), 1891-1896.

Pinto, S. S., Fritzen-Freire, C. B., Benedetti, S., Murakami, F. S., Petrus, J. C. C., Prudêncio, E. S. and Amboni, R. D. (2015). Potential use of whey concentrate and prebiotics as carrier agents to protect *Bifidobacterium-BB-12* microencapsulated by spray drying. *Food Res. Int.* **67**, 400-408.

Prabhu, G. (2006). US whey proteins in processed meats. *J. Dairy Sci.* **2** (6), 90-98.

Pugliese, A., Cabassi, G., Chiavaro, E., Paciulli, M., Carini, E. and Mucchetti, G. (2017). Physical characterization of whole and skim dried milk powders. *J. Food Sci. Technol.* **54** (11), 3433-3442.

Ranganna, S. (1986). "Handbook of analysis and quality control for fruit and vegetable products" (2 ed.). Tata McGraw-Hill Education. New Delhi. [ISBN 978-0-07-451851-9].

Reid, G., Charbonneau, D., Erb, J., Kochanowski, B., Beuerman, D., Poehner, R. and Bruce, A. W. (2003). Oral use of *Lactobacillus rhamnosus GR-1* and *L. fermentum RC-14* significantly alters vaginal flora: randomized, placebo-controlled trial in 64 healthy women. *FEMS Immunology Med. Microbiol.* **35** (2), 131-134. [doi:10.1016/S0928-8244(02)00465-0]

Ricke, S. and Pillai, S. (1999). Conventional and molecular methods for understanding probiotic bacteria functionality in gastrointestinal tracts. *Cric. Rev. Microbiol.* **25** (1), 19-38. [doi:10.1080/10408419991299176].

Robinson, R. and Tamime, A. (1993). Manufacture of yoghurt and other fermented milks. *In:* "Modern dairy technology".). pp. 1-48. UK. Springer.

Rupnar, P. (2006). Studies on preparation of paneer whey beverage using Kokam juice. M. Sc.(Ag.) Thesis. Mahatma Phule Krishi Vidyapeeth Univ., India.

Russell, T., Drake, M. and Gerard, P. (2006). Sensory properties of whey and soy proteins. *J. Food Sci.* **71** (6), 447-455.

Salji, J. P. and Ismail, A. A. (1983). Effect of initial acidity of plain yogurt on acidity changes during refrigerated storage. *J. Food Sci.* **48** (1), 258-259. [doi:10.1080/10408419991299176].

Salminen, S., Bouley, C., Boutron, M.-C., Cummings, J., Franck, A., Gibson, G., Isolauri, E., Moreau, M.-C., Roberfroid, M. and Rowland, I. (1998). Functional food science and gastrointestinal physiology and function. *Br. J. Nutr.* **80** (S1), 147-171. [doi:10.1079/BJN19980108].

Sarkar, S. (2008). Effect of probiotics on biotechnological characteristics of yoghurt: A review. *Br. Food J.* **3** (2), 78-89.

Savaiano, D. A. and Levitt, M. (1984). Nutritional and therapeutic aspects of fermented dairy products. *J. Dent. Child.* **51** (4), 305-308.

Seifu, E., Buys, E. and Donkin, E. (2005). Significance of lactoperoxidase system in the dairy industry and its potential application: a review. *Food Sci. Technol.* **16** (4), 137-154. [doi:10.1016/j.tifs.2004.11.002].

Shahani, K. M. and Chandan, R. C. (1979). Nutritional and healthful aspects of cultured and culture-containing dairy foods. *J. Dairy Sci.* **62** (10), 1685-1694. [doi:10.3168/jds.S0022-0302(79)83481-5].

Shrestha, A. K., Howes, T., Adhikari, B. P. and Bhandari, B. R. (2008). Spray drying of skim milk mixed with milk permeate: effect on drying behavior, physicochemical properties, and storage stability of powder. *Dry. Technol.* **26** (2), 239-247.

Shukla, F., Shilpa, M. and Thind, S. (2000). Bakery industry in India—Present quality control and future scenario: A review. *Beverage Food World*. **27**, 11-15.

Smit, G., Smit, B. A. and Engels, W. J. (2005). Flavour formation by lactic acid bacteria and biochemical flavour profiling of cheese products. *Microbiol. Rev.* **29** (3), 591-610. [doi:10.1016/j.fmrre.2005.04.002].

