EFFECT OF *Saccharomyces boulardii* ADJUNCT CULTURE ON THE QUALITY OF DAIRY AND NON-DAIRY (SOYA MILK) YOGHURT

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CERTIFICATION PAGE

This is to certify that this work: ***EFFECT OF Saccharomyces boulardii ADJUNCT CULTURE ON THE QUALITY OF DAIRY AND NON-DAIRY (SOYA MILK) YOGHURT*** was done by OBIAGELI SONIA OKAFOR of registration number 20164997948 in the Department of Biotechnology, School of Biological Science, in partial fulfillment of the requirements for the award of Master of Science (M.Sc) degree in Biotechnology.

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DEDICATION

This work is dedicated to my parents Mr. and Mrs. O. P. Ndu for their financial and moral support, to my sibling, Obinna for his understanding, love and prayers.

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ABSTRACT

The effect of adjunct culture of *Saccharomyces boulardii* on yoghurt from dairy and non-dairy milk was studied and treatment made from both samples by adding 1%, 2% and 3% of *Saccharomyces boulardii* with yoghurt starter culture (*Lactobacillus bulgaricus* and *Streptococcus thermophilus*). *S. boulardii* was incorporated into dairy and non-dairy milk, fermented for 8 hours at 40^0C and stored at 4^0C for a period of 21 days. After fermentation, physicochemical, proximate, nutritional and sensory evaluations were conducted respectively. The results showed that non-dairy samples without the addition of *S. boulardii* was lower at 18.95±0.5 and 17.05+0.7 in total solids and total solids non-fat while dairy samples without *S. boulardii*had lowest at 0.68±0.2 in total nitrogen. Statistical analysis ($p<0.05$) showed a significant difference on the samples in the various physicochemical evaluation. Dairy samples without *S. boulardii* had the highest fat and carbohydrate content at 2.6±0.5 and 12.14±0.1 respectively. Statistical analysis ($p<0.05$) showed a significant difference in the sample during the proximate evaluation. The result of the sensory evaluation revealed that dairy sample with 3% *S. boulardii*was best in aroma, texture, colour/appearance, taste and general acceptability at 8.4±0.4, 7.8±0.4, 8.2±0.3, 7.8±0.3 and 7.51±0.2 respectively. The samples were observed to be significantly different ($p<0.05$). Dairy samples without *S. boulardii* and 2% *S. boulardii* had highest at 224.65+0.7 and 126.53+0.9 for calcium and sodium respectively while non-dairy sample with 1% *S.boulardii* was highest at 560.54+1.6, 401.06+1.1, 162.98+1.0 & 3.97+0.3 in potassium, phosphorous, magnesium and manganese. Non-dairy samples had the highest at 90.91±0.01, 39.11+0.00, 131.9±0.09 and 27.11±0.01 in vitamin A, B_2, B_3 and B_9 while dairy sample had highest at 36.92±0.00 on B_{12}. The samples were significantly different ($p<0.05$) during the statistical analysis. Syneresis, water holding capacity, titratable acidity and microbial viability were analyzed during 21 days of storage time. Titratable acidity, water holding capacity of the sample increased with the increase in percentage of *S. boulardii*: while syneresis of the sample decreased with the increase in percentage of *S. boulardii*. The lactic acid bacteria had the highest growth with 3% *S. boulardii* at 8.83 and 8.54 Log CFU/ml for*L. bulgaricus* and *S. thermophilus* respectively. *S. boulardii* count was at the range of 10^6 and 10^5cfu/ml for dairy and nondairy samples respectively. The addition of *S. boulardii* improved the survivability of the bacteria starter culture.

Key Words: *Saccharomyces boulardii,* Yoghurt, Dairy Milk, Non- Dairy Milk.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

FIGURE **PAGE**

CHAPTER ONE

1.0 INTRODUCTION

1.1 BACKGROUND

The primary role of diet is to provide sufficient nutrients to meet the nutritional requirements of an individual. There is now increasing scientific evidence to support the hypothesis that some foods and food components have beneficial physiological and psychological effects over and above the provision of the basic nutrients. Thus, the concept of foods (functional foods) that were developed specifically to promote health or reduce the risk of disease was introduced. Functional food are foods that provide biological and therapeutic properties beyond their basic nutritional value (Hasler, 2002) which incorporate readily into diet food and proposed to reduce disease risk (Buckley. N. D., Champagne, C.P., Mastti, A. I., Wagar, L. E., Tompkins, T. A. & Green J. M, 2011).

Yoghurt is a semi-solid fermented milk product made by fermentation process of fresh milk using lactic acid starter culture composed of *Lactobacillus delbrueckii spp. bulgaricus* and *Streptococcus thermophilus* at a ratio of 1:1 to give acidity value of 0.7-1.1% lactic acid with pH approximate 3.8-4.6. Yoghurt made from cow's milk is widely consumed. On the other hand, there is a desire for alternatives to cow's milk due to problems relating to gastrointestinal intolerance and market demand for the formulation of novel dairy products (Rima, J., Santosh K. M., Gurvir, S. B., Singh, P. K. & Panwar, H, 2007). The fermented soy products represent an interesting alternative to the fermented milk products. The metabolisms of lactic acid bacteria (LAB) show some differences between soya milk and cow milk as a result of their different compositions. Soya milk contains galactooligosaccharides which are considered prebiotics, i.e. compounds supporting probiotic bacteria growth (Farnworth, 2007). The metabolism of saccharides and proteins caused by LAB can influence both nutritional value and final sensory quality of fermented milk product.

Yogurt is considered a functional food because of its probiotic components. Probiotics are defined by FAO/ WHO (2002) as "live microorganisms which, when administered in adequate amounts, confer a health benefit on the host". Most probiotic microorganisms belong to Lactic Acid Bacteria (LAB), such as *Lactobacillus* sp, *Bifidobacterium sp.* and *Enterococcus sp*. (Champagne, C. P., Ross, R. P., Saarela, M., Hansen, K. T. & Charalampopoulos, D, 2011).

This restricts probiotic foods to products that contain live microorganisms and improve health and well-being of humans or animals.

Probiotics must be viable and available at a high concentration (minimum 10^7cfu/g) to convey health benefits (Champagne *et al*., 2011). Nualkaekul and Salmeron, (2011) reported that many cultured dairy products currently in the market fail to meet these standards because the strains of probiotic bacteria used in production cannot survive the acidity of the product. According to Antonia, T., Aikaterini, P., Iliada, K., Lappa, V. K., Loulouda, A. B. & Nikolaos, K. (2019) the growth of the lactic acid bacteria starters is inhibited by unfavourable environmental conditions such as low pH-values and high salt concentrations (Sheehan, V. M., Ross, P. & Fitzgerald, G. F.,2007). Even if there are enough viable cells at production time, the acidity of the product can increase when it contains lactic acid producing bacteria, eventually inhibiting their own viability (Antonia *et al*., 2019).

Hattingh and Vilijeon (2001) stated that yeasts, in contrast, can grow under conditions unfavourable to many bacteria and therefore play a significant role in the ripening of some cheese varieties as well as the spoilage of dairy products (Martin- Rios, C., Demen-Meier, C., Gössling, S. & Cornuz, C., 2018: Hattingh and Vilijeon, 2001). The proteolytic and lipolytic activity of certain yeast species, possible microbial interactions, their inhibitory activity against spoilage organisms, their ability to produce vitamins and amino acids and the utilization of lactic acid with a resulting higher pH all make yeasts potentially viable organisms for use as starter cultures in the dairy industry (Martin *et al*., 2018). Although yeasts were always considered as contaminants in dairy products, causing spoilage during the fermentation process (Martin *et al*., 2018, Hattingh and Vilijeon, 2001), the above mentioned characteristics of the yeasts, indicate that they could be included as part of the starter cultures for yoghurt manufacture. This could be of considerable economic importance for the dairy industry.

In present study, the inclusion of *Saccharomyces boulardii* in yoghurt manufacture is investigated. *Saccharomycs boulardii* is a tropical strain of yeast first isolated from lychee and mangosteen fruit by Henri Boulard (Hui, 2004). It is related to *Saccharomyces cerevisiae*, but differing in several taxonomic metabolic, and genetic properties (Champagne *et al*., 2011). It has been discovered from studies that *Saccharomyces boulardii* maintains and restores the natural floral in the large and small intestine; so it is classified as a probiotic. It has been shown to be non-pathogenic; non-systematic (it remains in the gastrointestinal tract rather than spreading to other parts of the body). It grows mostly at a high temperature of 37^0C (Scott, 2002). *S.boulardii* is not part of the gut natural flora like some bacteria. It is resistant to stomach acid, bile and pancreatic juices. It tolerates differential pH levels which make its survivability

higher than that of bacteria (Davidson *et al*., 2000). *S. boulardii* is recognized to have probiotic effectiveness used alone or in combination with other probiotics to support digestion. It is useful as biotherapeutic agent in combination with antibiotics for treatment of Clostridum difficile diarrhea and colitis. Biotheraputic agents as with probiotic, must be given in sufficient concentration to exert therapeutic properties, remain stable and viable before use and survive in the intestinal ecosystem of the host to exert their therapeutic properties. Dietary supplementation with the probiotic organism such as *Lactobacillus* and *S. boulardii* was reported to help reduce some effect of aging (Nivien, Rios, C., Demen-Meier, C., Gössling, S. & Cornuz, C., 2006).

1.2 PROBLEM STATEMENT

Yoghurt is one of the oldest fermented dairy foods in the world. Its origins date back to the dawn of civilization. When humans began domesticating animals for milk production, milk's short shelf life required solutions for storing it. Yoghurt is made by introducing certain bacteria into fresh milk – typically *Streptococcus thermophilus* and *Lactobacillus delbrueckii* sub sp. *Bulgaricus*. Usually, both bacteria are present in yoghurt and form the yoghurt starter culture (Ranadheera *et al*., 2017). Their synergistic relationship is a key factor in the consistency of the final product. They are not natural inhabitants of the intestine and cannot survive the acidic conditions and bile concentrations in the gastrointestinal tract; hence, they do not do much to change the microbiota in the gut (Ranadheera, Rios, C., Demen-Meier, C., Gössling, S. & Cornuz, C.,2017).

Yoghurts also have added probiotics. These are live microorganisms that can help establish a healthy gut microbiota. The most widely used probiotics are the acidophilus strain, known as *Lactobacillus acidophilus*, and *Bifidobacterium*. These could be useful for people who have gastrointestinal problems such as irritable bowel syndrome (IBS) but their poor survival in yoghurt is attributed to the low pH of the environment and low acid-tolerance (LourensHattingh and Viljoen, 2002).

It is essential that probiotic products sold with any health indication meet the minimum criterion of a million viable probiotic cells per milliliter product at the expiry date. According to Kurmann and Rasic (1991) minimum dosage of probiotic cells per day for any beneficial effect on the consumer is considered to be 10^8–10^9 probiotic cfu/ml corresponding to an intake of 100g product containing 10^6–10^7 cfu/ml per day.

It has been reported (Shah, 2000) that probiotics, present in bio-yogurt, are unstable. People who are lactose intolerant are missing the enzyme lactase, which breaks down lactose, a sugar found in milk and dairy products. As a result, individuals who are lactose intolerant are unable to digest milk and dairy product, and may experience symptoms such as nausea, cramps, gas, bloating and diarrhea when exposed to such products.

Poor survival of probiotic bacteria in yogurt is attributed mainly to the low pH of the product environment. Since yeasts have the ability to metabolize organic acids, resulting in a decrease in acidity, the inclusion of yeasts as part of the normal microflora, in association with probiotic bacteria has been suggested with the intention to assure better survival. However, excessive gas and ethanol production initiated by some yeast species proved to be major constraints (Lourens-Hattingh and Viljoen, 2002).

1.3 AIM AND OBJECTIVES OF THE RESEARCH

The study is aimed at determining the effect of *Saccharomyces boulardii* on yoghurt quality produced with diary milk and soya milk.

Specific objectives of the research are:

1. To isolate and characterize freed dried orgaanisms in pellet form
2. Fermentation of dairy and soya milk in the presence of graded doses of *Saccharomyces boulardii*.
3. Evaluation of the effect of *Saccharomyces boulardii* on the physical and chemical quality of the yoghurt.
4. Evaluation of the effect of *Saccharomyces boulardii* inclusion on the proximate quality of the yoghurt.
5. Evaluation of the effect of *Saccharomyces boulardii* inclusion on the vitamins and minerals components of the yoghurt.
6. To determine the effect of *Saccharomyces boulardii* inclusion on the shelf-life of the yoghurt.
7. To determine microbial viability of the organisms after fermentation and storage

1.4 HYPOTHESIS

Null hypothesis: this states that *Saccharomyces boulardii* inclusion has no significant effect on the quality of the yoghurt.

Alternative hypothesis: this states that there is a significant effect on quality of yoghurt with added *Saccharomyces boulardii*.

1.5 JUSTIFICATION OF THE STUDY

S. boulardii is a live yeast used extensively as a probiotic (McFarland, 2010). Its mechanisms of action include regulation of intestinal microbial homeostasis, interference with the ability of pathogens to infect and colonize the mucosa, stabilization of the gastrointestinal barrier function and induction of enzymatic activity favouring absorption and nutrition (Im and Pothoulakis, 2010; Pothoilakis, 2009). The use of *Saccharomyces boulardii* in the fermentation of both dairy and non dairy milk could reduce the gastrointestinal diseases, lactose intolerance associated with dairy milk, reduce the phytate content of soymilk and induction of enzymatic activity and protease inhibitors.

1.6 SCOPE OF STUDY

This research work will specifically investigate the survivability and impact of adjunct culture of Saccharomyces boulardii on yoghurt produced with dairy and non dairy (soya) milk.

CHAPTER TWO

LITERATURE REVIEW

2.1 DEFINITION OF YOGHURT

Yoghurt is a semi-solid fermented milk product made by fermentation of fresh milk using lactic acid bacterial starter culture of *Lactobacillus delbrueckii spp. bulgaricus* and *Streptococcus thermophilus* in a ratio of 1:1 to give acidity value of 0.7-1.1% of lactic acid, with pH approximate of 3.8-4.6. As stated by Codex Alimentarius Commission (2003) both bacteria strains must remain active in the final product with at least 10million bacteria per gramme. The fermentation of lactose by these starter culture bacteria produces lactic acid and a series of other compounds which act on the milk protein to give yoghurt its texture and characteristic tart flavor (Code of Federal Regulation, 2016).

Yoghurt is one of the most popular fermented milk products and the consumption is increasing worldwide (Shiby and Mishra, 2013). The milk most commonly used for yoghurt is cow milk due to its common availability. Other milk from different sources (i.e. goat milk, camels, water buffalo milk and plants) can also be used to produce yoghurt. Milk used for yoghurt production may be raw, homogenized or pasteurized.

2.2 ORIGIN OF YOGHURT

The word "yoghurt" comes from the Turkish word "Yogurmak", which means thicken or coagulated. Yoghurt is an ancient food that has gone by many names over the millennia: Katyk (Armenia), dahi (India), zabadi (Egypt), mast (Iran), lebenraib (Saudi Arabia), laban (Iraq and Lebanon), roba (Sudan), iogurte (Brazil), culajade (Spain), coalhada (Portugal), dovga (Azerbaijan), and matsoni (Goegia, Russia and Japan) (Monreno *et al*., 2013).It is believed that milk products were incorporated into the human diet around 10,000-5000 BC, with the domestication of milk – producing animals (Monreno Rios, C., Demen-Meier, C., Gössling, S. & Cornuz, C., 2013). However, milk spoils easily, making it difficult to use. During thr earlier time, herdsmen in the Middle East carried milk in sacks which was made of intestinal gut. This lead to the discovery that contact with intestinal juice made the milk curdle and sour, preserving and conserving the dairy product for a period of time (Mchee, 2004).

Indian Ayurvedic scripts dating about 6000 BC, refer to the health benefits of consuming fermented milk products (Brothwell and Brothwell, 1997). Yoghurt was well known in the Greek and Roman empires, and the Greeks were the first to mention it in written references in

100BC, noting the use of yoghurt by barbarous nations (Batmanglij, 2007). The use of yoghurt by the medieval Turks was recorded in the books Diwan Lughat al-Turk by Mahmud Kashgari in 1984 and Kutadgu Bilig by K.H. Yusuf in 1983 (Fisberg and Mashado, 2015).

The Turks were the first to evaluate yoghurt's medicinal use for a variety of illnesses and symptoms, such as diarrhea and cramps, and alleviate the discomfort of sunburned skin. Genghis Khan, the founder of the Mongol empire, had a reputation for feeding his army yoghurt, a staple of the Mongolian diet based on the belief that it instilled bravery in warriors (Mchee, 2004). In 1542, King Francoise 1 of France introduced the dairy products to Western

Europe after being offered yoghurt as a treatment by the country's Turkish allies for bouts of severe diarrhea. It was later mixed with a variety of ingredients such as cinnamon, honey, fruits and sweets.

In 1905, Stamen Grigorov a Bulgarian medical student, first discovered *Bacillus bulgaricus* (known as *L. bulgaricus*), a lactic acid bacteria that is used in yoghurt starter culture till date.

According to Grigorov's research in 1909 a Russian Noble Laureate, Yilla Metchnikoff from the Pasteur institute in paris, suggested that *Lactobacilli* in yoghurt were associated with longevity in the Bulgarian peasants ((Mchee, 2004). During the early 20th century, yoghurts were sold in pharmacies as medicine due to its popularly known health benefits. Yoghurt became a commercial success after Isaac Carasso, from Barcelona started producing it with

Jam. In 1934 and 1941, the first yoghurt laboratory and factory were opened in France and United States respectively (Brothwell and Brothwell, 2007).

2.3. TYPES OF YOGHURT

Different types of yoghurt have been introduced into the dairy market in response to consumer preferences, changing life style, and health concerns (Tribby, 2009). Yoghurt can be placed into different categories according to manufacturing processes and variation in physical, chemical, flavour, and compositional modifications (Tamine and Robinson, 2007).

Traditional Unstrained Yoghurt: This kind of yoghurt tends to be thinner than yoghurts like Greek or Australian because it has not been strained and thus has more liquid content. Unstrained yoghurt is a great option for kids because they are easily turned off by the thicker consistency.

Greek Yoghurt: This yoghurt when compared to unstrained yoghurt has twice the protein, less sugar, fewer carbohydrate, and less liquid content.
Balkan- Style or Set- Style Yoghurt: In the production of this yoghurt, the warm cultured milk mixture is poured into containers, and then incubated without any further stirring. This Balkan- Style or set-style yoghurt has a characteristic thick texture.

Skryr, (Iceland Yoghurt): This is a version of yoghurt that undergoes a procedure of being strained 4 times to achieve a very thick consistency. It is traditionally made from skim milk. It contains more protein per serving than any other yoghurt.

Drinkable Yoghurt: Drinkable yoghurt comes in various varieties and flavours. They range from tart to sweet. Drinkable yoghurt has great texture, taste and benefits. They range from watery to ultra thick.

Goat Milk Yoghurt: This yoghurt has a smooth, richer and creamier texture than cow's milk yoghurt but certain individuals get turned off by its taste. Study show that 93% of infants allergic to cow's milk were able to drink goat's milk without reaction.

Sheep Milk Yoghurt: Sheep's milk yoghurt has a similar taste and texture as cow milk yoghurt but many do not usually react well to it. It is an excellent source of vitamin B, calcium and riboflavin. Sheep milk yoghurt is ideal for cooking because it doesn't break down like other yoghurt at high temperatures. The fat content of sheep milk is higher than cow milk.

Soy Yoghurt: Soy yoghurt starts with soy milk base made from soya beans and combined with live cultures to thicken up. It is dairy free, no cholesterol and appropriate for yoghurt lovers avoiding animal protein. This is produced using sweeteners and thickeners to achieve the thick consistency of yoghurt. Soy yoghurt is close to liquid and lacks in major flavour.

Almond Milk Yoghurt: Almond milk yoghurt is non-dairy yoghurt high in fiber and calcium and low in fat and sodium. This is a good option for people wanting to avoid lactose and dairy yoghurt. It has a similar texture to traditional yoghurt but it is thin and loose. Thickeners and sweeteners are added to improve its consistency. Almond milk yoghurt contains lower protein.

Prebiotic Yoghurt: This yoghurt contains substances called fructooligosaccharides, like inulin, in addition to probiotic cultures. These ingredients help the body to keep the probiotic bacteria alive in the body where they can reproduce in the intestine.

Kefir: This is a yoghurt drink made with traditional cultures and also has yeast added to it. The added yeast adds an extra tang to the flavor and has additional benefits to the health.

Kefir can be made from whole or low fat milk.

2.4. COMPOSITION OF STANDARD YOGHURT

According to the International Dairy Federation (IDF, 2000) fermented milk are milks prepared from milk and/or milk products (e.g any one or combinations of whole, partially or fully skimmed, concentrated or powdered milk, butter milk powder, concentrated or powdered whey), milk protein (such as whey protein, edible casein and caseinates), cream, butter or milk fat (all of which has been manufactured from raw materials that have been pasteurized) by the action of specific microorganisms, which results in a reduction of the pH with or without coagulation.

Codex Alimentarus Commission (CAC) and its committee, together with the IDF, have established regulations of international standards for fermented milks (Codex, 2003), describing fermented milk, concentrated fermented milk and flavoured fermented milks. Since drinkable fermented milk products that exist in the market were not covered in the 2003 Codex standard of identity, a drafted amendment to the Codex Standard of identity for fermented milks pertaining to drinks based on fermented milk was obtained in 2008 (Codex, 2008). The amendment proposed to have a minimum concentration of 40% fermented milk, with a minimum protein content of 1.08% and a minimum acidityof 0.1% (titratable acidity expressed as lactic acid (%w/w).

Fermented milk with minimum protein content of 2.7% as shown in Table 2.1 are usually classified into set yoghurt, stirred yoghurt, alternative culture yoghurt, cultured butter milk, cultured cream products, kefir, kumys and fermented special products (Codex, 2003). Each country has its own standard for milk or raw materials, starter culture, manufacturing procedure, quality requirements and legal requirements (Surono, 2011).

TABLE 2.1: CODEX STANDARD FOR FERMENTED MILKS

Composition	Fermented milk	Yoghurt alternative	Kfir
Milk Protein (% w/w)	Min. 2.7%	Min. 2.7%	Min. 2.7%
Milk Fat (% w/w)	Less than 10%	Less than 15%	Less than 10%
Titratable acidity i.e. lactic Acid (% w/w)	Min. 0.3%	Min. 0.6%	Min. 0.6%
Sum of Starter Culture ($cfu^{-1}g$ initial)	Min. 10^7	Min. 10^7	Min. 10^7
Labeled microorganisms ($cfu^{-1}g$ total)	Min. 10^6	Min. 10^6	Min. 10^6
Yeast ($cfu^{-1}g$ total)			Min. 10^4

Source: Codex Standard of Fermented Milks (Codex, 2003)

In the United States, the Code of Federal Regulations (CFR) of the Food and Drug Administration sets a standard of identity for yogurt. Three categories listed include: yoghurt, low-fat yoghurt and non-fat yoghurt (CFR, 2009).

TABLE 2.2: STANDARD COMPOSITION FOR YOGHURT IN THE UNITED STATES

Composition	Yoghurt	Low fat yoghurt	Non-fat yoghurt
Fat	>3.25	>0.5 - <2.0	<0.5
Protein	4.4	5.7	5.2
Carbohydrate	7.5	7.5	6.9
Milk Solids Non-fat	>8.25	>8.25	>8.25
Titratable Acidity	>0.9	>0.9	>0.9

Source: Surono, 2001.