Smith, T. M., Kolars, J. C., Savaiano, D. A. and Levitt, M. D. (1985). Absorption of calcium from milk and yogurt. *Am. J. Clin. Nutr*. **42** (6), 1197-1200.

Smithers, G. W., Ballard, F. J., Copeland, A. D., de Silva, K. J., Dionysius, D. A., Francis, G. L., Goddard, C., Grieve, P. A., McIntosh, G. H. and Mitchell, I. R. (1996). New opportunities from the isolation and utilization of whey proteins. *J. Dairy Sci.* **79** (8), 1454-1459.

Sodini, I., Remeuf, F., Haddad, S. and Corrieu, G. (2004). The relative effect of milk base, starter, and process on yogurt texture: a review. *Crit. Rev. Food Sci. Nutr.* **44** (2), 113-137.

Spalatelu, C. (2012). Biotechnological valorisation of whey. *Innov. Rom. Food Biotechnol.* **10** (3), 1-8.

Staff, M. (1998). Cultured milk and fresh cheeses. *Technol. dairy Product*. 123-144.

Stoliar, M. (2009). US whey ingredients in bakery products. *Polish J. Food Nutr. Sci.* **66** (3), 1-8.

Tamime and HC, A. D. (1980). Yogurt: technology and biochemistry. *J. Food Prot.* **43** (12), 939-977.

Tamime, A. (2002). Fermented milks: a historical food with modern applications–a review. *Eur. J. Clin. Nutr.* **56** (4), 2-15.

Tamime, A. and Robinson, R. (1999a). Yoghurt. *Sci. Technol.* **2** (4).

Tamime, A. Y. and Robinson, R. K. (1999b). "Yoghurt: science and technology". Woodhead Publishing. [1855733994].

Tamime, A. Y. and Robinson, R. K. (2007). "Tamime and Robinson's yoghurt: science and technology" (3rd ed.). Elsevier. England. [ISBN 978-1-845-69261-2].

Tamine, A. and Robinson, R. K. (1999). "Yoghurt: science and technology" (2nd ed.). Woodhead Publishing. England. [ISBN 0-8493-1785-1].

Tomarelli, R. and Bernhart, F. (1962). Biological assay of milk and whey protein compositions for infant feeding. *J. Nutr.* **78** (1), 44-50. [doi:10.1093/jn/78.1.44]

Tribby, D. (2008). Yogurt. *In:* "The sensory evaluation of dairy products" (2nd ed.). (C. M. C. Stephanie, D. MaryAnne and B. Floyd, Eds., Ed.). pp. 191-223. New York. Springer. [ISBN 978-0-387-77408-4].

Tull, A. (1997). "Food and nutrition". Oxford University Press. USA. [ISBN 978-0-198-32766-0].

Urbiene, S. and Leskauskaite, D. (2006). Formation of some organic acids during fermentation of milk. *Pol. J. Food Nutr. Sci*. **15** (56), 277-281.

Vakil, J. and Shahani, K. (1970). Carbohydrate metabolism of lactic acid cultures IV. Glycolytic and HMS pathway enzymes in *Streptococcus lactis* and their sensitivity to antibiotics. *Canad. J. Microbiol.* **36** (1), 285-295.

Varnam, A. and Sutherland, J. P. (2001). "Milk and milk products: Technology, chemistry and microbiology". Vol. 1. Springer Science & Business Media. United Kingdom. [ISBN 978-0-834-21955-7].

Venica, C. I., Perotti, M. C. and Bergamini, C. V. (2014). Organic acids profiles in lactose-hydrolyzed yogurt with different matrix composition. *Dairy Sci. Technol.* **94** (6), 561-580.

Walstra, P. (1999). "Dairy technology: principles of milk properties and processes". CRC Press. USA. [ISBN 978-0-824-74641-4].

Wohlrab, Y. and Bockelmann, W. (1992). Purification and characterization of a dipeptidase from *Lactobacillus delbrueckii subsp. bulgaricus. Int. Dairy J.* **2** (6), 345-361. [doi:10.1016/0958-6946(92)90026].

Wollowski, I., Rechkemmer, G. and Pool-Zobel, B. L. (2001). Protective role of probiotics and prebiotics in colon cancer. *Am. J. Clin. Nutr.* **73** (2), 451-455.

Yildiz, E. and Ozcan, T. (2019). Functional and textural properties of vegetable-fibre enriched yoghurt. *Int. J. Dairy Technol.* **72** (2), 199-207.