Heat treatment after fermentation of yoghurt results in an extension of shelf life, but kills the yoghurt cultures. IDF standard defines fermented milks as products without any form of heat treatment after fermentation (IDF, 2000). The current Codex Alimentarius trend is to differentiate between live product and heat-treated product by clear labeling, for instance,

"contains no active culture", for the consumer's information. IDF provides a recommended method for the enumeration of total viable count in yoghurt. The total viable count is proposed to be $10^8 cfu^{-1}g$ at the time of manufacture, and Codex standard for fermented milks (Codex, 2003) defines the viable count of yoghurt starter after the product has been stored under the storage condition specified in the labeling at the end of expired date as a minimum $10^6 cfu^{-1}g$ viable yoghurt starter.

The Prevention of Food Adulteration Act and Rules (PFA) set the standards of identity for yoghurt based on plain yoghurt, skimmed yoghurt, sweetened flavoured yoghurt and fruit

yoghurt (PFA, 2004). Various countries have their own standard of identifying yoghurt and other fermented milk, normally based on fat, solids nonfat content and acidity.

TABLE 2.3: PFA STANDARD FOR YOGURT

Particulars	Plain yoghurt	Skimmed yoghurt	Sweetened/ Flavoured yoghurt	Fruit yoghurt
Total Milk Solids (% w/w)	Min. 13.5	11.0	13.5	10.0
Milk Fat (%w/w)	Min. 3.0	Min.0.5	Min.3.0	Min.1.5
Sugar (%w/w)	Nil	Nil	Min. 6.0	Min.6.0
Titratable Acidity (%)		0.8 – 1.2		
Bacteria Count ($cfu^{-1}g$)	Min. 10^6	Min. 10^6	Min. 10^6	Min.10^6
Coliform Count ($cfu^{-1}g$)	*E.coli* shall be absent in the product			

Source: The Prevention of Food Adulteration Act & Rules, 2004.

Concentrated fermented milk is a fermented milk in which the protein content has been increased prior to or after fermentation to a minimum of 5.6% (PFA, 2004). Concentrated fermented milk includes strained yoghurt. Flavoured fermented milks are composite milk products, which contains a maximum of 50% (w/w) of non-dairy ingredients (such as nutritive and non nutritive sweeteners, fruits and vegetables as well as juices, purees, pulps and preservatives derived from cereals, honey comb, chocolate, nuts, coffee, spices and other harmless natural flavours) and/or flavours (Surono, 2011). The non dairy ingredients can be mixed in prior to or after fermentation. The current standard for identifying fermented milks concern; milk fat, milk protein content, amount of starter cultures; a minimum live and active culture content of labeled microorganisms added as supplement to the specific starter cultures as well as titratable acidity expressed as lactic acid (PFA, 2004)

2.5. TRADITIONAL YOGHURT

The traditional yoghurt is produced starting with milk in addition to starter culture bacteria of *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophillus* only. The bacteria feed on the milks sugar, known as lactose. The by-product of the fermentation process is lactic acid. The fermentation process is responsible for the tangy flavour of yoghurt; the production of lactic acid by *Lactobacillus delbrueckii subsp. bulgaricus* impacts a sour, acidic

taste and a mixture of various carbonyl compounds like acetone, diacetyl and acetaldehyde, the latter of which is considered the major flavour component according to Law in 1981 (Chaves, Rios, C., Demen-Meier, C., Gössling, S. & Cornuz, C. X2002). The lactic acid forces the milks protein or casein molecules to break down and recombine, transforming milk from a liquid into a delicate semisolid gel.

Traditional yoghurt has a high concentration of acetaldehyde due to the low utilization of this metabolite by yoghurt bacteria, which lack alcohol dehydrogenase, the main enzyme needed to convert acetaldehyde into ethanol (Chaves *et al*., 2002). During fermentation, acetaldehyde is produced directly from lactose metabolism as a result of pyruvate decarboxylation. However, lactic acid bacteria also have alternative metabolic pathways that can produce acetaldehyde (Chavez *et al*., 2002).

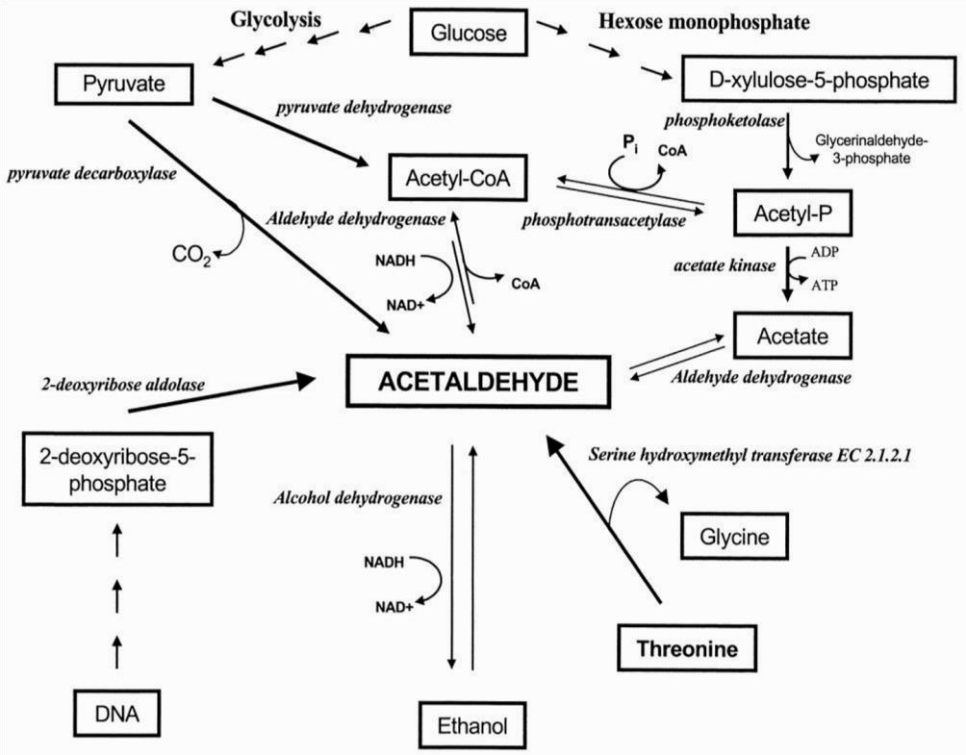

Fig 2.1: Production pathways of the compounds found in yogurt produced using bacteria *S. thermophilus* and *L. bulgaricus*. (Chaves, *et al.*, 2002).

Traditional yoghurt starters consist of weakly proteolytic *S. thermophilus* strain and proteolytic *L. delbrueckii subsp. bulgaricus* strain (Walstra *et al.*, 2006). During the early stage of fermentation, the initial pH of milk (ca. 6.7) is more favourable to the growth of *S. thermophilus*. These bacteria develop by using free amino acids and peptides available in milk. However, the contents of these free nitrogen sources are not sufficient to promote their full growth (Letort and Juillard, 2001), then *S. thermophilus* utilizes amino acids and peptides obtained from the proteolytic activity of *L. delbrueckii subsp. bulgaricus*. On the other hand, *S. thermophilus* produces lactic acid which consequently lowers the pH, hence retards its growth, and creates a favourable growth condition for *L. delbrueckii subsp. bulgaricus*. Furthermore, pyruvic acid, formic acid, folate, ornithine, several long_chain fatty acids and CO2 produced by *S. thermophilus* are the growth stimulants of *L. delbrueckii subsp. bulgaricus* (Angelov *et al.*, 2009). The proto_cooperation has an important role on the growth of *S. thermophilus* and *L. delbrueckii subsp. bulgaricus.* Although interaction between *S. thermophilus* and *L. delbrueckii subsp. bulgaricus* is often positive, absence of interaction or even negative effects can take place depending on the combination of bacterial strains, type and pre_heating process of base milk and fermentation conditions (Courtin and Rul, 2004).

2.5.1. PROPERTIES OF TRADITIONAL YOGHURT

2.5.1.1 MILK

The main ingredient in yoghurt is milk. The type of milk used depends on the type of yoghurt; whole milk for full fat yoghurt, low fat milk for low fat yoghurt, and skim milk for non fat yoghurt. Other dairy ingredients are allowed in yoghurt to adjust the composition, such as cream to adjust the fat content and nonfat dairy milk to adjust the solid contents. The solid content of finished yoghurt is often adjusted. (Chandan, 2006).

2.5.1.2 YOGHURT STARTER BACTERIA

2.5.1.2.1 *Streptococcus salivarius subsp. thermophilus*

According to the Taxonomic outline of the prokaryotes (Garrity *et al*., 2004), the species *S.thermophilus* belongs to the domain Bacteria, phylum *Firmicutes*, class *Bacilli*, order *lactobacillus*, family *Streptococcaeseae* and genus *Streptococcus* (Tannock, 2005). *Streptococcus salivarius subsp. thermophilus* is a Gram ‑ positive, catalase ‑ negative, thermophilic, facultatively anaerobic LAB. Young cells of *S. thermophilus* are spherical in shape and occur in chains. It is non-motile and does not form endospores. The cell wall is composed of N-acetylglucosamine (NAG) and N-acetylmuranic acid (NAM), which is bonded by ether bonds. This unique structure allows *S. thermophilus*to endure elevated temperatures. This bacterium has an optimum growth temperature of 40 – 45 ^{0}C and an optimum pH near 6.0 – 6.5 (Pearce, L., Flint, S. & Hubert, R, 2002).

S. thermophilus is widely used for the manufacturing of fermented dairy products and is considered as the second most important species of industrial lactic acid bacteria (LAB) after *Lactococcus lactis* (Iyer, R., Tomar, S. K., Uma Maheswari, T., & Singh, R, 2010). Live cultures of *S. thermophilus* make it easier for people who are lactose intolerant to digest dairy products. The bacteria break down lactose, the sugar in milk, that lactose-intolerant people find difficult to digest (Leboffe, 2012). One of the main functions of *S. thermophilus* is to provide a rapid acidification in milk. *S. thermophilus* is homofermentative and generates L (+) lactic acid as the main product from lactose metabolism. It metabolizes only the glucose moiety of lactose (Tamine and Robinson, 2007). *S. thermophilus* does not have the ability to metabolize galactose and thereby releasing this sugar from the cell into the medium. The rate of acidification is dependent on the strains metabolic rate that may be influenced by other factors, such as proteolytic system and urease activity (Iyer *et al.*, 2010). Most of *S. thermophilus*

strains show little or less proteolytic activity as a result of the lack of cell_enveloped proteases (PrtS). Traditionally, the level of free nitrogen sources available in milk is insufficient, and thus supplementation is usually required to support their full growth. One of the strategies used in the manufacturing of yoghurt is co_cultivation the non_proteolytic *S. thermophilus* with a suitable proteolytic culture, i.e. *L. delbrueckii subsp. bulgaricus* (Harnett, J., Davey, G., Patrick, A., Caddick, C., Pearce, L., & Fuquay, J. W, 2011). Several *S. thermophilus* strains also have capacity to produce aroma volatiles and exopolysaccharides (EPS). These strains are used to facilitate the distinctive flavor and texture characteristic of yoghurt (Zourari, A., Accolas, J. P., & Desmazeaud, M. J, 1992). Recently, the complete genome sequence and functional_genomic analyses of many *S. thermophilus* strains have been extensively published (Delorme, C., Bartholini, C., Luraschi, M., Pons, N., Loux, V., Almeida, M., Guédon, E., Gibrat, J. F., & Renault, P, 2011; Kang, X., Ling, N., Sun, G., Zhou, Q., Zhang, L., & Sheng, Q, 2012).

2.5.1.2.2 *Lactobacillus delbrueckii* subsp. *bulgaricus*

The subspecies *Lactobacillus bulgaricus* belongs to the domain Bacteria, phylum *firmicutes*, class *Bacilli*, order *Lactobacillales*, family *Lactobacillaceae*, genues *Lactobacillus* and species *L. delbrueckii*. Phylogenetically, *L. delbrueckii* subsp. *bulgaricus* is closely related (<10% sequence divergence) to *L. amylovorus, L. acidophilus, L. helveticus, L. acetotolerans, L. gasseri*, and *L. amylophilus* (Schleifer and Ludwig, 1995). The GC (guanine – cytosine) ratio of *L. delbrueckii* subsp. *bulgaricus* (49-51%) is somewhat higher than that found among other species (34 to 46%) within this phylogenetic tree (Wittouck, S., Wuyts, S., Meehan, C. J., Van Noort, V., & Lebeer, S, 2019). The genome size of *L. delbrueckii* subsp. *bulgaricus* has been determined to be 2.3 Mbp by pulse-field gel electrophoresis (Hao, P., Zheng, H., Yu, Y., Ding, G., Gu, W., Chen, S., Yu, Z., Ren, S., Oda, M., Konno, T., & Wang, S, 2011). Very few chromosomal genes (<15) have been sequenced from *L.delbrueckii* subsp. *bulgaricus*, however the complete sequence of a small cryptic plasmid and the partial sequence of a bacteriophage are known. Gene transfer systems for *L. delbrueckii* subsp. *bulgaricus* have been described. These include two conjugation-based gene transfer systems (Rantsiou, K., Phister, T., McKay, L., Dunny, G. & Mills, D, 1999) and electroporation (Serror, P., Sasaki, T., Ehrlich, S. D., & Maguin, E, 2002).

*Lactobacillus bulgaricus*are Gram-positive, facultatively anaerobic, non-motile and nonspore-forming, rod-shaped (cell size range = 0.5-0.8 x 2.0-9.0 mm) members of the industrially important lactic acid bacteria. Like other lactic acid bacteria, *L. bulgaricus* are acid tolerant,

cannot synthesize porphyrins, and possess a strictly fermentative metabolism with lactic acid as the major metabolic end product (Zheng, Z., Liao, P., Luo, Y., & Li, Z, 2014)

L. bulgaricus are part of the obligately homofermentative ("Group I") cluster, which produce D-lactic acid from hexose sugars via the Embden-Meyerhof pathway and are incapable of fermenting pentoses (Zheng *et al*., 2014). The *L. delbrueckii* species contains three subspecies, *L. delbrueckii* subsp. *delbrueckii*, *L. delbrueckii* subsp. *lactis*, and *L. delbrueckii* subsp. *bulgaricus*. *L. delbrueckii* subsp. *bulgaricus* grows on a relatively restricted number of carbohydrates and typically requires pantothenic acid and niacin (Wittouch *et al*., 2019). This bacterium has an optimum growth temperature in milk between 40 and 45 ^{0}C and an optimum pH near 5.0 – 5.5 (Rizzello, C. G., De Angelis, M., & John, W. F, 2011).

L. delbrueckii subsp. bulgaricus is one of the economically most important species of LAB, with a worldwide application in yoghurt manufacturing. Its basic sugar metabolism is obligate homofermentative. When grown in milk, *L. delbrueckiisubsp. bulgaricus* transports lactose into the cell in association with the expulsion of galactose similar to that found in *S. thermophilus*. Lactose is hydrolyzed by β-galactosidase with only glucose being metabolized but, in this case, the end product is D (–) lactic acid (Rizzello *et al.,* 2011). This form of lactic acid is less readily metabolized by humans than the L (+) isomer (Robinson and Fuquay, 2002). Unlike *S. thermophilus, L. delbrueckii subsp*. *bulgaricus* is more proteolytic, and thus it can hydrolyse caseins, especially β_casein, by means of cell_ enveloped proteases (PrtB) to generate free amino acids and oligopeptides (Robinson and Fuquay, 2002). A number of commercial yoghurt starters contain *L. delbrueckii subsp. bulgaricus* strains that produce substantial amount of volatiles and EPS. The complete genome sequences and functional_genomic analyses of certain *L. delbrueckii* subsp. bulgaricus strains have been published (Sun, Z., Chen, X., Wang, J., Zhao, W., Shao, Y., Guo, Z., Zhang, X., Zhou, Z., Sun, T., Wang, L., Meng, H., Zhang, H., & Chen, W, 2011).

2.5.2.1.2.3 INTERACTION BETWEEN *S. thermophilus* and *L. delbrueckii subsp. bulgaricus*

Even though *S. thermophilus* and *L. delbrueckii subsp. bulgaricus* are able to grow independently in milk, these bacteria perform a symbiotic interaction known as "proto_ cooperation" in mixed culture (Courtin and Rul, 2004). The proto_cooperation is based on the exchange of several metabolites which provide growth stimulating effects to each other (Fig

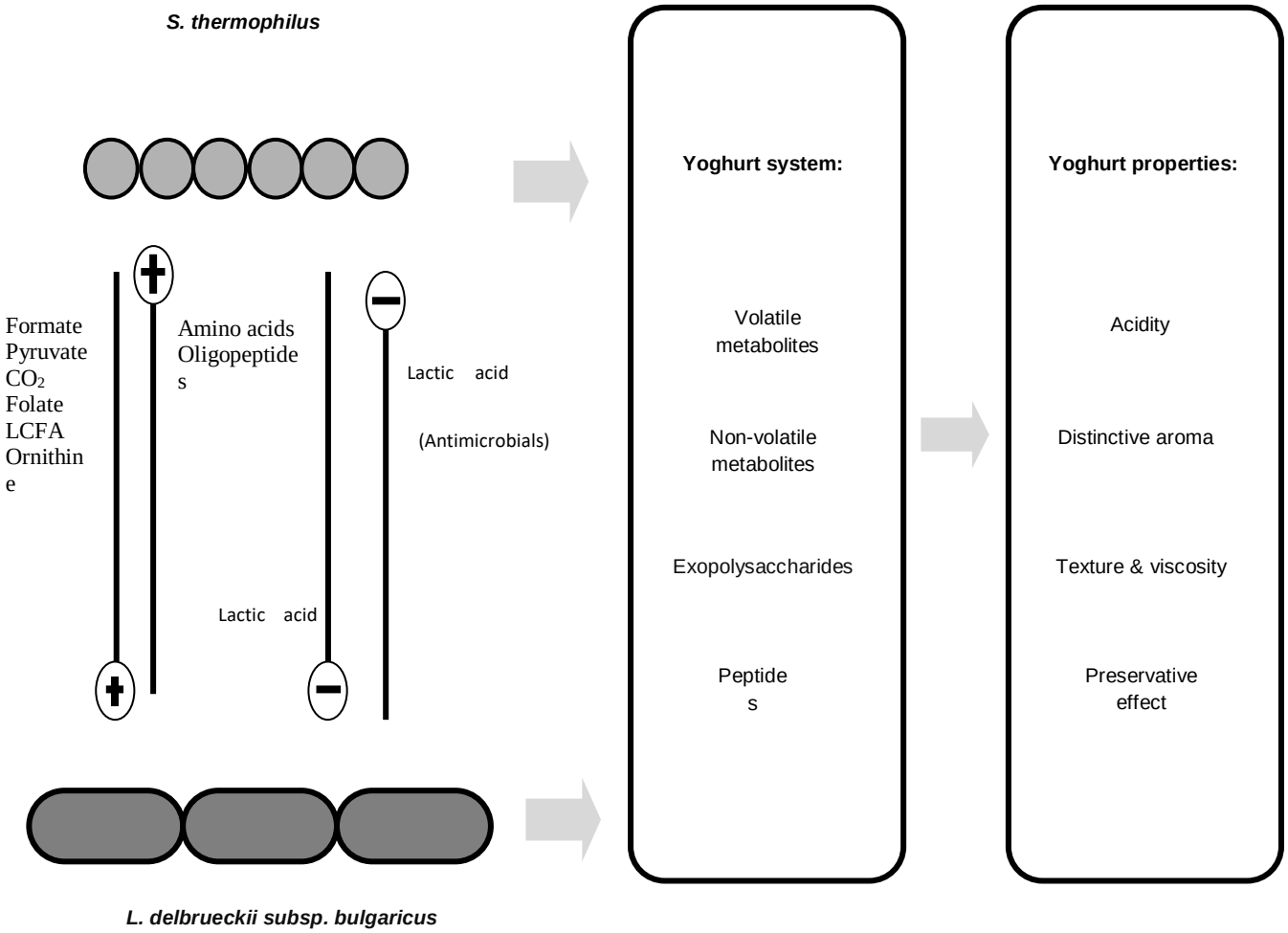

Fig. 2.2: Schematic representation of the proto - cooperation between *Streptococcus thermophilus* and *Lactobacillus delbrueckii subsp. bulgaricus* and their contribution to the characteristics of yoghurt. (Settachaimongkon, 2014).

2.5.2. YOGHURT MANUFACTURING PROCESS

The main steps involved in the yoghurt manufacture include; the standardization of milk (fat and protein content), homogenization, milk heat treatment, incubation/fermentation, cooling, and storage.

2.5.2.1 MILK STANDARDIZATION

Milk is often mixed with skim milk and cream to standardize (or adjust) the fat content to the desired level. Milk powders, including nonfat dry milk, whey protein concentrates, or milk protein concentrate, can be blended with the milk using a powder dispersion unit. Many commercial yoghurt products have milk solids contents of 14-15% (Tamime and Robinson, 2007). According to standards and regulations the minimum milk solids non-fat content for

many countries ranges from 8.2 to 8.6% (Tamime and Robinson, 2007). Over-stabilization results in a "jello-like" springy body of yoghurt while a weak "runny" body or whey separation can be produced due to under stabilization according to Vedamuthu, 1991(Lee and Lucey, 2010). In some countries, such as, the Netherlands and France, regulations do not allow the use of stabilizers for plain (unsweetened) yoghurt (Tamime and Robinson, 2007). In fruit yoghurts, stabilizers (e.g. pectin) are often added to the fruit preparation to help improve the yoghurt texture.

2.5.2.2 HOMOGENIZATION

Homogenization of the milk base is an important processing step for yoghurts containing fat. Milk is typically homogenized using pressures of 10-20 and 5 MPa for first and second stage pressures, respectively, and at a temperature range between 55 and 65°C. Homogenization results in milk fat globules being disrupted into smaller fat globules and the surface area of homogenized fat globules greatly increases. The use of homogenization prevents fat and whey separation (creaming) during fermentation or storage, increases whiteness, and enhances consistency of yoghurts (Lee and Lucey, 2010). When milk is homogenized, caseins and whey proteins form the new surface layer of fat globules, which increases the number of possible structure-building components in yoghurt made from homogenized milk (Walstra, 1998). Homogenized milk fat globules act like protein particles due to the presence of protein on the fat surface. Recently, ultra-high pressure homogenization at 200 or 300 MPa was investigated for the production of yoghurt. Compared with a conventional homogenization at 15 MPa, the use of ultrahigh pressure homogenization resulted in an increase in yoghurt firmness and water-holding capacity (Serra, M., Trujillo, A. J., Jaramillo, P. D., Guamis, B. & Ferragut, V, 2008, 2009). Ultra-high pressure causes whey protein denaturation as well as partial disruption of the casein micelles.

2.5.2.3. HEAT TREATMENT

Heating of milk is an important processing for the preparation of yoghurt since it greatly influences the physical properties and microstructure of yoghurt (Lucey, 2004). In yoghurt manufacture, milk is heated prior to culture addition. The temperature/time combinations for the batch heat treatments that are commonly used in the yoghurt industry include 85°C for 30 min or 90-95°C for 5 min (Tamime and Robinson, 2007). However, very high temperature short time (100°C to 130°C for 4 to 16 secs) or ultra-heat temperature (UHT) (140°C for 4 to 16 secs) are also sometimes used (Sodini *et al*., 2004). The heat treatment of milk is also used to destroy unwanted microorganisms, which provides less competition for the starter culture.