Young, S. (2007). Whey products in ice cream and frozen dairy desserts. *U.S. Dairy Export Council*. Retrieved from http:// www.thinkusadairy.org. . [Accessed December 1, 2017].

Younus, S., Masud, T. and Aziz, T. (2002). Quality evaluation of market yoghurt/dahi. *Pak. J. Nutr.* **1** (5), 226-230.

Youssef, M. and Barbut, S. (2011). Effects of two types of soy protein isolates, native and preheated whey protein isolates on emulsified meat batters prepared at different protein levels. *J. Meat Sci.* **87** (1), 54-60.

Zadow, J. (1994). "Utilisation of milk components: Whey". Springer. USA.

Zourari, A., Accolas, J. and Desmazeaud, M. (1992). Metabolism and biochemical characteristics of yogurt bacteria. A review. *Dairy Sci. Technol.* . **72** (1), 1-34. [doi:10.1051/lait:199211].

Appendices

Appendix-A

Sensory evaluation card

Name: ………………………….. Date: …………………….

Product: Whey based yoghurt

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

Quality description

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

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

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

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

Sample	Aroma	Color	Taste	Texture	OA
A					
B					
C					
D					
E					

Comments if any: …………………………………………………

Signature……………………..

Appendix B

Table B.1 Cost evaluation of 100ml of 14% SMP and 3.46% sugar incorporated whey based yoghurt

Particulars	Quantity (g)	Rate (Rs.)	Amount (Rs.)
SMP	14	625/kg	8.75
Sugar	3.46	90/kg	0.31
Overhead cost (20%)			1.81
Total			10.87

Appendix C

ANOVA results of sensory analysis

Table C.1 ANOVA (no blocking) for aroma of whey based yoghurt

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.	l.s.d
Sample	4	40.12	10.03	20.66	<.001	0.63
Panelist	9	2.42	0.27	0.55	0.825	0.89
Residual	36	17.48	0.49			
Total	49	60.02				

Table C.2 ANOVA (no blocking) for color of whey based yoghurt

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.	l.s.d
Sample	4	27.28	6.82	12.58	<.001	0.67
Panelist	9	3.78	0.42	0.77	0.64	0.95
Residual	36	19.52	0.54			
Total	49	50.58				

Table C.3 ANOVA (no blocking) for overall acceptance of whey based yoghurt

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.	l.s.d
Sample	4	21.40	5.35	10.14	<.001	0.66
Panelist	9	6.10	0.68	1.28	0.279	0.93
Residual	36	19.00	0.53			
Total	49	46.50				

Table C.4 ANOVA (no blocking) for taste of whey based yoghurt

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.	l.s.d
Sample	4	26.80	6.70	17.74	<.001	0.56
Panelist	9	5.60	0.62	1.65	0.139	0.79
Residual	36	13.60	0.38			
Total	49	46.00				

Table C.5 ANOVA (no blocking) for texture of whey based yoghurt

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.	l.s.d
Sample	4	29.88	7.47	14.52	<.001	0.65
Panelist	9	6.58	0.73	1.42	0.216	0.92
Residual	36	18.52	0.51			
Total	49	54.98				

Appendix D

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

	Product E	Product D
Mean	0.77	1.06
Variance	0.000633	0.0001
Observations	3	3
Hypothesized Mean Difference	0	
df	3	
t Stat	18.33526	
P(T<=t) one-tail	0.000177	
t Critical one-tail	2.353363	
P(T<=t) two-tail	0.000354	
t Critical two-tail	3.182446	

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

	Product E	Product D
Mean	1.02	1.75
Variance	0.0252	0.062533
Observations	3	3
Hypothesized Mean Difference	0	
df	3	
t Stat	4.288246	
P(T<=t) one-tail	0.011655	
t Critical one-tail	2.353363	
P(T<=t) two-tail	0.023309	
t Critical two-tail	3.182446	

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

	Product E	Product D
Mean	2.77	0.47
Variance	0.063333	0.013333
Observations	3	3
Hypothesized Mean Difference	0	
df	3	
t Stat	-14.3875	
P(T<=t) one-tail	0.000364	
t Critical one-tail	2.353363	
P(T<=t) two-tail	0.000728	
t Critical two-tail	3.182446	