Yoghurt starter cultures are sensitive to oxygen so heat treatment helps to remove dissolved oxygen assisting starter growth (Tamime and Robinson, 2007).

2.5.2.4. FERMENTATION PROCESS

After heat treatment, the milk base is cooled to the incubation temperature used for growth of the starter culture. An optimum temperature of the thermophilic lactic acid bacteria, i.e., *Streptococcus subsp. thermophilus* and *Lactobacillus delbrueckii subsp. bulgaricus,* is around 40-45°C. Bacterial fermentation converts lactose into lactic acid, which reduces the pH of milk. During acidification of milk, the pH decreases from 6.7 to ≤4.6. Gelation occurs at pH

5.2 to 5.4 for milk that was given a high heat treatment (Tamime and Robinson, 2007).

2.5.2.5. COOLING

When yoghurts have reached the desired pH (e.g. 4.6), yoghurts are partially cooled (-20°C) before fruit or flavoring ingredients are added. Yoghurt products are often blast chilled to <10°C (e.g., 5°C) in the refrigerated cold store to reduce further acid development (Tamime and Robinson, 2007). In the production of set yoghurt, yoghurts are directly transferred to a cold store or blast chilled in cooling tunnels.

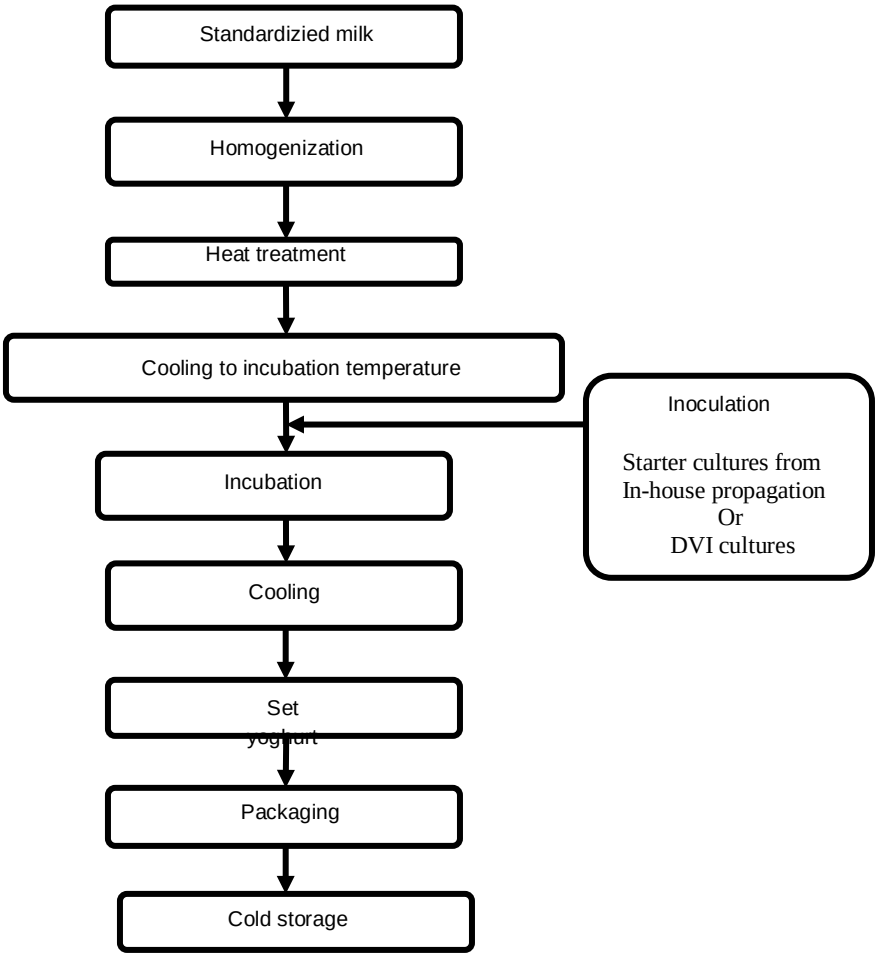

Fig. 2.3: Flowchart for the manufacturing of yoghurt. (Tamime and Robinson, 2007).

2.6 MODERN YOGHURT

Yoghurt has only recently gained popularity in various countries. This is due to many factors including the introduction of fruit and other flavorings into yoghurt, the convenience of it as a ready-made breakfast food or snack and the image of yoghurt as a low fat healthy food. Manufacturers have responded to the growth in the yoghurt market by introducing different types of yoghurt including low fat and non-fat, creamy, drinking, bio-yoghurt, organic, baby and frozen yoghurt. Unlike the traditional yoghurt which is sold in a plain and simple flavour, the modern yoghurt has wide assortment of flavours, these are typically fruit flavours such as strawberry or blueberry, cream pie, and chocolate. Cereal and nuts are also added in the yoghurt.

Yoghurt makers sell yoghurt with a varying level of fat, low fat yoghurt which contains between 0.5% and 4% fat is currently the best selling. Stabilizers are used in modern yoghurt to improve the body and texture by increasing firmness, preventing separation of whey (syneresis), and helping to keep the fruit uniformly mixed in the yoghurt. Most stabilizers used in modern yoghurt are alginates (arageenan), gelatins, guns (locust bean, sugar), pectins and starch. Modern yoghurt is made with different types of starter culture bacteria which include

lactobaccili and bifidobacteria. These bacteria serve as probiotics which aids digestion and other gastrointestinal diseases. And with research, no adverse effect has been recorded.

2.7. PROBIOTIC YOGHURT

The word "probiotic" derived from the Greek language, means "for life" (Lournes-Haltingh and Vilijoen, 2001) and has been defined in various ways. In 1991, Huis international Veld and Havenaar defined probiotics as being "a mono or mixed- cultured of life microorganisms which when applied to man or animal (e.g, as dried cells or fermented products) beneficially affects the host by improving the properties of the indigenous microflora (Lourens-Haltigh and Viligeon 2001). This definition implies that probiotics products for instance bioyoghurt contain microorganisms and improve the health status of the host by exerting beneficial effects on the gastrointestinal tract. Probiotics must be viable and available at a high concentration (minimum 10^7cfu/g) to convey health benefits (Champagne, C. P., Ross, R. P., Saarela, M., Hansen, K. T. & Charalampopoulos, D, 2011). World Health Organization (WHO) defines probiotics as live microorganisms that when administered in adequate amounts, confer a health benefits on the host (WHO, 2000).

Probiotics yoghurt may be produced using*L acidiophillus, B bifidum, B. longum, L reuteri, L plancarum, L. rhamnosus, B. animalis, B. infantis, B lactis or L. casei*, in any combination with or without the normal starter organism for traditional yoghurt. This probiotic yoghurt is also referred to as therapeutic yoghurt. Probiotic yoghurt production is more difficult without the presence of traditional starter organisms, this is because acid production by therapeutic starter is low and more care must be taken not to allow contamination according to Vaman in 1994 (Maleki, D., Azizi, A., Vaghef, E., Balkani, S. & Homayouni, A, 2015)

Many cultured dairy products in market fail to meet the standards because the strains of probiotic bacteria used in production cannot survive the acidity of the production (Kailasapatthy and Chin, 2000). Even if there are enough viable cells at production time, the acidity of the product can increase when it contains lactic acid producing bacteria, eventually inhibiting their own viability (Kailasapatthy and Chin, 2000). *L. acidophilus* and *Bifidobacterium sp*. are affected by the acidity of yoghurt and a decline in the production is inevitable as growth of *L. acidophilus* ceases at pH of 4.0, while that of *Bifidobacterum sp*. at pH 5.0, thus probiotics bacteria are unstable in yoghurt (Champagne *et al.*, 2011). Postacidification of yoghurt during the storage period is a large contributor to the death of probiotic cells in the product. There are other factors contributing to probiotic cell death

including hydrogen peroxide production by the yoghurt fermenting cultures which reduces the viability of probiotic bacteria (Champagne *et al.,* 2011)

2.7.1. THERAPEUTIC VALUE OF PROBIOTIC YOGHURT

The beneficial effects of consumption of fermented milk were once a debatable issue. Research conducted over the century have however, enhanced the understanding of the resulting therapeutic effects and it is currently widely recognized as wholesome (LourensHattingh and Vilijoen, 2001). A list of the main therapeutic benefits attributed to consumption of probiotics is indicated in the table below.

TABLE 2.4: Claimed therapeutic application of probiotic yoghurt in humans.

Therapeutics applications:
Prevention of urogenital infection
Alleviation of constipation
Protection against traveler's diarrhea
Prevention of antibiotic-induced diarrhea
Prevention of hypercholesterolaemia
Prevention against colon/bladder cancer
Prevention of osterporosis

Source: Lourens-Hattingh and Vilijoen, 2001

2.7.2. STRATEGIES FOR IMPROVING THE SURVIVAL OF PROBIOTICS IN YOGHURT

Certain approaches have been applied for improving the survival of probiotics during yoghurt production and storage. The most prevalent of which are the selection of appropriate strains on the basis of their acid and bile tolerances, supplementation of the milk with nutrients, addition of protective compounds, manipulation of starter cultures, selection of appropriate packaging materials, application of oxygen scavengers, performing two - stage fermentation and application of microencapsulation technique (Sarkar, 2010).

An alternative strategy to improve the survival of probiotics in yoghurt is to enhance their ability to cope with the harsh environments during production and storage. Stress adaptation is one of the strategies to improve the survival of probiotics by pre-treating (preculturing) them in a sublethal stress condition prior to exposure to a more harsh or lethal environment (Upadrasta, A., Stanton, C., Hill, C., Fitzgerald, G. & Ross, R. P, 2011). This approach allows probiotic cells to develop adaptive stress responses, i.e. a genotypic and/or phenotypic reaction to growth inhibition induced by environmental or physiological imbalances (De Dea Lindner, J., Canchaya, C., Zhang, Z., Neviani, E., Fitzgerald, G. F., Van Sinderen, D., & Ventura, M,

2007), leading to an increase in their survival compared to those that are directly shifted into the same lethal stress condition (Saarela, M., Rantala, M., Hallamaa, K., Nohynek, L., Virkajärvi, I., & Mättö, J, 2004). Adaptive responses on various types of stress, i.e. heat, cold, acid, bile, osmotic, oxygen, high pressure and nutrient starvation, in lactobacilli and bifidobacteria have been well investigated (Ruiz, L., Ruas_Madiedo, P., Gueimonde, M., De Los Reyes_Gavilán, C. G., Margolles, A., & Sánchez, B, 2011). These stress conditions are characterized due to the environmental challenges where probiotics are typically encountered,

i.e. during human gastrointestinal transit, industrial_scale production and in the food systems. Nevertheless, it should be mentioned that stress responses in LAB are expressed in a very specific process depending on the species, strains and particular types of stress (Van de Guchte, M., Serror, P., Chervaux, C., Smokvina, T., Ehrlich, S., & Maguin, E 2002).

Recent advances in post_genomics technologies, i.e. transcriptomics and proteomics, have extensively provided novel insights into how probiotics counteract with environmental stresses from a molecular perspective. Adaptive stress responses in probiotics are associated with the expression of a large number of genes, synthesis of stress_response proteins and alteration of various physiological features (Van de Guchte *et al*., 2002). As a consequence, stress adaptation not only enables to enhance the survival of probiotics but also induces substantial changes in their performance in a system. This information is important for the application of stress _ adapted probiotics in yoghurt since their metabolic activity may influence the biochemical and organoleptic characteristics of the product.

2.7.3. TECHNOLOGICAL ASPECTS OF PROBIOTICS IN YOGHURT

Nowadays, there has been a notable increase in the variety of probiotic dairy products including pasteurized milk, ice cream, frozen desserts, fermented milk, dairy based beverages, cheeses and infant milk powder (Tamime, 2005). Among the mentioned products, yoghurt is remarkably the most important food-carrier for the delivery of probiotics. Although the primary criteria for selection of probiotic strains are based on the clinical safety and functional properties (Vasiljevic and Shah, 2008), the following aspects must be considered from a technological standpoint: (i) the interaction between probiotics with traditional yoghurt starters, (ii) the ability of probiotics to grow in milk, (iii) the robustness of probiotics to withstand the manufacturing process and storage condition and (iv) the influence of probiotics on the sensory characteristics of yoghurt (Tamime, 2005).

There are several approaches by which probiotics can be added during the production of yoghurt: (i) probiotics are inoculated as an adjunct direct-vat inoculation (DVI) culture; (ii) probiotics are propagated in one batch of milk or suitable media in order to achieve a high biomass and then mixed together with yoghurt; and (iii) probiotics are used as a starter culture, e.g. *L. casei Shirota* (Yakult), or a part of the starter consortium, e.g. *L. acidophilus* and *B. bifidum* (ABT culture) (Tamime, 2005). However, many probiotic strains grow slowly in non-supplemented milk and the traditional fermentation temperature is often unsuitable for their growth, particularly the strains originating from the human GI tract (Walstra, P., Wouters, J. T. M., & Geurts, T. J. 2006). Thus, the suitable milk acidification with satisfactory sensory properties rarely occurs by pure culture of probiotics. Furthermore, the limited growth of probiotics in milk results in the risk of overgrowth of undesirable microorganisms which may cause undesirable flavor and texture in the fermented product (Mohammadi, R., Sohrabvandi, S., & Mohammad M. A. 2012).

In practice, it is common to used probiotics as an adjunct culture in combination with traditional yoghurt starters. The activity of yoghurt starters can create a favourable growth condition for probiotics. For example, *S. thermophilus* creates an anaerobic environment which subsequently stimulates the growth of *bifidobacteria* while *L. delbrueckii subsp. bulgaricus* sustains the amino acid requirement of probiotics by its proteolytic activity (Vasiljevic and Shah, 2008). On the other hand, organic acids and volatile compounds produced by the activity of probiotics may contribute to the organoleptic quality of yoghurt (Østlie, H. M., Treimo, J., & Narvhus, J. A. 2005). Thus, the interaction between yoghurt starters and probiotics is another important aspect that must be considered in order to select the most suitable combination regarding the functionality and sensory quality of product (Tamime, 2005).

The definition of probiotics underlines that these functional bacteria need to be viable, metabolically active and present in sufficiently high number at the time of consumption to ensure their beneficial health effects. It is recommended that a probiotic product should contain at least 10^6 cfu/g of viable probiotic cells throughout the entire shelf-life (Vasiljevic and Shah, 2008). However, numerous studies have demonstrated that many probiotic strains are not able to survive well in fermented milk (Sahan, N., Yasar, K., and Hayaloglu, A. A, 2000). The survival of probiotics can be adversely affected by certain metabolites including lactic acid, hydrogen peroxide, and bacteriocins produced by yoghurt starters (Mohammadi *et al.*, 2012). Besides, various factors are accountable for the viability loss of probiotics during yoghurt production and storage. These include sensitivity of the strains used, inoculation rate and level,

fermentation temperature, level of oxygen permeation through the package, presence of other competitive LAB, and application of food additives (Mohammadi *et al*., 2012).

2.8. BENEFITS OF YOGHURT

Interest in the role of probiotics for human health goes back at least as far as 1908 when Metchnikoff suggested that man should consume milk fermented with lactobacilli to prolong life (O'sullivan, M. G., Thornton, G., O'sullivan, G. C. & Acollins, J. K. 1992). It is only recently, however, that the interrelationship between intestinal microorganisms and the health benefits derived from it are beginning to be understood. It is generally recognized that an optimum 'balance' in microbial population in our digestive tract is associated with good nutrition and health (Rybkaa and Kailasapathy, 1995). The microorganisms primarily associated with this balance are *lactobacilli* and *bifidobacteria*. Factors that negatively influence the interaction between intestinal microorganisms, such as stress and diet, lead to detrimental effects in health. Increasing evidence indicates that consumption of 'probiotic' microorganisms can help maintain such a favourable microbial profile and results in several therapeutic benefits. In recent years' probiotic bacteria have increasingly been incorporated into foods as dietary adjuncts. Some studies using yogurt, individual LAB species, or both showed promising health benefits for certain gastrointestinal conditions, including lactose intolerance, constipation, diarrheal diseases, colon cancer, inflammatory bowel disease, Helicobacter pylori infection, and allergies. The benefits of yogurt consumption to gastrointestinal function are most likely due to effects mediated through the gut microflora, bowel transit, and enhancement of gastrointestinal innate and adaptive immune responses (Aldolfsson, O., Meydani, S. N. & Russell, R. M 2004). Probiotic yoghurt has shown to be beneficial in the maintainance of normal intestinal flora, enhancement of the immune system, reduction of lactose intolerance, reduction of serum cholesterol level and anti carcinogenic activities (Lourens-Hattingh and Vilijoen, 2001)

2.9. SHELF LIFE OF YOGHURT

Shelf-life is the period of time that a food may be stored before it starts to deteriorate or become potentially unsafe. It is influenced by the quality of the raw materials, the manufacturing processes, the way the product is packaged, storage conditions and whether it has been opened. Temperature control during all storage stages is particularly important for most foods – in the retail store and the home.

Storage time has an effect on the pH, titratable aciditity and microbial count of strained set yoghurt (Al-Kadamany, E., Toufeili, I., Khattar, M., Abou-Jawdeh, Y., Harakeh, S. and Haddad, T., 2002). There is a change in the sensory evaluation of yoghurt stored in the refrigerator after a period of weeks which showed a change in the taste, aroma and odour of the set yoghurt (Salvador and Fiszman, 2009). Yoghurt produced from whole milk or soya milk should be stored in the refrigerator for 14 days and freezer for 7 days or less at room temperature to avoid deleterious effect on the physicochemical, microbial and organoleptic properties of the product (Muhammad *et al*., 2009)

2.10 COMPOSITION OF COW MILK

The compositions of cow's milk vary widely between different breeds and during different stages of lactation. In the first few days after birth, a special type of milk called colostrum is excreted which is rich in fats and protein. Colostrum also contains important infection fighting antibodies which strengthens the immune system of young mammals. True milk occurs within a day's following birth (Parodi, 2004), the basic composition of cow milk is as follows:

Fat: 3.9% (2.4-5.5%)

Protein: 3.25% (2.3-4.4%)

Casein: 2.6% (1.7-3.5%)

Carbohydrates (lactose): 4.6% (3.8-5.3%)

Minerals: 0.65% (0.53-0.80%)

Organic acid: 0.18% (0.13-0.22%)

WATER: water dilutes the milk allowing its secretion from the body, without water, it would be impossible to express milk. Water content in cow milk is about 87.3% (85.5-88.7%)

CARBOHYDRATES: The major carbohydrate in mammalian milk is disaccharide (or sugar) called lactose. For lactose to be digested, it must be broken down in the intestine by the enzyme lactase to its components monosaccharides, glucose and galactose (Pruiff, 2003).

PROTEINS: Proteins provides energy and is required for the growth and repair of tissue

such as skin and muscle. Caseins are the primary group of proteins in cow's milk, making up about 80% of the total protein content (Farkye, 2003). The remaining protein is made up of whey proteins.

FATS: the principal fat in milk is a complex combination of lipids called triglycerols (esters of three fatty acids with one molecule of glycerol) (Paradi, 2004). There are more than 400 fatty acids in cow milk and these fatty acids are described as saturated and unsaturated; while unsaturated fatty acids are futher classified as monounsaturated or polyunsaturated. Most of fat in whole cow's milk is about 65% saturated fatty acid, 30% monounsaturated fatty acid and 5% polyunsaturated fatty acid.

MINERALS AND VITAMINS: Minerals found in cow's milk include sodium, potassium, calcium, magnesium, phosphorous and chloride, zinc, iron, selenium, iodine, and trace amount of copper and manganese (FSA, 2002). Vitamins include retinol, carotene, vitamin E, thiamine, riboflavin, niacin, vitamin B6, vitamin B12, folate, pentothenate, biotin, vitamin C and trace amounts of vitamin D (FSA, 2002).

FIBRE: Milk contains no dietary fibre.

2.11. COMPOSITION OF SOY MILK

2.11.1 SOYA BEAN

The soya bean, *Glycine max* (L.) *Merr*, is a member of the family *Fabaceae*, subfamily *Faboideae* (Mian, 2006). The soya bean plant has alternate, tri-foliate leaves except at the first two nodes. The leaves, stems and pods are usually covered with a gray or brown pubescence, which is very noticeable at maturity and is useful in variety identification. The seed is usually spherical in shape. The species originates from Asia, but is cultivated worldwide. Soya beans are classified as oilseeds, but also provide an important protein source for both human and animal consumption. Soybeans are a complete protein source, providing all eight amino acids essential for human health (Shekhar, H., Uddin, H., Zakir, H. & Kabir, Y, 2016).

2.11.2 CHEMICAL COMPOSITION OF SOYA BEAN

CARBOHYDRATES: The principal sugars of the soya bean are the disaccharide sucrose (5.0%), the trisaccharide raffinose (1.1%), and the tetrasaccharide stachyose (3.8%), which with 15% hemicellulose, 4% cellulose and 5.1% othercarbohydrates (such as glucose, arabinose, and verbascose) account for the total carbohydrate content of 34% of whole bean

(Obendorf and Kosina, 2011). Glucose or other reducing sugars are present in green or immature beans in substantial amounts, but they disappear as the beans approach maturity. Starch is seldom found in mature beans but frequently reported in immature beans (Dierking and Bilyeu, 2009). The most fiber, including hemicellulose and cellose, are present in soybean hull. Raffinose and stachyose are non-reducing sugars without food value unless they are hydrolyzed by strong acids into their components of galactose, glucose, and fructose (Dierking and Bilyeu, 2009). The two oligosaccharides are similar except one moregalactose residue is present in stachyose. Two enzymes, invertase and emulsin, can hydrolyze raffinose and stachyose. Invertase hydrolyzes the sucrose portion of the molecule to give melibiose and D-fructose. The enzyme emulsin, which contains an a-D-galactosidase, as well as, a pglucosidase, can hydrolyze the melibiose residue to yield galactose and sucrose. Bottom yeasts, which contain both enzymes, can completely hydrolyze raffinose (Dierking and Bilyeu, 2009). Therefore, these indigestible carbohydrates can be converted to digestible mono- or disaccharides with microbial action.

PROTEIN: Soya protein is essentially identical to the protein of other legume seeds and pulses. At least 60-70% of the total protein in the soyabean is stored in the protein bodies (Cheng, 1988). Minimum solubility of these proteins occur at pH 4.2, corresponding to the apparent isoelectrical point of the major proteins. Proteins present in soybean are trypsin inhibitor, hemagglutinin, glycinin, conglycinin and lipoxygenases (Monero, 2008). The trypsin inhibitor possesses a single polypeptide chain and is compact, low in asymmetry and rigid in structure. Hemagglutinin is a glycoprotein. The absence of cystine crosslinks in this glycoprotein suggests that the molecule may be fairly flexible and subject to conformational changes very easily (Monero, 2008).

FAT: The primary fatty acids present in soyabean oil are unsaturated fatty acids, such as oleic acid (22.8%), linoleic and linolenic acids (57.6%), and 14% of the palmitic and stearic acids, while the rest are 0.3% saturated fatty acids with a carbon number less than 14 (Wang *et al*., 2011). These fatty acids and glycerol consist of 16.5% lipid in mature dry soybean.

Soyabeans are a rich source of sphingolipids (Wang, Feng-Qing; Yao, Kang; Wei, Dong-Zhi., 2011).