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

	Product E	Product D
Mean	82.78	75.60
Variance	0.442633	0.266133
Observations	3	3
Hypothesized Mean Difference	0	
df	4	
t Stat	-14.7787	
P(T<=t) one-tail	6.100000	
t Critical one-tail	2.131847	
P(T<=t) two-tail	0.000122	
t Critical two-tail	2.776445	

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

	Product E	Product D
Mean	4.72	5.39
Variance	0.065833	0.314533
Observations	3	3
Hypothesized Mean Difference	0	
df	3	
t Stat	1.88163	
P(T<=t) one-tail	0.078221	
t Critical one-tail	2.353363	
P(T<=t) two-tail	0.156443	
t Critical two-tail	3.182446	

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

	Product E	Product D
Mean	4.33	4.07
Variance	0.0033333	0.0033333
Observations	3	3
Hypothesized Mean Difference	0	
df	3	
t Stat	-5.656854	
P(T<=t) one-tail	0.0024063	
t Critical one-tail	2.1318467	
P(T<=t) two-tail	0.00481267	
t Critical two-tail	2.77644510	

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

	Product E	Product D
Mean	3.77	6.60
Variance	0.0637	0.284133
Observations	3	3
Hypothesized Mean Difference	0	
df	3	
t Stat	8.330735	
P(T<=t) one-tail	0.001813	
t Critical one-tail	2.353363	
P(T<=t) two-tail	0.003625	
t Critical two-tail	3.182446	

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

	Product E	Product D
Mean	17.26	24.45
Variance	0.442633	0.266133
Observations	3	3
Hypothesized Mean Difference	0	
df	4	
t Stat	14.77866	
P(T<=t) one-tail	6.100000	
t Critical one-tail	2.131847	
P(T<=t) two-tail	0.000122	
t Critical two-tail	2.776445	

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

	Product E	Product D
Mean	29.35	24.42
Variance	0.663433	0.651033
Observations	3	3
Hypothesized Mean Difference	0	
df	4	
t Stat	-4.37607	
P(T<=t) one-tail	0.005956	
t Critical one-tail	2.131847	
P(T<=t) two-tail	0.011911	
t Critical two-tail	2.776445	

Appendix E

Table E.1 8 solution for optimization of SMP and sugar for best syneresis, appearance, texture and overall acceptability

Number	SMP	Sugar	Syneresis	Appearance	Texture	Overall acceptability	Desirability
1	14.00	2.00	28.03	7.52	7.70	6.97	0.887
2	14.00	2.03	28.06	7.51	7.70	6.96	0.877
3	13.96	2.00	28.16	7.51	7.69	6.95	0.875
4	14.00	2.14	28.18	7.49	7.69	6.95	0.873
5	14.00	2.22	28.27	7.47	7.67	6.95	0.871
6	13.89	2.00	28.48	7.49	7.67	6.95	0.868
7	14.00	2.65	28.63	7.38	7.62	6.90	0.858
8	14.00	3.46	28.97	7.21	7.51	6.82	0.825

Appendix F

Table F.1 List of equipment used

Physical Apparatus	
Heating arrangement	Refrigerator
Electric balance (Phoenix)	Hot air oven (Vitco, India)
Incubator (Vitco, India)	Titration apparatus
Gerber centrifuge	Thermometer
Stainless steel vessels	Gerber butyrometer
Kjeldahl digestion and distillation set	Muffle furnace (Accumax India)
Daily routine glassware	Desiccator
Vaccum pump (Accumax India)	

Table F.2 List of chemicals used

Chemicals	Specifications
Sodium bicarbonate	Loba Chemie Pvt. Ltd (India)
Boric acid	Central Drug House Pvt. Ltd (India)
Starter culture	STI 15 (Denmark)
40% Formaldehyde	Thermo Fisher Scientific
Sodium hydroxide	HiMedia Laboratories Pvt. Ltd (India)
Oxalic acid	
Mixed indicator solution	
Saturated potassium oxalate	Merck Life Science Pvt. Ltd (India)
Sulphuric acid	Merck Life Science Pvt. Ltd (India)
Carrez- I and carrez- II	

Appendix G

Table G.1 Multi-response optimization constraints of whey based yoghurt.