2.11.3 SOYA MILK

Soya milk is plant-based milk produced from soybeans. It is produced by soaking and grinding soya beans, boiling the mixture, and filtering out remaining particulates. It is a stable mixture

of oil, protein and water. Soya milk is mostly used as a substitute for dairy milk by individuals who are lactose intolerant. Soya milk is an excellent source of high quality protein and vitamins B. Soya milk is not a rich source of calcium, this is why most soy milk product is fortified with calcium. Soya milk natuarally contains isoflavones, plant chemicals that help lower LDL (bad cholesterol) if taken as part of a heart healthy eating plan.

TABLE 2.5: Composition of soy milk (per 100g)

(per 100g)	Content
Water	93.3
Protein	2.8
Fat (total lipids)	2.0
Saturated fatty acid	0.214
Monounsaturated fatty acid	0.326
Polyunsaturated fatty acid	0.833
Carbohydrates	1.8
Fibre	1.3
Ash	0.27

Source: USDA. Nutrient Database for Standard Reference (2019).

2.12. *Saccharomyces boulardii*

Saccharomyces boulardii is a tropical species of yeast first isolated from lychee and mangosteen fruit in 1923 by French scientist Henri Boulard. Although early reports described distinct taxonomic, metabolic, and genetic properties (Malgoire, J. Y., Bertout, S., Renaud, F., Bastide, J. M. & Mallié, M, 2005) *S. boulardii* is a strain of S. cerevisiae, sharing >99% genomic relatedness (Khatri, I., Tomar, R., Ganesan, K., Prasad, G. S. & Subramanian, S. 2017). Metabolically and physiologically, *S. boulardii* differs considerably from *S. cerevisiae*, particularly, when it concerns growth yield and resistance to temperature and acidic stresses (Fietto, J. L. R., Araujo, R. S. & Valadao, F. N. 2004). Whereas most *S. cerevisiae* strains grow and metabolize at a temperature of 30°C, *S. boulardii* is a thermotolerant yeast that grows optimally at 37°C, i.e. the physiological temperature of the host. Studies have demonstrated that *S. boulardii* appears to be more resistant than the *S. cerevisiae* strain W303 when exposed to a simulated gastric environment (Fietto *et al.*, 2004). *S. boulardii* is sometimes used as a probiotic with the purpose of introducing beneficial active cultures into the large and small intestines, as well as conferring protection against pathogenic microorganisms in the host

(McFarland, 2010). Boulard first isolated this yeast after he observed natives of Southeast Asia chewing on the skin of lychee and mangosteen in an attempt to control the symptoms of cholera. In healthy patients, *S. boulardii* has been shown to be nonpathogenic and nonsystemic (it remains in the gastrointestinal tract rather than spreading elsewhere in the body).

S. boulardii is often marketed as a dietary supplement (McFarland, 2010). Several mechanisms of action have been identified directed against the host as well as pathogenic microorganisms and include regulation of intestinal microbial homeostasis, interference with the ability of pathogens to colonize and infect the mucosa, modulation of local and systemic immune responses, stabilization of the gastrointestinal barrier function and induction of enzymatic activity favoring absorption and nutrition (Im and Pothoulakis, 2010; Pothoulakis, 2009; Czerucka, D., Piche, T. & Rampal P. 2007).

2.12.1. MECHANISMS OF ACTION OF *SACCHAROMYCES BOULARDII*

A very active research in this field has provided interesting data on several mechanisms of action of *S. boulardii*. They may be classified into three main areas: anti-microbial action, trophic action, and immunoregulation.

ANTI-MICROBIAL ACTION

Within the intestinal luminal *S. boulardii* exerts several anti-microbial activities that could be divided in two groups:

Direct anti-toxin effects: The anti-toxin action elicited by *S. boulardii* is mainly due to small peptides produced by the yeast. A 54kDa serine protease is able to inhibit enterotoxin and cytotoxic activities of *C. difficile* by degradation of toxin A and B and receptors sites of toxin A on the enterocyte cell surface. Others *Saccharomyces* strains fail to show these activities (Castagliuolo, I., Riegler, M. F., Valenick, L., Lamont, J. T. & Pothoulakis, C, 1999). A 120 kDa protein that has a nonproteolytic activity, competes specifically with the hyper-secretion caused by the toxins of *Vibrio (V.) cholera* decreasing cyclic adenosine monophosphate in the enterocytes (Czerucka *et al.*, 2007). Finally, *S. boulardii* produces a phosphatase able to dephosphorylate endotoxins (such as lipopolysaccharide of *E. coli* 055B5) and inactivates its cytotoxic effects (Buts, J. P., Dekeyser, N., Stilmant, C., Delem, E., Smets, F. & Sokal, E., 2006). This mechanism may account for the protection afforded in cases of sepsis.

Inhibition of growth and invasion of pathogens: In vitro, *S. boulardii* directly inhibits the growth of several pathogens (*Candida albicans, E. coli, Shigella, Pseudomonas aeruginosa, Staphylococcus aureus, Entamoeba hystolitica*), and cell invasion by *Salmonella typhimurium* and *Yersinia enterocolitica* (Mumy, K. L., Chen, X., Kelly, C. P. & Mccormick, B. A. 2008). The yeast may also act by enhancing the integrity of the tight junction between enterocytes, thus preserving intestinal integrity and function (Wu, X., Vallance, B. A., Boyer, L., Bergstrom, K. S., Walker, J., Madsen, K., O'kusky, J. R.,

Buchan, A. M. & Jacobson, K. 2008). *S. boulardii* is also able to reduce the translocation of pathogens in rat and pig animal models (Karen, M., Yuksel, O., Akyürek, N., Ofluoğlu, E., Cağlar, K., Sahin, T. T., Paşaoğlu, H., Memiş,

L., Akyürek. N. & Bostanci, H.., 2010), and it can also interfere with pathogenic attachment to intestinal receptor sites (Tasteyre, A., Barc, M. C., Karjalainen, T., Bourlioux, P. & Collignon, A ,2002). In enteropathogenic E. coli (EPEC) infection, *S. boulardii* acts as a decoy by causing bacterial cells to bind to its surface rather than to enterocytes. In vitro, *S. boulardii* inhibits the adhesion of *C. albicans* to epithelial cell lines, this effect is also observed with the extracts of *S. boulardii* (Murzyn*et al*., 2010). Data on the effects of *S. boulardii* against common viruses responsible for diarrhea (such as Rotavirus, Adenovirus, Norovirus) are still very limited.

TROPHIC ACTION

When *S. boulardii* is given to antibiotic-shocked animal or patients with diarrhea, normal microbiota is re-established more rapidly. *S. boulardii* has no effect on microbiota composition in healthy humans (Barc, M. C., Charrin-Sarnel, C., Rochet, V., Bourlioux, F., Sandré, C., Boureau, H., Doré, J. & Collignon, A. 2008). This effect is tightly linked to a stimulation of short chain fatty acids (SCFA) production, especially butyrate (Schneider, S. M., Girard-Pipau, F., Filippi, J., Hebuterne, X., Moyse, D., Hinojosa, G. C.,

Pompei, A.& Rampal, P., 2005). The production of SCFAs is significantly decreased in patients receiving antibiotics. The effect on butyrate production is particularly relevant considering the important role of this compound for the regulation of many intestinal functions including, the stimulation of enterocytes growth and differentiation, fluid absorption, immune stimulation, anti-inflammatory effects, enteric neurons growth and differentiation (Schneider *et al*., 2005). These effects may be especially relevant in the management of antibiotic-associated diarrhea (AAD). *S. boulardii* exerts trophic effects and enhances enzymes expression on microvilli of the host, it improves disaccharidases activity, enhance the absorption of D-glucose coupled to

Na+ by the symport glucose/Na+ and expression of the sodium-glucose cotransporter-1 (SLGT-1) The yeast stimulates the activity of sucrase at levels high enough to be effective in the treatment of congenital sucraseisomaltase deficiency (Buts *et al*., 2002)

S. boulardii stimulates mucosal peptidase activity and endoluminal peptide hydrolysis in suckling rat small intestine, and it was showed that this yeast is also able to produce and secrete in the intestinal lumen a leucine aminopeptidase, belonging to the Zn2+- metalloprotease family, with proteolytic activity on endoluminal N-terminal of oligopeptides. This effect could be potentially important in preventing reactions to food antigens when mucosal permeability is increased (Buts *et al*., 2002). S. boulardii stimulates the production of glycoproteins in the brush border of microvilli such as hydrolases, transporters, secretory IgA, and the receptor for polymeric immunoglobulins (Ozkan, T. B., Sahin, E., Erdemir, G. & Budak F., 2007). The production of intestinal polyamines induced and stimulated by *S. boulardii* is one of its most relevant and specific mechanisms of action. The polyamines spermidine, spermine, and putrescine enhance the expression of brush border enzymes (such as hydrolases, proteases, and transport molecules) (Buts, J. P., Dekeyser, N., Stilmant, C., Delem, E., Smets, F. & Sokal, E. 2006). *S. boulardii* activates expression of peroxisome proliferator-activated receptor-γ (PPAR-γ) that protects from gut inflammation and inflammatory bowel diseases of the host.

IMMUNOREGULATION

S. boulardii inhibits mitogen-activated protein kinase (MAP kinase) and nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) signal transduction pathways and decreases the secretion of IL-8 and reducing inflammatory diarrhea (Pothoulakis, 2009). *S. boulardii* also secretes a small (<1kDa) heat-stable and water soluble anti-inflammatory factor termed "*S. boulardii* anti-inflammatory factor" that inhibits the NF-κB-dependent signaling pathway in the presence of *C. difficile* toxin (Sougioultzis, S., Simeonidis, S., Bhaskar, K. R., Chen, X., Anton, P. M., Keates, S., Pothoulakis, C. & Kelly, C. P. 2006). The yeast also decreases enterocyte apoptosis, probably as a result of the decrease of the synthesis of Tumor necrosis factor-α (TNF-α). It has been recently postulated that *S. boulardii* inhibits dendritic cell-induced activation of naïve T cells (Baumgart, 2007). It also modifies the migration of lymphocytes in a model of inflammatory bowel disease (IBD) (Dalmasso, G., Cottrez, F., Imbert, V., Lagadec, P., Peyron, J. F., Rampal, P. 2006). Recent research demonstrated that the supernatant of *S. boulardii* cultures modifies the capacity of lymphocytes to adhere to endothelial cells, leading to improved cell rolling and adhesion (Dalmasso *et al*., 2006). There is a very clear and marked stimulation of production of IgA and specific IgG anti-toxins A and

B by *C. difficile*, as demonstrated by the increased content in the intestinal lumen and in crypt cells. This may be explained by a trophic effect exerted on the mucosa or by direct immunostimulation (Ozkan *et al*., 2007). Another mechanism indirectly involved in the immune-regulation effect exerted by *S. boulardii* is the modulation of intestinal permeability. Increased intestinal permeability is frequently observed in different situations such as following shock, burn injury, obstructive jaundice, intestinal resection, hepatic transplant, or intestinal obstruction (IO) (Quirino, I. P., Correia, M. D. & Cardoso, V. N. 2007). A recent study has demonstrated that in a murine IO model the oral pretreatment with viable or heat killed cells of *S. boulardii*, preserves intestinal integrity and modulates inflammation, preventing bacterial translocation and intestinal lesions (Generoso, S. V., Viana, M. L., Santos, R. G., Arantes, R. M., Martins, F. S., Nicoli, J. R., Machado, J. A., Correia, M. I. & Cardoso, V. N. 2010). Finally, it has been demonstrated that *S. boulardii* modulates the nitrogen oxide pathway through the inhibition of the inducible nitric oxide synthase (iNOS), contributing to a general down regulation of intestinal inflammation and an anti-secretory stimuli on transepithelial ion transport (Girard, P., Pansart, Y., Lorette, I. & Gillardin, J. M. 2003).

2.12.2 PROPERTIES OF *SACCHAROMYCES BOULARDII* THAT CAN DETERMINE ITS EFFICACY.

Saccharomyces boulardii survives at body temperature (37°C): unique advantage of being one of the few yeasts that do best at human body temperatures (Graff, S., Chaumeil, J. C., Boy, P., Lai-Kuen, R. & Charrueau, C. 2008). In lyophilized form, *S. boulardii* survives gastric acid and bile. As is the case with all yeasts, *S. boulardii* is naturally resistant to antibiotics (Graff *et al.*, 2008). *S. boulardii* is resistant to proteolysis (But 2009). When given orally, achieves steady-state concentrations within three days and is cleared within 3–5 days after it is discontinued (Elmer, G. W., Martin, S. W., Horner, K. L., McFarland, L. V. & Levy, R. H , 1999). Some types of fiber (psyllium) increased *S. boulardii* levels by 22% (Elmer *et al.*, 1999). *Saccharomyces boulardii* survives passage to its target organ (most commonly the colon): although much of the oral dose is destroyed (usually stool levels are 100–1000 times lower than the oral dose), surviving oral doses have been found to be effective (usually at levels over 108 organisms/gram stool) (Gorbach, 2000).

2.12.3 FACTORS THAT DETERMINE EFFICACY OF *SACCHAROMYCES BOULARDII* AS A PROBIOTIC

The efficacy of *S. boulardii* as a probiotic involves many factors, including the intrinsic properties of the yeast, its pharmacokinetics, product to product variation, and stability, number of strains used in the probiotic preparation and dose of the probiotic used. There are many different *Saccharomyces* products commercially sold as probiotics and *S. boulardii* is usually available in capsules of either lyophilized or heat-dried preparations (McFarland, 2010). The choice of a high-quality probiotic product is one of the most important factors that determine efficacy of the probiotic. The quality of these products from different sources may vary and many of the commercially available products may lack regulated quality control programs (Martins, F. S., Nardi, R. M., Arantes, R. M., Rosa, C. A., Neves, M. J. & Nicoli, J. R 2005; Weese, 2003). Even if the label states it contains *S. boulardii*, a variation in efficacy may occur due to lower than stated dose or inaccurate strain composition (Martins, F. S., Veloso, L. C., Arantes, R. M. & Nicoli, J. R. 2009). Selecting high-quality probiotic products can be difficult without access to specific quality control assays for commercially available probiotic products. Products from companies that sponsor original clinical trials may be more reliable as the sponsorship could indicate a higher degree of commitment to high-quality products (McFarland, 2010).

The stability of the probiotic product may significantly affect its potency over time. Lyophilized products are stable at room temperature, have the advantage of portability and maintain high viability counts over prolonged periods (Graff *et al.*, 2008). Heat-dried preparations must be refrigerated and may not be stable at room temperature (McFarland, 2010). Although mixtures of probiotics containing *S. boulardii* are available in the market, no randomized controlled trials have been performed showing that these mixtures are superior to the single-strain preparations. Preclinical studies in animal models have found promising results in probiotic mixtures containing *S. boulardii* (Bisson J.F., Hidalgo S., Rozan P., Messaoudi M. 2010). However, possible antagonism between the different probiotics may attenuate the therapeutic responses of the probiotic strains (Kajander, K., Myllyluoma, E., Rajilic-Stojanovic, M., Kyronpalo, S., Rasmussen, M., & Jarvenpaa, S. 2008). The dose of *S. boulardii* used can affect its efficacy as a probiotic (McFarland, 2010).

2.12.3 FUNCTIONS OF *SACCHAROMYCES BOULARDII* AS A PROBIOTIC

ANTIBIOTIC-ASSOCIATED DIARRHEA (AAD)

AAD is defined as otherwise unexplained diarrhea that occurs in association with the administration of antibiotics (Bartlett, 2002). Measures to prevent AAD include the use of probiotics. Many meta-analyses have concluded that *S. boulardii* is effective in preventing AAD (McFarland, 2009), most probiotic meta-analyses have focused on one type of disease indication (e.g. antibiotic associated diarrhea) with a variety of *S. boulardii* strains.

CLOSTRIDIUM DIFFICILE INFECTION (CDI)

Probiotics represent a promising approach as an adjunctive therapy for CDI. Several pieces of evidence suggest that *S. boulardii* represents the most effective probiotic that can prevent or, together with other agents, treat antibiotic-associated diarrhea and recurrent CDI (McFarland, 2006) through many mechanisms. Animal models of CDI respond to this yeast and case reports or small case series of patients with recurrent CDI treated with *S. boulardii* showed improvement (McFarland, 2010). There are only very limited data from one small observational trial in children suggesting that *S. boulardii* may be effective in CDI (Buts *et al*., 1993). However, according to guidelines no compelling evidence exists to support routine use of probiotics for prevention or treatment of CDI (Cohen *et al*., 2010) especially since some of these studies did not control the dose or duration of either vancomycin or metronidazole for treatment of CDI and since scarce data exist on the use of *S. boulardii* for recurrent CDI in humans.

ACUTE DIARRHEA

Using *S. boulardii* showed that this probiotic may be effective in treating acute adult diarrhea due to a variety of causes and can significantly lower diarrhea severity (Mansour-Ghanaei *et al*., 2003). A meta-analysis indicated that *S. boulardii* significantly reduces the duration of acute childhood diarrhea and the risk of prolonged diarrhea (Billoo *et al*., 2006). The absence of blinding as well as other factors such as ambulatory care may explain why *S. boulardii* had no effect in a European RCT (Canani *et al*., 2007). The findings from RCTs and guidelines from professional pediatric societies indicate that *S. boulardii* may be an effective adjunct therapy in managing acute gastroenteritis in children (Guarino *et al*., 2008).

PERSISTENT DIARRHEA

Results from clinical trials indicate that *S. boulardii* improves outcomes in children with persistent diarrhea (Gaon *et al*., 2003). The relative significant reduction in persistent diarrhea with *S. boulardii* was approximately 50% (Castaneda *et al*., 1995). These results indicate that *S. boulardii* is useful in the management of persistent diarrhea in children.

ENTERAL NUTRITION-RELATED DIARRHEA

Diarrhea is a significant problem in patients on total enteral nutrition (TEN) and may involve changes in intestinal short chain fatty acids (SCFAs) (Schneider *et al*., 2005). *S. boulardii*induced increase of fecal SCFA concentrations may explain the preventive effects of this yeast on TEN-induced diarrhea (Schneider *et al.,* 2005).

TRAVELER'S DIARRHEA

Taking *Saccharomyces boulardii* by mouth appears to prevent traveler's diarrhea.*Saccharomyces boulardii* has a high reduction effect to traveler's diarrhea (McFarland, 2006). Various researches has indicated that *Saccharomyces boulardii* may be more effective in preventing traveler's diarrhea, rather than treating diarrhea once it becomes symptomatic.

HELICOBACTER PYLORI INFECTION

Saccharomyces boulardii supplementation improved eradication rates and reduced treatmentrelated side effects and symptoms in individuals infected with *H. pylori* (Tong, J. L., Ran, Z. H., Shen, J., Zhang, C. X. & Xiao, S. D. 2007). *S. boulardii* induces morphologic changes in *H. pylori* cells consistent with cellular damage (Vandenplas, Y., Brunser, O. & Szajewska, H 2009). *S. boulardii* may not be effective in eradicating H. pylori itself, but it is effective in reducing the side effects of the standard triple therapy.

CROHN'S DISEASE

Taking *Saccharomyces boulardii* seems to reduce the number of bowel movements in people with Crohn's disease. Early research also shows that taking *Saccharomyces boulardii* along with mesalamine can help people with Crohn's disease stay in remission longer. But taking *Saccharomyces boulardii* alone does not seem to help people with Crohn's disease stay in remission longer (Garcia *et al*., 2008). Remission is a period of time during which symptoms of disease are controlled. *S. boulardii* reduce colonic permeability thereby reducing the risk of bacterial translocation in patients (Garcia *et al*., 2008).

ULCERATIVE COLITIS

Probiotics have been used as an adjunct treatment in an attempt to induce remission in patients with active ulcerative colitis flares (Cain and Karpa, 2011). Research shows that adding *Saccharomyces boulardii* to standard mesalamine therapy can reduce symptoms in people with mild-to-moderate ulcerative colitis. (Guslandi, 2010). *S. boulardii* and rifaximin has been effective in preventing early flare ups of ulcerative colitis (Guslandi, 2010). Overall, based upon current consensus, the level of evidence for use of probiotics either to maintain remission or induce remission of ulcerative colitis symptoms is presently limited to a 'C' rating (Floch *et al*., 2008).

IRRITABLE BOWEL SYNDROME

Recent evidence suggests a role of the microflora in IBS pathogenesis (Parkes, G. C., Brostoff, J., Whelan, K. & Sanderson, J. D, 2008). Research shows that taking *Saccharomyces boulardii* improves quality of life in people with diarrhea-predominant or mixed-type IBS. But Saccharomyces boulardii doesn't seem to improve most IBS symptoms such as stomach pain, urgency, or bloating (Parkes *et al*., 2008). Addition of *S. boulardii* to mebeverine provides superior results in IBS treatment and exerts beneficial effects on the quality of life and IBS symptoms (Guslandi, 2011).

PARASITIC INFECTIONS

Little is known about the efficacy of *S. boulardii* against protozoal infections but this probiotic seems to have a beneficial effect in amebiasis, giardiasis and infection with Blastocystis hominis (Mansour-Ghanaei *et al*., 2003).

HIV-RELATED DIARRHEA

Patients with HIV-associated diarrhea seem to be one group that requires a higher than typical dose of *S. boulardii*. *S. boulardii* has been shown to significantly increase the recovery rate of stage IV AIDS patients suffering from diarrhea versus placebo. On average, patients receiving *S. boulardii* gained weight, while the placebo group lost weight over the 18-month trial (Saint-Mac, 1995). No adverse reactions were observed in these immunocompromised patien

CHAPTER THREE

MATERIALS AND METHODS

3.1 COLLECTION OF SAMPLES

Diary milk (Peak milk powder, WAMCO Nigeria) was purchased from Eke-Awka market in Awka, Anambra State, Nigeria.

Soya Beans was purchased from Eke-Awka market in Awka, Anambra State. Nigeria.

Yoghurt Starter containing *Lactobacillus bulgaricus* and *Streptococcus thermophilus* (1:1) (Yogourmet, Lyo-San Inc., Canada) was purchased from Shoprite Supermarket Lekki, Lagos State.Nigeria

Flora Norm cotaining *Saccharomyces boulardii* (Prisma Pharmaceutical Ltd.) was obtained from NAFDAC Agulu, Anambra State.Nigeria.

3.2 STERILIZATION OF MATERIALS

The work bench was first disinfected by swabbing with cotton wool soaked in 70% Ethanol. The glass wares used were washed with detergent, rinsed properly in several changes of tap water and further rinsed with distilled water. The glass wares used included test tubes, pipettes, conical flasks, beakers and McCartney bottles. They were air dried and wrapped with aluminum foil and sterilized in the hot air oven at 170^{o}C for 3 hours. The agar media were dispersed in conical flasks, plugged with cotton wool and wrapped with aluminum foil and thereafter sterilized in the autoclave at 121^{o}C for 15minutes. Aseptic technique was applied in the working environment by ensuring that all work was done near the naked flame of Bunsen burner.

3.3 CULTURE MEDIA

3.3.1. Potato Dextrose Broth and Agar (PDB and PDA):

Cultivation of yeast and mold was carried out using Potatoes Dextrose Media (Murray, P. R., Baron, E. J., Jorgensen, J. H., Landry, M. L. & Pfaller, M.A, 2007).

PRINCIPLES

Potato starch, potato infusion and dextrose support luxuriant growth of fungi. Lowering the pH of the medium to approximately 3.5 with sterile tartaric acid achieves the inhibition of bacterial growth. It is important, however, to avoid heating the medium after it has been acidified because this action results in the hydrolysis of the agar and impairs its ability to solidify.

PROCEDURE

For Broth: Twenty-four grammes of the commercially available Potatoes Dextrose Broth were dissolved in 1,000ml of distilled water. This was heated with frequent agitation and boiled for 1 minute to completely dissolve. The mixture was autoclaved at 121^0C for 15mins.

For Agar: thirty-nine grammes of the commercially available Potatoes Dextrose Agar were dissolved in 1,000ml of distilled water. This was heated with frequent agitation and boiled for 1 minute to completely dissolve. The mixture was autoclaved at 121^0C for 15mins.

3.3.2. De Man Rogosa Sharpe (MRS) Broth and Agar

MRS media is a media for the cultivation of *Lactobacillus species* that permits low selectivity (Downes and Ito, 2001).

PRINCIPLE

Lactobacilli MRS media supports luxuriant growth of all *Lactobacilli* from oral cavity, dairy products, foods, faeces and other sources. Proteose peptone and beef extract supply nitrogenous and carbonaceous compounds. Yeast extract provides vitamin B complex and dextrose is the fermentable carbohydrate and energy source. Polysorbate 80 supplies fatty acids required for the metabolismof *Lactobacilli*. Sodium acetate and ammonium citrate inhibit *Streptococci*, moulds and many other microorganisms.

PROCEDURE

For Broth: A 51.15g of the commercially available Man Rogosa Sharpe (MRS) was dissolved in 1,000ml of distilled water. This was heated with frequent agitation and boiled for 1 minute to completely dissolve. The mixture was autoclaved at 121^0C for 15mins.

For Agar: A 67.15g of the commercially available formulation was dissolved in 1,000ml of distilled water. This was heated with frequent agitation and boiled for 1 minute to completely dissolve. The mixture was autoclaved at 121^0C for 15mins.

3.3.3. M 17 Broths and Agar

M17 media is used for isolating and enumerating lactic *streptococci* in yogurt, cheese starters and other dairy products (Downes and Ito, 2001)

PRINCIPLE

Lactic *Streptococci* are nutritionally fastidious and require complex media for optimal growth. Disodium-β-glycerophosphate maintains the pH above 5.7. The maintenance of pH is very important as the lower pH results in injury and reduced recovery of lactic *Streptococci*. Glycerophosphate does not form precipitate with calcium which is needed for theplaque assay of lactic bacteriophages. Peptic digest of animal tissue, casein enzymix hydrolysate, papaic digest of soyabean meal, yeast extract, beef extract, providecarbonaceous, nitrogenous compounds, vitamin B complex and other essential growth factors. Lactose is the fermentable carbohydrate and ascorbic acid is stimulatory for the growth of lactic *Streptococci*.

Magnesium sulphate provides essentialions to the organisms.

PROCEDURE

For Broth: A 42.25g of the commercially formulated M17 was dissolved in 1,000ml of distilled water. This was heated with frequent agitation and boiled for 1 minute to completely dissolve. The mixture was autoclaved at 121^0C for 15mins.

For Agar: A 33.25g of the commercially formulated M17 and 19g of Disodium-β-glycerophosphate were dissolved in 1,000ml of distilled water. This was heated with frequent agitation and boiled for 1 minute to completely dissolve. The mixture was autoclaved at 121^0C for 15mins.

3.3.4. CHROMOGENIC COLIFORM AGAR (CCA)

Coliform Broth is recommended for isolation and cultivation of coliform organisms from cream, yogurt and raw milk (Jorgensen *et al*., 2015)

PRINCIPLE

Chromogenic Coliform Agar is a selective medium recommended for the simultaneous detection of *Escherichia coli* andtotal coliforms in water samples. The medium contains three chromogenic substrates. The enzyme ß-D-galactosidase produced by coliforms cleaves 6chloro-3-indoxyl-β-D-galactopyranoside to form pink to red coloured colonies. The enzyme ß-D-glucuronidase produced by *E.coli*, cleaves 5-bromo-4chloro-3-indoxyl-β-D-glucuronic acid. Colonies of *E.coli* give dark blue to violet coloured colonies due to cleavage of both chromogens. Addition of L-Tryptophan improves the indole reaction thereby increasing the detection reliability. Tryptone, sodium pyruvate and sorbitol provide nitrogenous substances, fermentable carbohydrate and other essential growth nutrients for the organisms. Phosphates buffer the medium. The media formulation helps even sublethally injured coliforms to recover and grow rapidly. Tergitol-7 inhibits gram-positive as well as some gram-negative bacteria other than coliforms.

PROCEDURE

For Broth: A 57.14g of the commercially available Chromogenic Coliform Agar was dissolved in 1,000ml of distilled water. This was heated with frequent agitation and boil for 1 minute to completely dissolve. The mixture was autoclaved at 121^0C for 15mins.

For Agar: A 30.92g of the commercially available Chromogenic Coliform Agar was dissolved in 1,000ml of distilled water. This was heated with frequent agitation and boil for 1 minute to completely dissolve the powder. Autoclaving or Overheating is not required. It is cooled to 45°C.

3.4. ACTIVATION OF ORGANISMS

3.4.1. *Lactobacillus bulgaricus*

The 5g satchet of yoghurt starter (containg *L. bulgaricus* and *S. thermophilus*) was swabbed with 70% Ethanol and cut using sterile scissors. A 2.5g of the yoghurt starter was collected and used to inoculate the MRS broth (Merck KGaA). The culture was maintained at 37^0C for 16hrs in an aseptic environment to obtain cells at the stationary phase. The cells were then harvested by centrifugation (Selecta Medifridger centrifuge, Spain) and the pellet was washed once in sterile distilled water and re-suspended in 100 mL distilled water. The bacterial cells were then

standardized with a spectrophotometer (Spectronic 20, Milton Roy Company, PACISA, Madrid, Spain) using a wavelength of 600 nm.

3.4.2. *Streptococcus thermophilus*

The 5g satchet of yoghurt starter (containg *L. bulgaricus* and *S. thermophilus*) was swabbed with 70% Ethanol and cut using sterile scissors. A 2.5g of the yoghurt starter was collected and used to inoculate the M17 broth (Merck KGaA, Darmstadt, Germany). The culture was maintained at 40^0C for 16hrs in an aseptic environment to obtain cells at the stationary phase. The cells were then harvested by centrifugation (Selecta Medifridger centrifuge, Spain) and the pellet was washed once in sterile distilled water and re-suspended in 100 mL distilled water. The bacterial cells were then standardized using spectrophotometer (Spectronic 20, Milton Roy Company, PACISA, Madrid, Spain) using a wavelength of 600 nm.

3.4.3. *Saccharomyces boulardii*

The 500mg sachet of flora norm (containing *S. boulardii*) was swabbed with 70% Ethanol and cut using sterile scissors. The floranorm was used to inoculate the PDA broth. The culture was maintained at 25^0C for 24hrs in an aseptic environment environment to obtain cells at the stationary phase. The cells were then harvested by centrifugation (Selecta Medifridger centrifuge, Spain) and the pellet was washed once in sterile distilled water and re-suspended in 100 mL distilled water. The bacterial cells were then standardized using spectrophotometer (Spectronic 20, Milton Roy Company, PACISA, Madrid, Spain) using a wavelength of 600 nm.

3.5. CHARACTERIZATION AND IDENTIFICATION OF ORGANISMS

3.5.1. COLONIAL MORPHOLOGY

The bacterial colonies were first described and characterized by their morphological appearances (i.e. colony shape, edge or margin, pigmentation, elevation, colony surface, consistency and optical characteristics) on the plate. The isolates were subsequently identified using the Bergey's manual of Determinative Bacteriology (Buchanan and Gibbons, 1974).

3.5.2 CELLULAR MORPHOLOGY

3.5.2.1 Gram Staining

A drop of sterile distilled water was placed in the middle of a clean, grease free glass slide. A sterile wire loop was then used to pick a bacterial colony and a smear of the bacterial isolate was made on the slide. The smear was allowed to air dry and then heat fixed by passing the

reverse side of the slide quickly through the flame three times. The slides were then flooded with crystal violet (primary stain) for 60 seconds, the stain was drained off and washed gently with distilled water after which it was flooded with Lugol's iodine (mordant) for 60 seconds and again drained off and washed gently with distilled water. It was then flooded with 95 % alcohol for 15 seconds after which it was then rinsed with distilled water and finally counter stained with safranin for 30 seconds. The slide was then gently washed with distilled water and air dried. The slide was observed under oil immersion lens (× 100). Gram positive cells stained purple while Gram negative ones stained pink or red (Fawole and Oso, 2007).

3.5.2.2 Motility test

The hanging drop method as described by Fawole and Oso (2007) was used. Loop full of sterile distilled water was placed on a cover slip. A small portion of each bacterial isolate from 24 hours old culture was transferred to the drop of water on the cover slip using a sterile inoculating loop and a smooth suspension made by thoroughly mixing it. Vaseline was applied around the edges of the cover slip and it was carefully covered with a clean cavity glass slide. Cover-slip was pressed down to make an air tight seal. The cover slip was subsequently observed inverted upon the cavity slide under the ×40 objective lens. Motile bacterial cells were seen moving rapidly in the field.

3.5.3 BIOCHEMICAL TESTS

All the bacteria and yeast isolates were subjected to a number of biochemical tests which were as follows.

3.5.3.1 Catalase test

Most aerobic microorganisms produce catalase enzyme that is capable of reacting with hydrogen peroxide (H_2O_2) to release oxygen. A few drops (i.e. 2 to 3 drops) of 3% hydrogen peroxide were placed in the middle of each clean slide and sterilized inoculating loop was used to pick bacterial isolate from pure culture. This was used to make a smear on the slide containing hydrogen peroxide. The occurrence of effervescence caused by liberation of oxygen indicated production of catalase by the bacteria (Fawole and Oso, 2007).

3.5.3.2 Citrate Utilization

The Simmon's citrate agar slant in test tubes was used to carry out this test. Colony was picked with a sterilized inoculating loop by flaming and was used to streak the slants in the test tubes.

The test tubes were incubated at 37ºC for 72 hours and the colour change from green to blue was observed which indicated a positive result. No change indicated a negative result (Fawole and Oso, 2007).

3.5.3.3 Coagulase test

Smears of various bacterial isolates were made on different slides and a drop of plasma was added. The slide was rocked for about 10 seconds and observed immediately for indications of agglutination or clumping. The clumping of organism in about 10seconds indicated a positive result (Fawole and Oso, 2007).

3.5.3.4 Triple Sugar Ion (TSI)

TSI agar was prepared as instructed by the manufacturer. Exactly 5ml of TSI powder was dispensed into each test tube and plugged with cotton wool, wrapped with aluminium foil and autoclaved at 121ºC for 15minutes. The test tubes were placed in a slanting position and allowed to solidify. Isolates were inoculated aseptically by stabbing the butt and streaking the slant. This process was repeated for all the slants with different colonies of bacteria. The tubes were incubated at 37ºC for 24 hours. Yellow colouration of the agar indicated acid producing organisms while red colouration indicated alkali producing organisms (Fawole and Oso, 2007).

3.5.3.5 Oxidase test

This test indicates the presence of cytochrome c oxidase that is able to reduce oxygen (O_2) and artificial electron acceptors (Prescott,*et al*., 2008). A drop of 1% tetra methyl-pphenylenediamine hydrogen chloride was dropped on a filter paper. Fresh culture of the isolate was then rubbed on the filter paper and observed. A positive result was indicated by a purple colour change within 10 seconds (Fawole and Oso, 2007).

3.5.3.6 Indole Production

The test demonstrates the ability of some bacteria especially Gram negative to decompose tryptophan (an amino acid) to indole which is present in tryptone soya broth. Sterilized Tryptone soya broth (TSB) was inoculated with bacterial isolates in test tubes and incubated at 37ºC for 72 hours. After the incubation period, 2ml of chloroform was added to the broth cultures in each test tube and was shaken gently; thereafter 2ml of kovac's reagent was added to each test tube and also shaken gently. The test tubes were returned to the test tube rack and

were allowed to stand for about 20 minutes. A red colouration on the surface layer indicated indole production by the bacterial isolate which is a positive result (Fawole and Oso, 2007).

3.5.3.7 Methyl Red-Voges Proskauer (MR-VP) Test

Test tubes containing sterilized MR-VP broth were inoculated with the isolate in duplicate and incubated at 37^{o}C for 72hours. After incubation, the MR-test and VP-test were performed on Methyl Red. To one of the test tubes, 5 drops of methyl red indicator was added and a change in colour was watched out for. A bright red colour on the surface indicated a positive result while a yellow or orange colour indicated a negative result (Fawole and Oso, 2007).

3.5.3.8 Voges Proskauer

To the second test tube, 1ml of 5% α-naphthol solution was added followed by 1ml of potassium hydroxide (KOH) solution. The mixture was shaken and allowed to stand for some minutes and observed. A red colour within 5 minutes was indicative of a positive reaction (Fawole and Oso, 2007).

3.5.3.9 Starch Hydrolysis

Soluble starch was added to already prepared nutrient agar in the ratio 2g soluble starch to 1 liter of nutrient agar and it was sterilized in the autoclave. The medium was thereafter poured into Petri dishes, inoculated and then incubated at 37^{o}C for 48hours. After incubation, the plates were flooded with Gram's iodine solution and observed. A clear zone around a distinct colony indicated hydrolysis of starch (a positive result) while a blue-black colouration gave a negative result (Fawole and Oso, 2007).

3.5.3.10 Oxygen Relationship

Using a sterile inoculating loop, isolates were stabbed to a depth of about 6cm in sterile nutrient agar slants prepared in test tubes. The test tubes were then incubated at 37^{o}C for 24hours and observed. Aerobes grew at the surface while facultative anaerobes grew throughout the length of the slab (Fawole and Oso, 2007).

3.5.3.11 Urease production

This is carried out to check the ability of organism to hydrolyze urea (an organic nitrogen source) to ammonia and carbon dioxide by the production of an enzyme called urease. This distinguishes between certain members of the Enterobacteriaceae. Christein's agar was

inoculated with the bacterial isolates and incubated at 37°C for 7days. It was observed daily for colour change. A change in colour from light orange to pink indicated a positive result (Fawole and Oso, 2007).

3.5.3.12 Nitrate reduction

Nutrient broth was prepared according the manufacturer's instruction by dispensing 1.3g of the powder into 100ml distilled water in an Erlenmeyer flask. The medium was heated over a Bunsen burner flame to homogenize it, and then 1ml of Sodium Nitrate (NaNO3) solution was added to the broth after which it was distributed into McCartney bottles and autoclaved at 121°C for 15 minutes. The broths in the bottles were subsequently inoculated with the test organisms and incubated at 37°C for 72 hours. After 3-day incubation, few drops of 1% αNaphtol was added to the culture and observed. A brownish ring on the top layer of the culture medium indicated a positive result (Fawole and Oso, 2007).

3.5.3.13 Sugar Fermentation

The sugars used were lactose, glucose, sucrose, maltose, mannitol and fructose. Phenol red was used as an indicator; 10 ml of sterilized nutrient broth was dispensed into sterile test tubes and inoculated with the test organism. Five grammes (5g) of each sugar was weighed and poured into the test tubes, the test tubes were shaken gently to dissolve the sugars, and then 5 drops of phenol indicator was introduced into the test tube and shaken to mix. Sterile Durham tubes were inserted in an inverted position into each of the test tubes, the mouth of the test tubes were then plugged with cotton wool and incubated at 37°C for 7 days. The test tubes were examined after 24 hours for colour change, acid and gas production until the 7th day. On the 7th day, test tubes were then observed for acid and/or gas production in the Durham tubes (Fawole and Oso, 2007).

3.6. PREPARATION OF SOYA BEAN POWDER

Five hundred grammes of soya bean was soaked overnight in 3ltrs of water. The soybean was blanched in a cooking pot for 15 min. The bean was dehulled and oven dried at 80^0C for 21h. The dried bean was roasted for 10mins under medium heat and milled into powder using an industrial processor. The Soya bean powder was stored in a dry airtight jar and kept in the refrigerator.

3.7 EXPERIMENTAL DESIGN

Four (4) portions of each the Dairy milk (D) and Non-Dairy (soya) milk (ND) were measured out and labeled: $D_{10/0}$, $D_{9/1}$, $D_{8/2}$ and $D_{7/}$[1]; and $ND_{10/0}$, $ND_{9/1}$, $ND_{8/2}$ and $ND_{7/3}$ respectively for Dairy milk and Non-Dairy (soya) milk. Each of the labeled portions was treated as milk sample throughout the experiment. The subscript represents the different doses of the conventional yoghurt starter culture (1:1 ratio of *L. bulgaricus* and *S. thermophilus*) and *S. boulardii* adjunct culture used correspondingly as shown in the Table below.

Table 3.1 Percentage (%) inclusion of culture and adjunct culture in the milk samples

	Percentage (%) inclusion	
Milk sample	*L. bulgaricus & S. thermophiles (1.1)*	*S. boulardii adjunct culture*
$D_{10/0}$	10:0	0:0
$D_{9/1}$	9:0	0:1
$D_{8/2}$	8:0	0:2
$D_{7/3}$	7:0	0:3
$ND_{10/0}$	10:0	0:0
$ND_{9/1}$	9:0	0:1
$ND_{8/2}$	8:0	0:2
$ND_{7/3}$	7:0	0:3

3. 8 PREPARATION OF YOGHURT FROM THE MILK SAMPLES

Yoghurt was prepared from the two samples; dairy milk and soya bean powder as follows; Eight (8) sterilized 250ml capacity flask (representing the eight milk samples) were

appropriately labeled and the contents added as shown in Table 3.2 below. Flasks 1-4 were homogenized and pasteurized at 90^0C for 10 minutes and rapidly cool to 40^0C by suspending the flask in an ice bath for each treatment. Soya bean was homogenized for 10 minutes and sieved to remove lumps and debris. The milk was pasteurized at 110^0C for 10 minutes and rapidly cooled to 40^0C by suspending the flask in an ice bath for each treatment. The milk samples were inoculated with the yoghurt starter culture (1:1 of *L.bulgaricus* and *S. thermophilus*) and adjunct *S. boulardii* as shown in Table 3.3 below. After inoculation, the flasks were placed in an incubator of 40^0C for 8hrs after which the desired custard consistency was reached (Falade, K. O., Ogundele, O. M., Ogunshe, A. O., Fayemi, O.E. & Ocloo, F. C, 2015). After 8hrs, the flask was brought out of the incubator without shaking or stirring and placed in the refrigerator for 21 days at 4^0C

TABLE 3.2 Formulation of milk samples for yoghurt preparation

	Flask							
	1	2	3	4	5	6	7	8
Contents	$D_{10/0}$	$D_{9/1}$	$D_{8/2}$	$D_{7/3}$	$ND_{10/0}$	$ND_{9/1}$	$ND_{8/2}$	$ND_{7/3}$
Sterile H_2O (ml)	100	100	100	100	100	100	100	100
D milk (g)	40	40	40	40	-	-	-	-
ND milk (g)	-	-	-	-	40	40	40	40

TABLE 3.3 Percentage inclusions of 1:1 *L.bulgaricus*/*S. thermophilus* and adjunct *S. boulardii*

	Milk samples							
Cultures	$D_{10/0}$	$D_{9/1}$	$D_{8/2}$	$D_{7/3}$	$ND_{10/0}$	$ND_{9/1}$	$ND_{8/2}$	$ND_{7/3}$
1:1 %	10	9	8	7	10	9	8	7
Adjunct %	0	1	2	3	0	1	2	3
Total inclusion %	10	10	10	10	10	10	10	10

3.9 DETERMINATION OF PHYSIOCHEMICAL PARAMETERS

3.9.1 pH

The pH was determined by the method described by AOAC (2005)

PRINCIPLE

A pH meter has a glass electrode for measuring the pH. The glass bulb specifically select hydrogen-ion concentration. On immersion in the solution to be tested, hydrogen ions in the test solution exchange for other positively charged ions on the glass bulb, creating an electrochemical potential across the bulb. The electronic amplifier detects the difference in electrical potential between the two electrodes generated in the measurement and converts the potential difference to pH units. The pH meter is calibrated with solutions of known pH, typically before each use, to ensure accuracy of measurement. To measure the pH of a solution, the electrodes are used as probes, which are dipped into the test solutions and held there sufficiently long for the hydrogen ions in the test solution to equilibrate with the ions on the surface of the bulb on the glass electrode. This equilibration provides a stable pH measurement.

PROCEDURE

This was determined using a pH meter. The pH meter was first switched on and the electrode dipped into phosphate buffers of pH of 4 and 9 to confirm the accuracy of the meter and standardize it. The electrode was then withdrawn and the standby knob was switched off. The electrode was rinsed with distilled water and wiped dry using clean wipes and thereafter dipped into the yoghurt sample. The standby knob was then switched on to put the meter on. The reading was then taken when the pointer appeared to be stable. The electrode was withdrawn and then returns knob was depressed to place the pointer at 7. The electrode was then rinsed and wiped dry with clean wipes and this was repeated for the other yoghurt samples.

3.9.2 TITRATABLE ACIDITY

The titratable acidity was determined by method described by AOAC (2005)

PRINCIPLE

The titratable acidity is expressed as % lactic acid and is determined by titration of a known amount of reconstituted milk with 0.1 N NaOH using phenolphthalein as indicator.

PROCEDURE

The amounts of acid in the yoghurt drinks were determined by titrimetric method. The titratable acidity of yoghurt sample was determined by titration with 0.1N sodium hydroxide solution

which was prepared by dissolving 2.0g of NaOH pellets in 500ml of distilled water. A 10g of sample was weighed into a clean conical flask and diluted with 10ml of distilled water. Three drops of phenolphthalein indicator were added to the diluted sample and titrated against 0.1N sodium hydroxide solution. A faint but permanent pink colour change marked the end point. Titratable acidity was expressed as the amount or volume of 0.1N sodium hydroxide that neutralized the sample, i.e. the volume of 0.1N NaOH titrated against sample to raise its pH to 7.0.

3.9.3. TOTAL SOLIDS

The total solids was determined by methods described by AOAC (2005)

PRINCIPLE

By evaporating yoghurt under controlled conditions, the total solids content can be determined accurately.

PROCEDURE

The total solids content of each yoghurt brand was also determined after oven drying at 105^{o}C for 24 hours as described for moisture content below in 3.10.1. The percentage was calculated with the formula below.

$$\% \text{ Total solids} = \frac{\text{Final weight}}{\text{Initial weight}} \times 100.$$

3.9.4 DETERMINATION OF TOTAL SOLIDS-NON-FAT

The total solids-not-fat was determined as described by AOAC (2005). It was obtained by taking the difference between % Total Solids and % Fat content. % Fat content was determined as stated in 3.10.4. That is:

% Solids-Not-Fat = % Total Solids - % Fat content.

3.9.5 DETERMINATION OF TOTAL NITROGEN

The macro kjeldah method as described by AOAC (2005) was used to determine the crude protein content.

PRINCIPLE

Sample is digested with boiling sulphuric acid. The nitrogen of sample transformed to ammonium sulphate. The acid digest is made strongly alkaline using NaOH the ammonia released is distilled into boric acid solution. It is then titrated with standard sulphuric acid.

PROCEDURE

Two grammes each sample was introduced into the digestion flask. Ten grammes of copper sulphate and sodium sulphate in the ratio of 5:1 and 25ml of concentrated sulphuric acid was added to the digestion flask. The flask was placed into digestion block in fume cupboard and heated until frothing ceased given a clear and light blue colouration. The mixture was allowed to cool transferred into volumetric flask with washing and then made up to 100 ml with distilled water.