Name	Goal	Lower Limit	Upper Limit	Importance
SMP	is in range	8	14	***
Sugar	is in range	2	6	***
Syneresis	minimize	22.6	59.83	*****
Appearance	maximize	5.2	7.4	***
Texture	maximize	4.8	8.2	***
Overall acceptability	maximize	5.2	7.4	**

Appendix H

Table H.1 Analysis of variance (ANOVA) for syneresis

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	1146.30	2	573.15	166.03	< 0.0001	**significant**
A-SMP	1146.28	1	1146.28	332.04	< 0.0001	
B-Sugar	0.0289	1	0.0289	0.0084	0.9294	
Residual	27.62	8	3.45			
Lack of Fit	26.73	6	4.46	10.06	0.0932	**not significant**
Pure Error	0.8858	2	0.4429			
Cor Total	1173.92	10				

*Significant at $p<0.05$, DF: Degree of freedom

Table H.2 Model summary statistics for syneresis

Source	Sequential p-value	Lack of Fit p-value	Adjusted R^2	Predicted R^2	
Linear	< 0.0001	0.0932	0.9706	0.9506	**Suggested**
2FI	0.7494	0.0795	0.9669	0.9165	
Quadratic	0.3275	0.0754	0.9704	0.8983	
Cubic	0.0983	0.1277	0.9895	0.8445	**Aliased**

* Case(s) with leverage of 1.0000: PRESS statistic not defined.

Table H.3 Analysis of variance (ANOVA) for appearance

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	4.55	2	2.27	18.30	0.0010	**significant**
A-SMP	3.16	1	3.16	25.44	0.0010	
B-Sugar	1.39	1	1.39	11.17	0.0102	
Residual	0.9939	8	0.1242			
Lack of Fit	0.6473	6	0.1079	0.6224	0.7238	**not significant**
Pure Error	0.3467	2	0.1733			
Cor Total	5.54	10				

*Significant at $p<0.05$, DF: Degree of freedom

Table H.4 Model summary statistics for appearance

Source	Sequential p-value	Lack of Fit p-value	Adjusted R^2	Predicted R^2	
Linear	0.0010	0.7238	0.7758	0.6956	**Suggested**
2FI	0.7974	0.6624	0.7464	0.5046	
Quadratic	0.3738	0.6698	0.7604	0.4523	
Cubic	0.5990	0.4852	0.7163	-0.5843	**Aliased**

* Case(s) with leverage of 1.0000: PRESS statistic not defined.

Table H.5 Analysis of variance (ANOVA) for texture

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	9.01	2	4.51	17.97	0.0011	**significant**
A-SMP	8.84	1	8.84	35.24	0.0003	
B-Sugar	0.1728	1	0.1728	0.6891	0.4305	
Residual	2.01	8	0.2508			
Lack of Fit	1.26	6	0.2099	0.5622	0.7526	**not significant**
Pure Error	0.7467	2	0.3733			
Cor Total	11.02	10				

*Significant at p<0.05, DF: Degree of freedom

Table H.6 Model summary statistics for texture

Source	Sequential p-value	Lack of Fit p-value	Adjusted R²	Predicted R²	
Linear	0.0011	0.7526	0.7724	0.6686	**Suggested**
2FI	0.4616	0.7263	0.7606	0.5227	
Quadratic	0.6913	0.6129	0.7109	0.3014	
Cubic	0.4233	0.5896	0.7283	-0.0312	**Aliased**

* Case(s) with leverage of 1.0000: PRESS statistic not defined.

Table H.7 Analysis of variance (ANOVA) for overall acceptability

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	3.23	2	1.62	7.22	0.0161	**significant**
A-SMP	2.91	1	2.91	13.02	0.0069	
B-Sugar	0.3200	1	0.3200	1.43	0.2661	
Residual	1.79	8	0.2239			
Lack of Fit	1.04	6	0.1741	0.4663	0.8017	**not significant**
Pure Error	0.7467	2	0.3733			
Cor Total	5.03	10				

*Significant at $p<0.05$, DF: Degree of freedom

Table H.8 Model summary statistics for overall acceptability

Source	Sequential p-value	Lack of Fit p-value	Adjusted R^2	Predicted R^2	
Linear	0.0161	0.8017	0.5545	0.3605	**Suggested**
2FI	0.4347	0.7835	0.5363	0.0823	
Quadratic	0.7741	0.6539	0.4140	-0.3613	
Cubic	0.6166	0.4523	0.2925	-3.4096	**Aliased**

* Case(s) with leverage of 1.0000: PRESS statistic not defined.

Color plates

Plate 1 Incubation

Plate 2 Whey based yoghurt

Plate 3 Sensory evaluation

Plate 4 Sensory evaluation