A 10ml of the mixture was poured into the distillation apparatus and 10ml of 40% boric acid added. The released ammonia by boric acid was allow to continue until 10ml of boric acid is treated with 0.02m of hydrochloric acid until the green colour change to purple. The nitrogen in the sample was then determined. The percentage nitrogen of the sample was calculated as;

$$\% \text{ Nitrogen} = \frac{\text{molar mass of N2} \times \text{acid conc. (0.02m)} \times \text{volume made} \times \text{titre value}}{10 \times 1000 \times \text{sample weight (2g)}} \times 100$$

3.9.6 DETERMINATION OF WATER HOLDING CAPACITY (WHC)

Water holding capacity (WHC) Water holding capacity was measured as described by Parnell-Clunies, *et al*., (1986). Yogurt incubated in the sterile centrifuge tubes were centrifuged at 10 °C at 13500 × g for 30 min (Marathon 21000R, Fischer Scientific). The supernatant fluid was drained for 20 min by inverting tubes at 24 °C ±1. Water holding capacity was expressed as percent pellet weight over original yogurt weight.

$$\%\text{WHC} = \frac{\text{Drained tube weight - Tube weight}}{\text{Initial Yoghurt weight}} \times 100$$

3.9.7 DETERMINATION OF SYNERESIS

Susceptibility of yoghurt to syneresis was determined by centrifuging 10g of sample at 2500rmp for 5mins and weighing the supernatant (Guzman-Gonzalez, *et.al*, 2000). Then measuring the weight of the supernatant recovered. Percentage syneresis was calculated as.,

$$\text{Syneresis (\%)} = \frac{\text{weight of supernatant}}{\text{Weight of sample}} \times 100$$

3.10. DETERMINATION OF PROXIMATE PARAMETERS

3.10.1. MOISTURE CONTENT

The moisture content was determined as described by AOAC (2005).

PRINCIPLE

This method relys on measuring the mass of water in a known mass of sample. The moisture content is determined by measuring the mass of a food before and after the water is removed by evaporation.

PROCEDURE

A clean crucible was oven dried and weighed as (W1), then 10ml of the yoghurt was dispensed into it and both the crucible and the yoghurt sample were weighed and recorded as (W2). The crucible and its content was then dried at 105^{o}C in an oven for 24 hours after which it was removed and weighed again as (W3) which gave a constant and final weight.
This was done in duplicates and the average or mean was taken. The loss in weight represents the moisture content and the percentage was calculated as follows;

$$\text{\% Moisture content} = \frac{\text{Initial weight} - \text{Final weight}}{\text{Initial weight}} \times 100.$$

3.10.2 ASH CONTENT

Total ash was determined according to AOAC (2005).

PRINCIPLE

When a known weight of sample is ignited to ash, the weight of the ash thus obtained is expressed in terms of percentage of the weight of sample.

PROCEDURE

A clean oven dried crucible was weighed (W1). A 10 ml of the yoghurt sample was added into the clean dried crucible and weighed as W2. The crucible and its contents was then transferred into the muffle furnace set at 600^{o}C for about 6 hours, the colour change to ash showed that it was fully incinerated. The crucible and its content was removed from the furnace and placed inside desiccators to cool, after cooling it was then weighed as W3. The ash content of each of the samples was calculated as follows;

Weight of dried crucible = W1

Weight of dried crucible + sample = W2

Weight of dried crucible + sample after ashing = W3

Weight of sample (WS) = (W2- W1) g

Weight of Ash (WA) = (W3 – W1) g

% Ash = W3 –W1/ W2 – W1 × 100 i.e. WA /WS × 100

3.10.3 PROTEIN CONTENT DETERMINATION

The macro kjeldah method as described by AOAC (2005) was used to determine the crude protein content.

The nitrogen in the sample was then determined as shown in 3.9.5. The percentage nitrogen of the sample was calculated as;

$$\% \text{ Nitrogen} = \frac{\text{molar mass of N2} \times \text{acid conc. (0.02m)} \times \text{volume made} \times \text{titre value}}{10 \times 1000 \times \text{sample weight (2g)}} \times 100$$

% Crude protein = % Nitrogen × 6.25. A conversion factor of 6.25 (equivalent to 0.16 g nitrogen per gram of protein) is used for many applications.

3.10.4 FAT CONTENT DETERMINATION

The soxhlet solvent extraction method as described by AOAC (2005) was used to determine the fat content.

PRINCIPLE:

Lipid in food present in various forms like monoglycerides, diglycerides, triglycerides and sterol and free fatty acid and phospholipid and carotenoids and fat-soluble vitamins. Lipid is soluble in organic solvent and insoluble in water, because of this, organic solvents like hexane, petroleum ether have the ability to solubilize fat and fat is extracted from food in combination with the solvent. Later the fat is collected by evaporating the solvent.Almost all the solvent is distilled off and can be reused.

When a definite quantity of H_2SO_4 and amyl alcohol are added to a definite volume of yoghurt, the protein will be dissolve and fat globule will be set free which remain in liquid state. On centrifugation, fat will easily be separated.

PROCEDURE

In this method 2g of the sample was weighed into a flat bottom flask of known weight with the extractor mounted on it. The thimble was held half way into the extractor and the weighed sample was carefully transferred into the thimble and the thimble was plugged with cotton wool. The extraction was carried out at the temperature of 65^0C for 8hours. The solvent (petroleum ether) was removed by evaporation and then, the remaining part of the flask was dried in the oven at 80^0C for 30minutes and was finally cooled in a desiccator. The flask was reweighed and the percentage fat was calculated as;

$$\% \text{ Fat} = \frac{\text{Weight of extracted fat}}{\text{Weight of sample used}} \times 100$$

3.10.5 DETERMINATION OF CARBOHYDRATE CONTENT

The content of carbohydrate was determined by difference as described by Onyeike *et al.*, (2017)

CHO = 100 - %(ash + protein + fat +moisture)

3.11 MICRONUTRIENT ANALYSES

3.11.1 MINERAL EVALUTION

Mineral analysis was conducted using Varian AA240 Atomic Absorption Spectrophotometer according to American Public Health Association (APHA) (1995).

PRINCIPLE:

Atomic absorption spectrophotometer is based on the sample being aspirated into the flame and atomized when the AAS's light beams is directed through the flame into the monochromator, and onto the detector that measures the amount of light absorbed by the atomized element in the flame. Since minerals have their own characteristic wavelength, a source lamp composed of that element is used, making the method relatively free from spectral or radiational interference. The amount of energy of the characteristic wavelength absorbed in the flame is proportional to the concentration of the element in the sample.

PROCEDURE:

The sample was thoroughly shaken and 20ml of it is transferred into a glass beaker of 250ml volume, to which 3ml of conc. Nitric acid is added and heated to boil till the sample turned light yellow. The mixture is cooled and filtered with a filter paper. A series of standard mineral solution prepared and the stock mineral solution were calibrated.

3.11.2 VITAMIN EVALUATION

Determination of vitamin A

Vitamin A was determined by the calorimetric method of Kirk and Sawyer (1991). One gramme each of the sample and standard was mixed with 30 ml of absolute alcohol and 3 ml of 50% KOH solution was added to it and boiled gently for 3 min under reflux. After washing with distilled water, vitamin A was extracted with 50 ml of diethyl ether. The extract was evaporated to dryness at low temperature and then dissolved in 10 ml of isopropyl alcohol. A 1 ml each of standard vitamin A solution and that of the dissolved extract were transferred to separate curvettes and their absorbance read in a spectrophotometer at 425 nm with a reagent blank at zero. Calculated as thus:

$$Vitamin\ A = Absorbance\ of\ sample \div Absorbance\ of\ Standard \times Concentration\ of\ standard$$

Determination of vitamin B_1 and B_2

A 1 g of sample was weighed into a conical flask and was dissolved with 100 ml of deionized water. The mixture was shaken thoroughly and heated for 5 min and allowed to cool and filtered. The filtrate was poured into a cuvette and their respective wavelengths for the vitamins set to read absorbance using spectrophotometer

Vitamin B_1 = 261 nm

Vitamin B_2 = 242 nm

Vitamin B_1 and B_2 are calculated using the formular below:

$$\frac{\text{Absorbance of sample x volume of curvette x D.F}}{\text{Extinction coefficient}}$$

Determination of vitamin B_3

A 5 g of sample was dissolved in 20 ml of anhydrous glacial acetic acid and slightly warmed. 5 ml of acetic anhydride was added and mixed 3 drops of crystal violet solution was added as indicator. A 0.1 m perchloric acid was used to titrate to a greenish blue colour.

B_3 mg/l =

$$\frac{\text{Absorbance of sample}}{\text{Absorbance of std}} \times \text{Conc of std}$$

Determination of vitamin B_6

A 0.1 ml was dissolved in a mixture of 1 ml of anhydrous glacial acetic and 1 ml of 0.1 m of mercury II acetate solution. A 1 ml of 0.1 m of perchloric acid as added. The absorbance read at 542 nm against blank.

B_6 mg/l =

$$\frac{\text{Absorbance of sample}}{\text{Absorbance of std}} \times \text{Conc of std}$$

Determination of vitamin B_9

Weighed equivalent 0.2 ml of sample was taken into separator. In separator, 5 ml of water was added, mixed well and extract with 5 ml chloroform. Discard the water layer then taken chloroform in dry 50 ml volumetric flask by passed through anhydrous sodium sulphate and made up to 50 ml with chloroform.

Procedure: A 5 ml of sample and blank solution was taken into test tube. In each test tube, added 2 ml of 0.2% solution of phenyl hydrazine (in hydrochloric acid and alcohol in ratio of 1:5 v/v) and mixed well. After that heat on water bath to almost dryness and cool at room temperature. A 15 ml solution mixture (ammonia and alcohol in ratio of 1:1) was added in each test tube. Absorbance recorded at 635 nm against blank.

$$\frac{\text{Absorbance of sample}}{\text{Absorbance of std}} \times \text{Conc of std}$$

Determination of vitamin B_{12}

A 25 mg of sample was dissolved in 250 ml of deionized water. A 1 ml of dissolved extract and equal volume of standard vitamin B_{12} were transferred to separate cuvettes and respective absorbance measured in a UV spectrophotometer at 361.

B_{12} mg/l =

$$\frac{\text{Absorbance of sample x volume of curvette x D.F}}{\text{Extinction coefficient}}$$

Determination of vitamin E

This was determined by the Futter-Mayer colorimetric method with association of vitamin chemist (Kirk and Sawyer, 1991). A 1 g of the sample was mixed with 10 ml of ehanolic sulphuric acid and boiled gently under reflux for 30 min. it was transferred to a separating funnel and treated with 30 ml diethyl ether and recovering ether layer each time, the ether extracted was transferred to a dessicator and dried under 30 min and later evaporated to dryness at room temperature. The dried extract was dissolved in 10 ml of pure ethanol. A 1 ml of the dissolved extract and equal volume of standard vitamin E were transferred to separate curvettes. After continuous addition of 5 ml of absolute alcohol and 1 ml of concentrated nitric acid solution, the mixtures were allowed to stand for 15 min and respective absorbance measured in a UV spectrophotometer at 410 nm with blank reagent at zero.

E mg/l =

$$\frac{\text{Absorbance of sample}}{\text{Absorbance of std}} \times \text{Conc of std}$$

3.12. MICROBIOLOGICAL ANALYSIS

3.12.1. TOTAL VIABILITY COUNTS OF *L. bulgaricus,*

The viable cells count of *L. bulgaricus* was measured during storage time. The sample was mixed thoroughly by shaking vigorously so that uniform consistency is obtained. A 1g of the sample was added into 9 ml dilution of MRS broth to get 1:10 dilution. A 1 ml of suspension of 1:10 was added to 9 ml dilution of MRS broth to have 1:100 dilutions. The dilution continued till a 1:100000 dilutions were reached. A 1 ml portions of 1:1000 and 1:10000 dilutions were pipetted into sterile petri dishes. MRS agar of 15ml was added to each plate. The plates were Inverted and incubated at 37°C for 24 hr. After incubation, the plates were examined for typical colonies of the bacteria.

3.12.2. TOTAL VIABILITY COUNT OF *S. thermophilus*

The viable cells count of *S. thermophilus* was measured during storage time. The sample was mixed thoroughly by shaking vigorously so that uniform consistency is obtained. A 1g of the sample was added into 9 ml dilution of M17 broth to get 1:10 dilution. A 1 ml of suspension of 1:10 was added to 9 ml dilution of M17 broth to have 1:100 dilutions. The dilution continued till a 1:100000 dilutions were reached. A 1 ml portion of 1:1000 and 1:10000 dilutions was pipetted into sterile petri dishes. M17 agar of 15ml was added to each plate. The plates were Inverted and incubated at 37°C for 24 hr. After incubation, the plates were examined for typical colonies of the bacteria.

3.12.3. TOTAL VIABILITY COUNT OF *S. boulardii*

The viable cells count of *S. boulardii* was measured during storage time. The sample was mixed thoroughly by shaking vigorously so that uniform consistency is obtained. A 1g of the sample was added into 9 ml dilution of PDA broth to get 1:10 dilution. A 1 ml of suspension of 1:10 was added to 9 ml dilution of PDA broth to have 1:100 dilutions. The dilution continued till a 1:100000 dilutions were reached. A 1 ml portion of 1:1000 and 1:10000 dilutions was pipetted into sterile petri dishes. The pH of potato dextrose agar was adjusted aseptically to 3.5 by adding tartaric acid solution at the time of pouring plates. PDA agar of 15ml was added to each plate. The plates were Inverted and incubated at 25°C for 24 hr.

After incubation, the plates were examined for typical colonies of the bacteria.

3.12.4. TOTAL COUNT FOR COLIFORM AND *E. coli*

The viable cells count of *coliform* and *E. coli* was measured during storage time. The sample was mixed thoroughly by shaking vigorously so that uniform consistency is obtained. A 1g of the sample was added into 9 ml dilution of CCA broth to get 1:10 dilution. A 1 ml of suspension of 1:10 was added to 9 ml dilution of CCA broth to have 1:100 dilutions. The dilution continued till a 1:100000 dilutions were reached. A 1 ml portion of 1:1000 and 1:10000 dilutions was pipetted into sterile petri dishes. CCA agar of 15ml was added to each plate. The plates were Inverted and incubated at 30°C for 24 hr. After incubation, the plates were examined for typical colonies of the bacteria.

3.13 SENSORY ANALYSES

The yoghurt prepared was evaluated by twenty member panelists consisting of yoghurt consumers selected at random from National Agency for Food and Drug Administration and Control. The sensory evaluation was to determine the level of Sourness/Taste, Body/Texture, Color/Appearance, Odor and Overall Acceptability, by using a 9-point hedonic scale where 1 to 9 represent dislike extremely (1) to like extremely (9) as delineated byWichchukit and O'Mahony (2015). The samples were dispensed in disposable cups (labeled). The panelists were given a questionnaire (Appendix XIV) for records and a disposable spoon for analysis.

The questionnaires were collected and collated.

Statistical Analysis:

One-way Analysis of Variance (ANOVA) was used to determine difference in the various treatments carried out.

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 RESULTS

4.1.1 PHYSICOCHEMICAL EVALUATION OF YOGHURT SAMPLES

The four (4) portions of dairy milk was designed as D10/0, D9/1, D8/2, and D7/3, while the four (4) portions of non-dairy milk was designed as ND10/0, ND9/1, ND8/2, and ND7/3. The result of the physicochemical evaluation of yoghurt produced from dairy milk (cow milk) and nondairy milk (soy milk) is as presented in Table 4.1. The percentage values for total solid content in the different portions of dairy milk shows a range of 19.82 ± 0.5 – 21.88 ± 0.6, and follows a descending order of D8/2 > D9/1 > D10/0 > D7/3, while nondairy milk shows a range of 18.45 ± 0.5 – 19.83 ± 0.4, and follows a descending order of ND7/3 > ND8/2 > ND9/1 > ND10/0 (Table 4.1).

The percentage values for TS Non-Fat in the different portions of dairy milk shows a range of 17.24 ± 0.5 – 20.28 ± 0.7, and follows a descending order of D8/2 > D9/1 > D7/3 > D10/0, while nondairy milk shows a range of 17.05 ± 0.7 – 18.63 ± 0.6, and follows a descending order of ND7/3 > ND8/2 > ND9/1 > ND10/0 (Table 4.1).

The percentage values for total nitrogen content in the different portions of dairy milk shows a range of 0.68 ± 0.2 – 0.84 ± 0.2, and follows a descending order of D7/3 > D8/2 > D9/1 > D10/0, while nondairy milk shows a range of 1.3 ± 0.2 – 1.4 ± 0.2, and follows a descending order of ND7/3 > ND9/1 > ND8/2 > ND10/0 (Table 4.1).

Table 4.1: Physicochemical Composition of Dairy + Non-Dairy Yoghurt from milk samples

	PHYSICOCHEMICAL COMPOSITION (%) OF SAMPLES							
PARAMETER	D10/0	D9/1	D8/2	D7/3	ND10/0	ND9/1	ND8/2	ND7/3
Total Solid	19.84±0.3^{a}	19.96±0.5^{a}	21.88±0.6^{c}	19.82±0.5^{a}	18.45±0.5^{d}	18.93±0.6^{d}	19.68±0.5^{a}	19.83±0.4^{a}
TS Non-Fat	17.24±0.5^{a}	17.96±1.1^{a}	20.28±0.7^{b}	17.83±0.7^{a}	17.05±0.7^{a}	17.73±0.5^{a}	18.28±0.4^{c}	18.63±0.6^{c}
Total Nitrogen	0.68±0.2^{a}	0.70±0.1^{a}	0.77±0.1^{a}	0.84±0.2^{a}	1.3±0.2^{b}	1.4±0.1^{b}	1.4±0.1^{b}	1.4±0.2^{b}

Values are means ± SD of triplicate determinations

Values in the same row with the same superscript are not significantly different at 5% level ($p>0.05$).

4.1.2 PROXIMATE EVALUATION OF YOGHURT SAMPLES

Table 4.2 shows the proximate evaluation of yoghurt samples from dairy milk and nondairy milk. The percentage values for moisture content in the different portions of dairy milk shows a range of 78.13 ± 0.1 – 80.17 ± 0.05, and follows a descending order of D7/3 > D10/0 > D9/1 > D8/2, while nondairy milk shows a range of 80.32 ± 0.02 – 83.42 ± 0.03, and follows a descending order of ND7/3 > ND10/0 > ND9/1 > ND8/2.

The percentage values for ash content in the different portions of dairy milk shows a range of 0.8 ± 0.1 – 1.2 ± 0.3, and follows a descending order of D8/2 > D9/1 > D7/3 > D10/0, while nondairy milk shows a range of 1.2 ± 0.1 – 1.6 ± 0.1, and follows a descending order of ND9/1 > ND8/2 > ND10/0 > ND7/3 (Table 4.2).

The percentage values for protein content in the different portions of dairy milk shows a range of 4.3 ± 0.1 – 5.3 ± 0.6, and follows a descending order of D7/3 > D8/2 > D9/1 > D10/0, while nondairy milk shows a range of 7.9 ± 0.4 – 9.2 ± 0.3, and follows a descending order of ND7/3 > ND8/2 > ND9/1 > ND10/0 (Table 4.2).

The percentage values for fat content in the different portions of dairy milk shows a range of 1.6 ± 0.3 – 2.6 ± 0.3, and follows a descending order of D10/0 > D9/1 > D7/3 > D8/2, while nondairy milk shows a range of 1.2 ± 0.3 – 1.4 ± 0.3, and follows a descending order of ND8/2 > ND10/0 > ND7/3 > ND9/1 (Table 4.2).

The percentage values for carbohydrate content in the different portions of dairy milk shows a range of 11.78 ± 0.1 – 14.26 ± 0.4, and follows a descending order of D8/2 > D9/1 > D10/0 >

Table 4.2: Proximate Composition of Dairy + Non-Dairy Yoghurt from milk samples

	PROXIMATE COMPOSITION (%) OF SAMPLES							
CONSTITUENT	D10/0	D9/1	D8/2	D7/3	ND10/0	ND9/1	ND8/2	ND7/3
Moisture	80.16±0.06^{a}	80.04±0.01^{a}	78.13±0.1^{b}	80.17±0.05^{a}	82.55±0.15^{c}	81.07±0.03^{d}	80.32±0.02^{a}	83.42±0.03^{e}
Ash	0.8±0.1^{a}	1.0±0.1^{a}	1.2±0.3^{a}	0.9±0.1^{a}	1.4±0.1^{a}	1.6±0.1^{b}	1.4±0.2^{a}	1.2±0.1^{a}
Protein	4.3±0.1^{a}	4.4±0.1^{a}	4.8±0.5^{b}	5.3±0.6^{c}	7.9±0.4^{d}	8.8±0.4^{e}	9.1±0.4^{f}	9.2±0.3^{g}
Fat	2.6±0.3^{a}	2.0±0.4^{b}	1.6±0.3^{c}	2.0±0.4^{b}	1.4±0.2^{d}	1.2±0.3^{d}	1.4±0.3^{d}	1.2±0.4^{d}
Carbohydrate	12.14±0.1^{a}	12.56±0.2^{a}	14.26±0.4^{b}	11.78±0.1^{c}	6.75±0.2^{d}	7.38±0.3^{e}	7.82±0.3^{e}	4.27±0.3^{f}

Values are means ± SD of triplicate determinations

Values in the same row with the same superscript are not significantly different at 5% level ($p>0.05$).

4.1.3. MICRONUTRIENT EVALUATION OF YOGHURT SAMPLES

Table 4.3 shows the mineral content of yoghurt samples from dairy milk and nondairy milk. The concentration of calcium in the different portions of dairy milk shows a range of 218.71 ± 1.2 – 224.65 ± 0.7 (mg/l), and follows a descending order of D10/0 > D8/2 > D7/3 > D9/1, while nondairy milk shows a range of 186.95 ± 0.8 – 192.04 ± 1.4 (mg/l), and follows a descending order of ND9/1 > ND8/2 > ND7/3 > ND10/0 (Table 4.3).

The concentration of potassium in the different portions of dairy milk shows a range of 296.76 ± 0.4 – 301.01 ± 2.7 (mg/l), and follows a descending order of D9/1 > D8/2 > D7/3 > D10/0, while nondairy milk shows a range of 556.11 ± 2.1 – 560.54 ± 1.6 (mg/l), and follows a descending order of ND9/1 > ND7/3 > ND8/2 > ND10/0 (Table 4.3).

The concentration of phosphorus in the different portions of dairy milk shows a range of 192.59 ± 1.5 – 194.1 ± 1.8 (mg/l), and follows a descending order of D10/0 > D7/3 > D8/2 > D9/1, while nondairy milk shows a range of 392.16 ± 2.5 – 401.06 ± 1.1 (mg/l), and follows a descending order of ND9/1 > ND7/3 > ND10/0 > ND8/2 (Table 4.3).

The concentration of magnesium in the different portions of dairy milk shows a range of 7.79 ± 2.8 – 8.14 ± 3.1 (mg/l), and follows a descending order of D9/1 > D8/2 > D10/0 > D7/3, while nondairy milk shows a range of 154.44 ± 1.7 – 162.98 ± 1.0 (mg/l), and follows a descending order of ND9/1 > ND8/2 > ND7/3 > ND10/0 (Table 4.3).

The concentration of manganese in the different portions of dairy milk shows a range of 0.21 ± 2.3 – 0.27 ± 0.1 (mg/l), and follows a descending order of D10/0 > D7/3 > D8/2 > D9/1, while nondairy milk shows a range of 3.12 ± 1.1 – 3.97 ± 0.3 (mg/l), and follows a descending order of ND9/1 > ND7/3 > ND8/2 > ND10/0 (Table 4.3).

The concentration of sodium in the different portions of dairy milk shows a range of 122.52 ± 0.2 – 126.53 ± 0.9 (mg/l), and follows a descending order of D8/2 > D10/0 > D7/3 > D9/1, while nondairy milk shows a range of 7.25 ± 2.3 – 7.82 ± 1.8 (mg/l), and follows a descending order of ND8/2 > ND10/0 > ND7/3 > ND9/1 (Table 3).

The concentration of iron in the different portions of dairy milk shows a range of 0.004 ± 2.8 – 0.005 ± 1.7 (mg/l), and follows a descending order of D8/2> D10/0 > D7/3 > D9/1, while nondairy milk shows a range of 17.02 ± 1.3 – 18.15 ± 2.0 (mg/l), and follows a descending order of ND10/0 > ND8/2 > ND7/3 > ND9/1 (Table 3).

The concentration of zinc in the different portions of dairy milk shows a range of 0.32 ± 1.3 – 0.42 ± 1.9 (mg/l), and follows a descending order of D10/0 > D8/2 > D9/1 > D7/3, while nondairy milk shows a range of 2.04 ± 1.9 – 2.16 ± 3.0 (mg/l), and follows a descending order of ND10/0 > ND8/2 > ND9/1 > ND7/3 (Table 4.3).

Table 4.3 Mineral Content of Dairy + Non-Dairy Yoghurt from milk samples

MINERALS (mg/l)	SAMPLES							
	D10/0	D9/1	D8/2	D7/3	ND10/0	ND9/1	ND8/2	ND7/3
Calcium	224.65±0.7[a]	218.71±1.2[b]	224.05±0.5[a]	221.34±2.1[c]	186.95±0.8[d]	192.04±1.4[e]	190.84±0.7[f]	187.98±0.2[g]
Potassium	296.76±0.4[a]	301.01±2.7[b]	300.78±0.1[c]	299.23±1.5[d]	556.11±2.1[e]	560.54±1.6[f]	557.32±0.4[g]	559.43±1.5[h]
Phosphorous	194.1±1.8[a]	192.59±1.5[b]	193.05±4.1[c]	193.76±0.5[d]	397.67±2.2[e]	401.06±1.1[f]	392.16±2.5[g]	398.77±1.0[h]
Magnesium	7.89±2.6[a]	8.14±3.1[b]	7.92±1.3[c]	7.79±2.8[d]	154.44±1.7[e]	162.98±1.0[f]	157.81±0.6[g]	154.98±2.3[e]
Manganese	0.27±0.1[a]	0.21±2.3[b]	0.22±3.1[c]	0.25±0.3[d]	3.12±1.1[e]	3.97±0.3[f]	3.54±0.1[g]	3.71±1.7[h]
Sodium	125.34±2.0[a]	122.52±0.2[b]	126.53±0.9[c]	124.23±0.8[d]	7.41±2.1[e]	7.25±2.3[f]	7.82±1.8[g]	7.35±3.1[h]
Iron	0.005±1.1[a]	0.004±2.8[b]	0.005±1.7[a]	0.005±0.1[a]	18.15±2.0[c]	17.02±1.3[d]	17.76±2.0[e]	17.63±2.7[f]
Zinc	0.42±1.9[a]	0.34±0.9[b]	0.39±2.1[b]	0.32±1.3[b]	2.16±3.0[c]	2.07±1.9[d]	2.11±1.4[c]	2.04±1.9[d]

Values are means ± SD of triplicate determinations
Values in the same row with the same superscript are not significantly different at 5% level ($p>0.05$).

4.1.4: Vitamine Evaluation of Diary + Non-Diary Yoghurt (produced) from milk samples

The result of vitamin evaluation of yoghurt samples from dairy milk and nondairy milk is as presented in table 4.4. The concentration of vitamin A in the different portions of dairy milk shows a range of 28.38 ± 0.01 – 32.143 ± 0.02 (mg/l), and follows a descending order of D7/3 > D10/0 > D8/2 > D9/1, while nondairy milk shows a range of 82.31 ± 0.01 – 90.91 ± 0.01 (mg/l), and follows a descending order of ND10/0 > ND7/3 > ND8/2 > ND9/1 (Table 4.4).

The concentration of vitamin B_1 in the different portions of dairy milk shows a range of 2.11 ± 0.01 – 2.43 ± 0.00 (mg/l), and follows a descending order of D10/0 > D8/2 > D9/1 > D7/3, while nondairy milk shows a range of 8.12 ± 0.00 – 9.06 ± 0.01 (mg/l), and follows a descending order of ND7/3 > ND10/0 > ND8/2 > ND9/1 (Table 4.4).

The concentration of vitamin B_2 in the different portions of dairy milk shows a range of 18.75 ± 0.00 – 19.85 ± 0.04 (mg/l), and follows a descending order of D9/1 > D7/3 > D10/0 > D8/2, while nondairy milk shows a range of 36.85 ± 0.00 – 39.11 ± 0.00 (mg/l), and follows a descending order of ND9/1 > ND8/2 > ND7/3 > ND10/0 (Table 4.4).

The concentration of vitamin B_3 in the different portions of dairy milk shows a range of 2.15 ± 0.00 – 3.6 ± 0.00 (mg/l), and follows a descending order of D9/1 > D8/2 > D7/3 > D10/0, while nondairy milk shows a range of 126.94 ± 0.00 – 131.9 ± 0.01 (mg/l), and follows a descending order of ND7/3 > ND9/1 > ND8/2 > ND10/0 (Table 4.4).

The concentration of vitamin B_6 in the different portions of dairy milk shows a range of 12.01 ± 0.02 – 12.98 ± 0.03 (mg/l), and follows a descending order of D8/2 > D9/1 > D7/3 > D10/0, while nondairy milk shows a range of 17.3 ± 0.00 – 23.3 ± 0.02 (mg/l), and follows a descending order of ND7/3 > ND9/1 = ND8/2 > ND10/0 (Table 4.4).

The concentration of vitamin B_9 in the different portions of dairy milk shows a range of 11.61 ± 0.00 – 13.02 ± 0.02 (mg/l), and follows a descending order of D7/3 > D10/0 > D8/2 > D9/1, while nondairy milk shows a range of 24.03 ± 0.04 – 27.11 ± 0.01 (mg/l), and follows a descending order of ND7/3 > ND8/2 > ND9/1 > ND10/0 (Table 4.4).

The concentration of vitamin B_{12} in the different portions of dairy milk shows a range of 35.91 ± 0.02 – 36.92 ± 0.00 (mg/l), and follows a descending order of D7/3 > D8/2 > D9/1 > D10/0, while nondairy milk shows a range of 4.27 ± 0.02 – 5.82 ± 0.00 (mg/l), and follows a descending order of ND7/3 > ND8/2 > ND9/1 > ND10/0 (Table 4.4).

The concentration of vitamin E in the different portions of dairy milk shows a range of 3.24 ± 0.01 – 4.27 ± 0.02 (mg/l), and follows a descending order of D9/1 > D7/3 > D10/0 > D8/2, while nondairy milk shows a range of 10.98 ± 0.00 – 14.15 ± 0.00 (mg/l), and follows a descending order of ND9/1 > ND7/3 > ND10/0 > ND8/2 (Table 4.4).

Table 4.4: Vitamin Evaluation of Dairy + Non-Dairy Yoghurt (produced) from milk samples

VITAMIN (mg/l)	SAMPLES							
	D10/0	D9/1	D8/2	D7/3	ND10/0	ND9/1	ND8/2	ND7/3
Vitamin A	30.13±0.01^{a}	28.38±0.01^{b}	29.45±0.04^{c}	32.143±0.02^{d}	90.91±0.01^{e}	82.31±0.01^{f}	85.94±0.00^{g}	89.70±0.02^{h}
Vitamin B_1	2.43±0.00^{a}	2.21±0.02^{b}	2.31±0.01^{c}	2.11±0.01^{d}	8.76±0.05^{e}	8.12±0.00^{f}	8.32±0.01^{g}	9.06±0.01^{h}
Vitamin B_2	19.07±0.03^{a}	19.85±0.04^{b}	18.75±0.00^{c}	19.51±0.01^{d}	36.85±0.00^{e}	39.11±0.00^{f}	37.23±0.02^{g}	36.91±0.01^{h}
Vitamin B_3	2.15±0.00^{a}	3.6±0.00^{b}	3.05±0.00^{c}	2.45±0.01^{d}	126.94±0.00^{e}	129.55±0.03^{f}	128.95±0.00^{g}	131.9±0.01^{h}
Vitamin B_6	12.01±0.02^{a}	12.81±0.01^{b}	12.98±0.03^{b}	12.48±0.01^{c}	17.3±0.00^{d}	17.3±0.01^{d}	17.3±0.01^{d}	23.3±0.02^{e}
Vitamin B_9	12.68±0.01^{a}	11.61±0.00^{b}	11.81±0.01^{b}	13.02±0.02^{c}	24.03±0.04^{d}	24.39±0.02^{e}	25.35±0.00^{f}	27.11±0.01^{g}
Vitamin B_{12}	35.91±0.02^{a}	36.11±0.00^{b}	36.23±0.04^{c}	36.92±0.00^{d}	4.27±0.02^{e}	4.59±0.01^{f}	4.91±0.01^{g}	5.82±0.00^{h}
Vitamin E	3.86±0.00^{a}	4.27±0.02^{b}	3.24±0.01^{c}	4.17±0.00^{b}	12.74±0.02^{d}	14.15±0.00^{e}	10.98±0.00^{f}	12.74±0.00^{g}

Values are means ± SD triplicate determinations
Mean +SD. Values in the same row with the same superscript are not significantly ($p<0.05$) different.

4.1.5 SHELF LIFE EVALUATION OF YOGHURT SAMPLES

Fig 4.1 shows the result of change in pH of yoghurt samples from dairy milk and nondairy milk. The pH value at day 1 in the different portions of dairy milk shows a range of 4.38 – 4.63, and follows a descending order of D10/0 > D9/1 > D8/2 > D7/3, while nondairy milk shows a range of 4.72 – 4.91, and follows a descending order of ND10/0 > ND9/1 > ND8/2 > ND7/3 (Fig 4.1).

The pH value at day 7 in the different portions of dairy milk shows a range of 4.18 – 4.47, and follows a descending order of D10/0 > D9/1 > D8/2 > D7/3, while nondairy milk shows a range of 4.68 – 4.83, and follows a descending order of ND10/0 > ND9/1 > ND8/2 > ND7/3 (Fig 4.1).

The pH value at day 14 in the different portions of dairy milk shows a range of 3.97 – 4.27, and follows a descending order of D10/0 > D9/1 > D8/2 > D7/3, while nondairy milk shows a range of 4.5 – 4.79, and follows a descending order of ND10/0 > ND9/1 > ND8/2 > ND7/3 (Fig 4.1).

The pH value at day 21 in the different portions of dairy milk shows a range of 3.97 – 4.2, and follows a descending order of D10/0 > D9/1 > D8/2 > D7/3, while nondairy milk shows a range of 4.42 – 4.74, and follows a descending order of ND10/0 > ND9/1 > ND8/2 > ND7/3 (Fig 4.1).

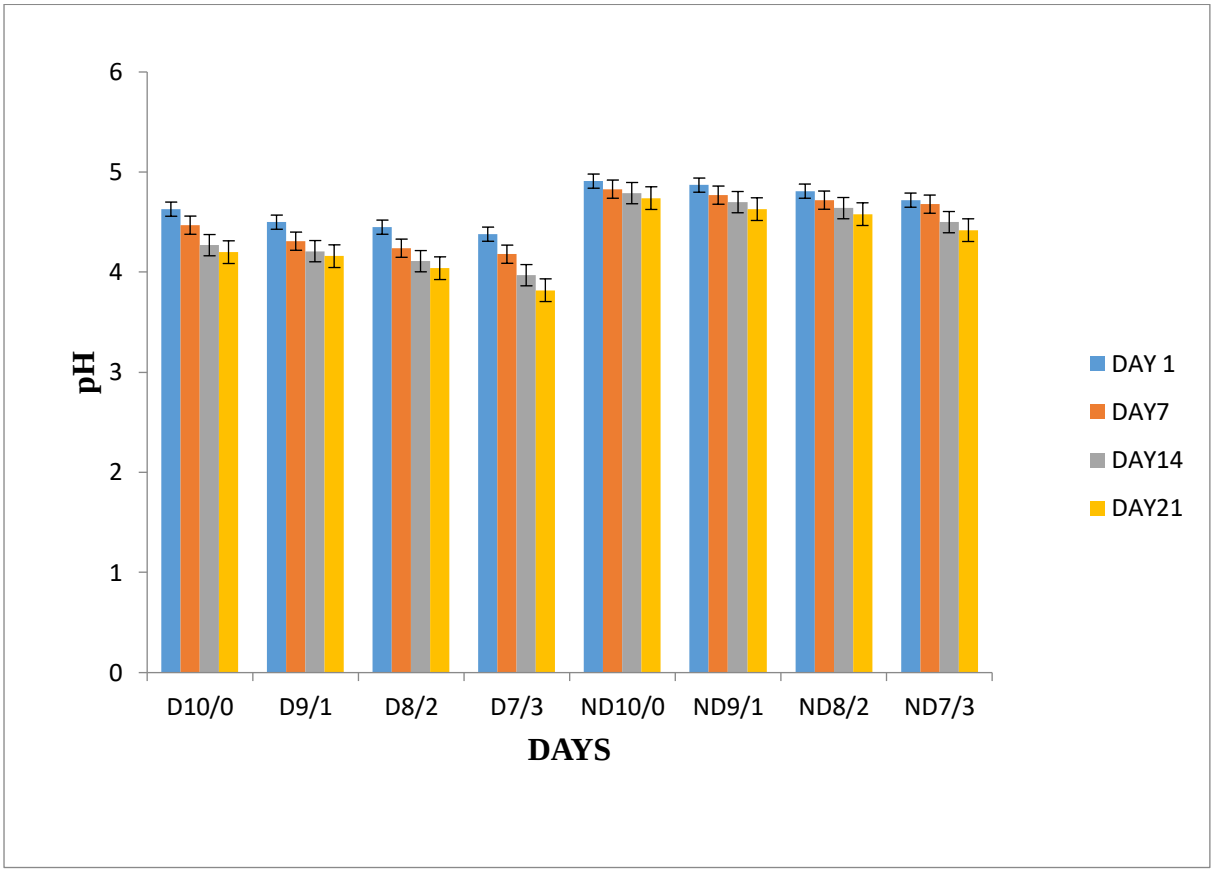

Fig 4.1: Change in pH of dairy + non dairy yoghurt from milk samples during the storage

4.1.6: CHANGE IN TITRATABLE ACIDITY

The result of change in titratable acidity is as presented in fig 4.2. The value for titratable acidity at day 1 in the different portions of dairy milk shows a range of 7.92 – 10.98, and follows a descending order of D7/3 > D8/2 > D9/1 > D10/0, while nondairy milk shows a range of 7.92 – 8.55, and follows a descending order of ND7/3 > ND8/2 > ND9/1 > ND10/0 (Fig 4.2).

The value for titratable acidity at day 7 in the different portions of dairy milk shows a range of 8.16– 11.61, and follows a descending order of D7/3 > D8/2 > D9/1 > D10/0, while nondairy milk shows a range of 8.1 – 8.92, and follows a descending order of ND8/2 > ND7/3 > ND9/1 > ND10/0 (Fig 4.2).

The value for titratable acidity at day 14 in the different portions of dairy milk shows a range of 10.44 – 12.24, and follows a descending order of D7/3 > D8/2 > D9/1 > D10/0, while nondairy milk shows a range of 8.64 – 9.63, and follows a descending order of ND7/3 > ND8/2 > ND9/1 > ND10/0 (Fig 4.2).

The value for titratable acidity at day 21 in the different portions of dairy milk shows a range of 10.89 – 12.78, and follows a descending order of D7/3 > D8/2 > D9/1 > D10/0, while nondairy milk shows a range of 9.18 – 10.08, and follows a descending order of ND7/3 > ND8/2 > ND9/1 > ND10/0 (Fig 4.2).

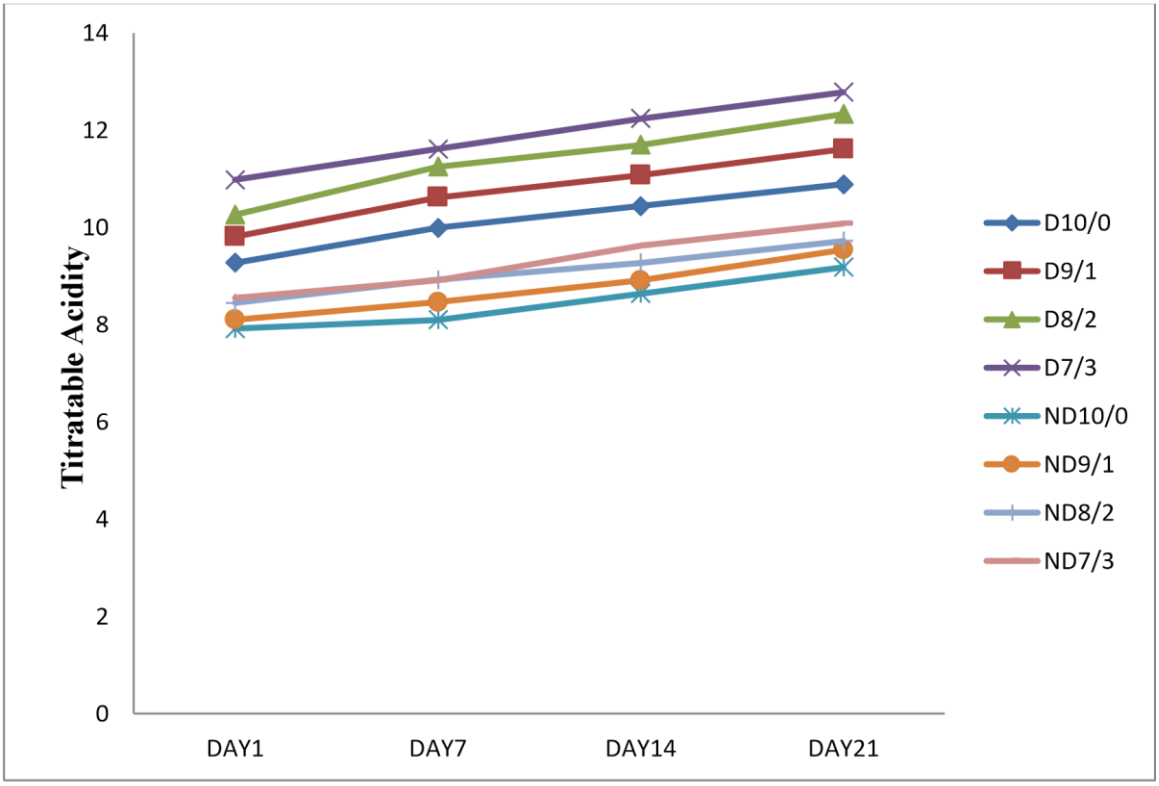

Fig 4.2: Change in titratable acidity evaluation of dairy + non dairy yoghurt from milk samples during the storage

4.1.7: CHANGE IN WATER HOLDING CAPACITY

Fig 4.3 shows the change in water holding capacity evaluation. The result shows the value for water holding capacity at day 1 in the different portions of dairy milk shows a range of 59 – 68, and follows a descending order of D7/3 > D8/2 > D9/1 > D10/0, while nondairy milk shows a range of 49 – 57, and follows a descending order of ND7/3 > ND8/2 > ND9/1 > ND10/0 (Fig 4.3).

The value for water holding capacity at day 7 in the different portions of dairy milk shows a range of 57 – 66, and follows a descending order of D7/3 > D8/2 > D9/1 > D10/0, while nondairy milk shows a range of 48 – 57, and follows a descending order of ND7/3 > ND8/2 > ND9/1 > ND10/0 (Fig 4.3).

The value for water holding capacity at day 14 in the different portions of dairy milk shows a range of 56 – 63, and follows a descending order of D7/3 > D8/2 > D9/1 > D10/0, while nondairy milk shows a range of 47 – 56, and follows a descending order of ND7/3 > ND8/2 > ND9/1 > ND10/0 (Fig 4.3).

The value for water holding capacity at day 21 in the different portions of dairy milk shows a range of 50 – 61, and follows a descending order of D7/3 > D8/2 > D9/1 > D10/0, while nondairy milk shows a range of 46 – 54, and follows a descending order of ND7/3 > ND8/2 > ND9/1 > ND10/0 (Fig 4.3).

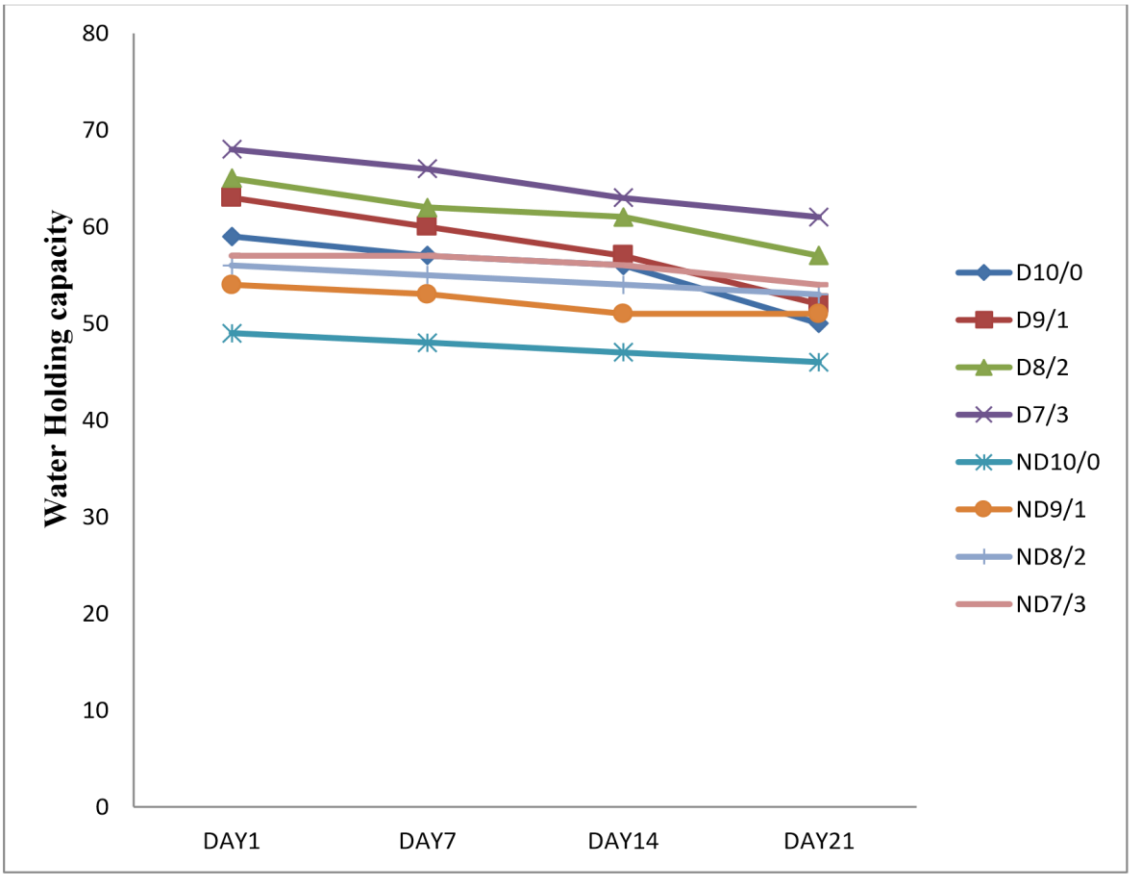

Fig 4.3: Change in Water Holding Capacity evaluation of dairy + non dairy yoghurt from Milk samples during the storage

4.1.8: CHANGE IN SYNERESIS

The result of change in syneresis is as presented in fig 4.4. The value for syneresis evaluation at day 1 in the different portions of dairy milk shows a range of 4 – 11, and follows a descending order of D10/0 > D9/1 > D8/2 > D7/3, while nondairy milk shows a range of 27 – 34, and follows a descending order of ND10/0 > ND9/1 > ND8/2 > ND7/3 (Fig 4.4).

The value for syneresis evaluation at day 7 in the different portions of dairy milk shows a range of 5 – 13, and follows a descending order of D10/0 > D9/1 > D8/2 > D7/3, while nondairy milk shows a range of 29 – 35, and follows a descending order of ND10/0 > ND9/1 > ND8/2 > ND7/3 (Fig 4.4).

The value for syneresis evaluation at day 14 in the different portions of dairy milk shows a range of 7 – 14, and follows a descending order of D10/0 > D9/1 = D8/2 > D7/3, while nondairy milk shows a range of 30 – 35, and follows a descending order of ND10/0 > ND9/1 > ND8/2 > ND7/3 (Fig 4.4).

The value for syneresis evaluation at day 21 in the different portions of dairy milk shows a range of 8 – 14, and follows a descending order of D10/0 > D9/1 > D8/2 > D7/3, while nondairy milk shows a range of 30 – 36, and follows a descending order of ND10/0 > ND9/1 > ND8/2 > ND7/3 (Fig 4.4).

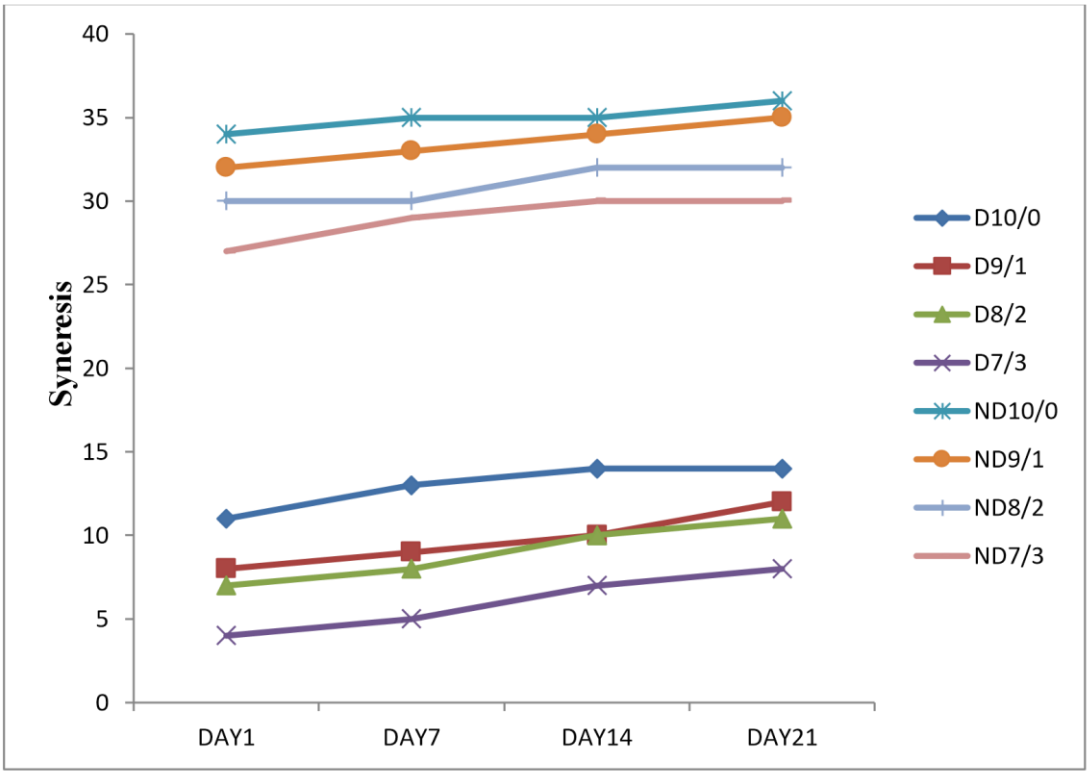

Fig 4.4: Change in Syneresis of dairy + non dairy yoghurt from milk samples during the storage

4.1.9: MICROBIAL VIABILITY OF YOGHURT SAMPLES *Lactobacillus bulgaricus* (Log.CFU/ml)

Fig 4.5 shows the result of the change in viable count of *Lactobacillus bulgaricus* of the yoghurt. The value for viable count of *L.bulgaricus* at day 1 in the different portions of dairy milk shows a range of 8.62 – 8.83 (Log CFU/ml), and follows a descending order of D7/3 > D8/2 > D9/1 > D10/0, while nondairy milk shows a range of 7.59 – 7.79 (Log CFU/ml), and follows a descending order of ND7/3 > ND8/2 > ND9/1 > ND10/0 (Fig 4.5).

The value for viable count of *L.bulgaricus* at day 7 in the different portions of dairy milk shows a range of 8.30 – 8.79 (Log CFU/ml), and follows a descending order of D7/3 > D8/2 > D9/1 > D10/0, while nondairy milk shows a range of 7.44 – 7.70 (Log CFU/ml), and follows a descending order of ND7/3 > ND8/2 > ND9/1 > ND10/0 (Fig 4.5).

The value for viable count of *L.bulgaricus* at day 14 in the different portions of dairy milk shows a range of 8.10 – 8.75 (Log CFU/ml), and follows a descending order of D7/3 > D8/2 > D9/1 > D10/0, while nondairy milk shows a range of 7.59 – 7.79 (Log CFU/ml), and follows a descending order of ND7/3 > ND8/2 > ND10/0 > ND9/1 (Fig 4.5).

The value for viable count of *L.bulgaricus* at day 21 in the different portions of dairy milk shows a range of 7.94 – 8.63 (Log CFU/ml), and follows a descending order of D7/3 > D8/2 > D9/1 > D10/0, while nondairy milk shows a range of 6.86 – 7.45 (Log CFU/ml), and follows a descending order of ND7/3 > ND8/2 > ND9/1 > ND10/0 (Fig 4.5).

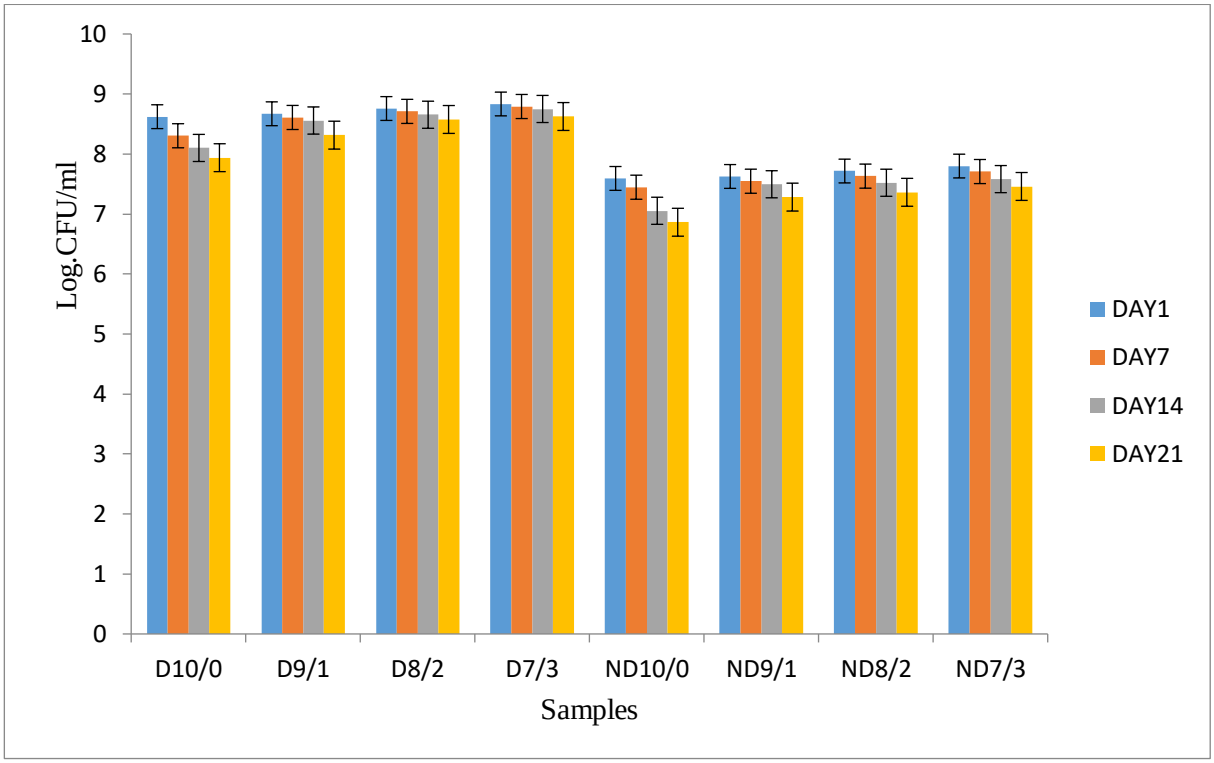

Fig 4.5: Change in viable count of *L.bulgaricus* of dairy & non dairy yoghurt from milk samples during the 21 storage time

4.1.10 VIABLE COUNT OF *Streptococcus thermophiles* (Log.CFU/ml) OF YOGHURT SAMPLES

The change in viable count of *Streptococcus thermophiles* (log.cfu/ml) of yoghurt from dairy milk (cow milk) and non-dairy milk (soy milk) within 21 day of storage time are presented in from milk samples during storage

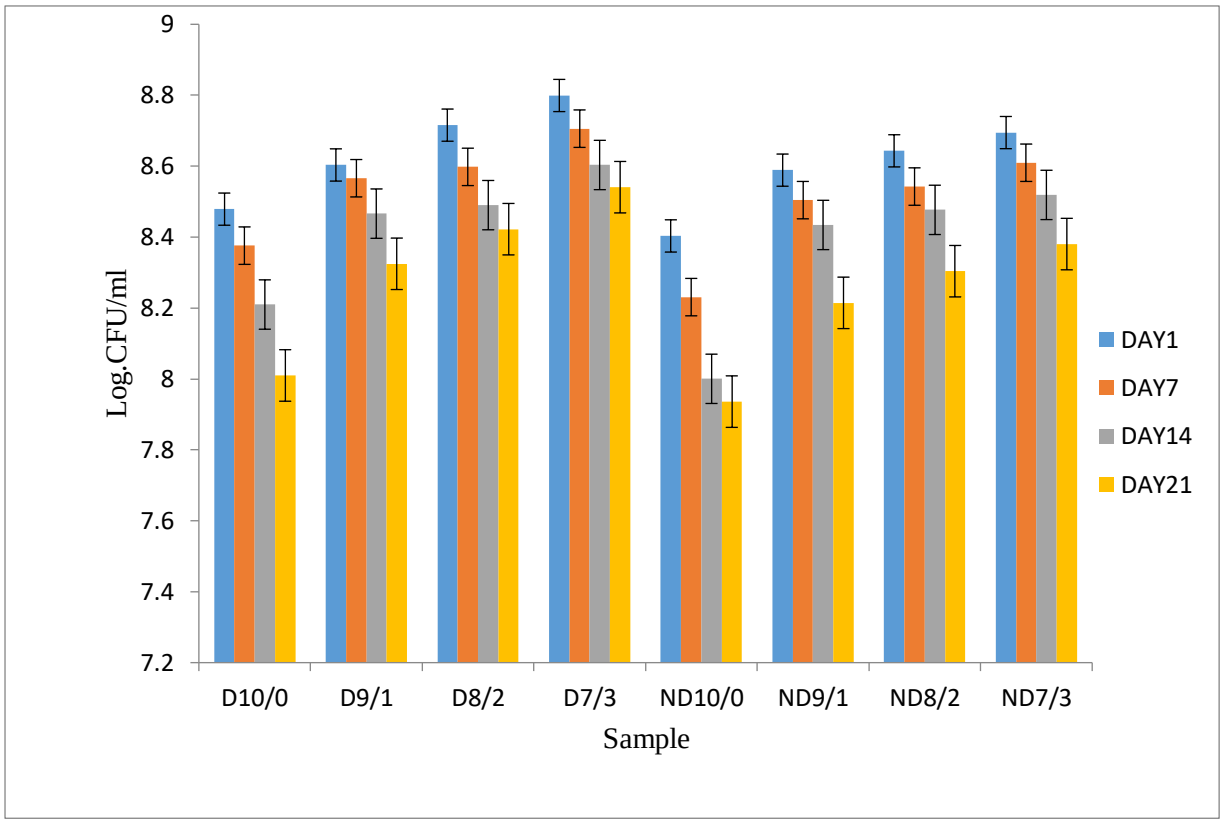

Fig 4.6: Change in viable count of *S. thermophilus* of dairy & non dairy yoghurt from milk samples during the 21 days storage time

4.1.12 VIABLE COUNT OF *Saccharomyces boulardii*(Log.CFU/ml) OF YOGHURT SAMPLES

The change in viable count of *Saccharomyces boulardii* (log.cfu/ml) of yoghurt from dairy milk (cow milk) and non-dairy milk (soy milk) within 21 day storage time are presented in Fig. 4.7.

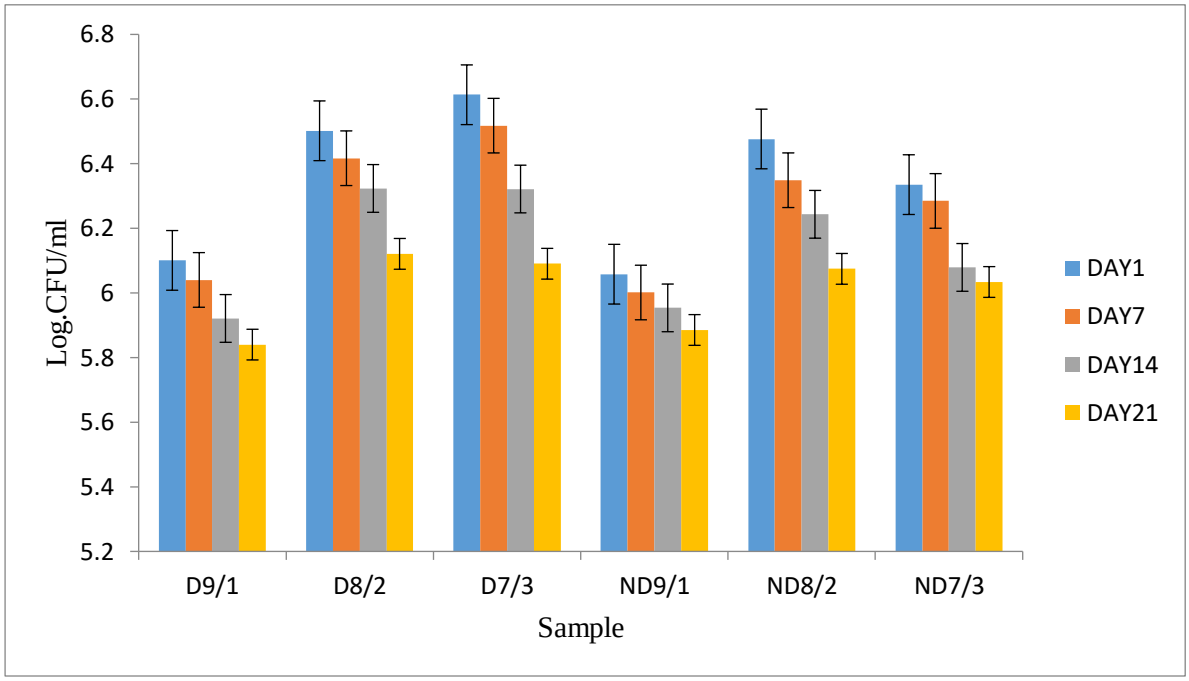

Fig 4.7: Change in viable count of *S. boulardii* of dairy & non dairy yoghurt from milk samples during the 21 days storage time.

4.1.13 SENSORY EVALUATION OF YOGHURT SAMPLES

The sensory score of yoghurt samples from dairy milk and nondairy milk is as presented in Table 5. The value for odour in the different portions of dairy milk shows a range of 6.5 ± 0.5 – 8.4 ± 0.4, and follows a descending order of D7/3 > D10/0 > D9/1 > D8/2, while nondairy milk shows a range of 5.9 ± 0.4 – 7.2 ± 0.3, and follows a descending order of ND10/0 > ND7/3 > ND9/1 > ND8/2 (Table 4.5).

The value for texture in the different portions of dairy milk shows a range of 6.3 ± 0.4 – 7.8 ± 0.4, and follows a descending order of D7/3 > D9/1 > D8/2 > D10/0, while nondairy milk shows a range of 6.1 ± 0.1 – 6.6 ± 0.4, and follows a descending order of ND8/2 > ND9/1 > ND7/3 > ND10/0 (Table 4.5).

The value for colour/appearance in the different portions of dairy milk shows a range of 7.7 ± 0.2 – 8.2 ± 0.3, and follows a descending order of D7/3 > D9/1 > D8/2 > D10/0, while nondairy milk shows a range of 7.0 ± 0.3 – 7.3 ± 0.4, and follows a descending order of ND9/1 > ND10/0 = ND7/3 > ND8/2 (Table 4.5).

The value for taste in the different portions of dairy milk shows a range of 5.7 ± 0.2 – 7.6 ± 0.3, and follows a descending order of D7/3 > D9/1 > D10/0 > D8/2, while nondairy milk shows a range of 5.7 ± 0.4 – 6.3 ± 0.3, and follows a descending order of ND7/3 > ND9/1 > ND8/2 > ND10/0 (Table 4.5).

The value for odour in the different portions of dairy milk shows a range of 6.5 ± 0.5 – 8.4 ± 0.4, and follows a descending order of D7/3 > D10/0 > D9/1 > D8/2, while nondairy milk shows a range of 5.9 ± 0.4 – 7.2 ± 0.3, and follows a descending order of ND10/0 > ND7/3 > ND9/1 > ND8/2 (Table 4.5).

The value for general acceptability in the different portions of dairy milk shows a range of 6.8 ± 0.4 – 7.5 ± 0.2, and follows a descending order of D7/3 > D10/0 > D8/2 > D9/1, while nondairy milk shows a range of 5.7 ± 0.4 – 6.2 ± 0.1, and follows a descending order of ND10/0 > ND7/3 > ND9/1 > ND8/2 (Table 4.5).

Table 4.5: Sensory Score of Dairy + Non-Dairy Yoghurt from milk samples

	SENSORY SCORES OF SAMPLES							
PARAMETER	D10/0	D9/1	D8/2	D7/3	ND10/0	ND9/1	ND8/2	ND7/3
Odour	7.9±0.5^{a}	7.3±0.3^{a}	6.5±0.5^{b}	8.4±0.4^{c}	7.2±0.3^{a}	6.7±0.4^{b}	5.9±0.4	6.9±0.6^{b}
Texture	6.3±0.4^{a}	7.1±0.4^{b}	6.8±0.3^{b}	7.8±0.4^{c}	6.1±0.1^{a}	6.5±0.3^{a}	6.6±0.4^{b}	6.4±0.3^{a}
Color/Appearance	7.7±0.2^{a}	7.9±0.4^{a}	7.7±0.4^{a}	8.2±0.3^{c}	7.1±0.3^{a}	7.3±0.4^{a}	7.0±0.3^{a}	7.1±0.3^{a}
Taste	6.1±0.3^{a}	6.6±0.4^{a}	5.7±0.2^{b}	7.6±0.3^{c}	5.7±0.4^{b}	6.1±0.2^{a}	5.9±0.2^{a}	6.3±0.3^{a}
General Acceptability	7.2±0.3^{a}	6.8±0.4^{b}	6.9±0.4^{b}	7.5±0.2^{c}	6.2±0.1^{d}	5.9±0.3^{d}	5.7±0.4^{e}	6.1±0.3^{d}

Values are means ± SD of triplicate determinations

Values in the same row with the same superscript are not significantly different at 5% level ($p>0.05$).

4.2 DISSCUSION

4.2.1 PHYSICO-CHEMICAL COMPOSITION OF THE YOGHURT

As shown in table 4.1, total solid contents of the dairy samples which ranged from 19.82±0.5 to 21.88±0.6% were relatively higher than that of the non-dairy samples with the range, 18.45±0.5 to 19.83±0.4%. However, the values were similar to the values, 18% and 19.45% obtained by Igbabul *et al*., (2014) and Galeboe *et al*. (2018) respectively for cow milk yoghurts. Akeem, S. A., Yerumoh, O., Leight, O., Bamgbala, K., Okeke, G., Sokunbi, F & Olayiwole.

I (2013), Ahmed, I., Gulzar, M., Shahazad, F., Yaqub, M. & Zhoor, T. (2013) and Akalin, A. S., Unal, G., Dinkci, N. & Hayaloglu, A. A., (2012) on their parts reported much lower values of 13.26%, 10.40% and 14.78% respectively for the same sample. Relative to the values obtained in this work for soymilk yoghurt, lower values, 10.4% and 11.45% were reported by Falade *et al*., (2010) and Abou-Dobara, M. I., Magdy, M. I & Nawal, M. R (2016) respectively working on soymilk yoghurt. As stated by Galeboe *et al.* (2018), the total solid content of yoghurt is influenced by the total solid of material (milk) from which it is produced. Increased solid content improves the nutritional value of yoghurt (Obakeng, G., Eyassu, S. & Sekwatimonang, B, 2018). The addition of adjunct culture of *S. boulardii*, apart from at D7/3 increased the total solid content of both dairy and soy milk yoghurt. This increase became statistically significant ($p<0.05$) at D8/2, ND8/2 and ND7/3 respectively for dairy and non dairy samples.

Total solid non-fat content result (Table 4.1) showed that D8/2(20.28%) had the highest value of TSNF. D9/1, D7/3 and ND9/1 were similar. The dairy samples were fairly higher than the non dairy samples. The result obtained from this study agrees with the result of Igbabul *et al.*, (2014), which reported a total solid non-fat content of 18.31% from cow yoghurt. The result from the study was higher than the values, 14.37% and 11.51% reported by Njoya and Mahbou (2016) and Ekram and El-zubeir (2011) respectively for pineapple flavoured milk. Earl (1988) stated that total solids non-fat is essential to achieve the required texture and viscosity of the final product (Igbabul *et al.*, 2014). Increase in total solid non-fat aids in the production of firmer yoghurt and improves its stability (Galeboe *et al*., 2018). The addition of *S. boulardii* had an increasing effect on the total solids non-fat content of the samples. The increase was significant ($P<0.05$) at D8/2, ND8/2 and ND7/3 for both dairy and non-dairy samples respectively.

Total nitrogen content from table 4.1 shows that in dairy samples, the values ranged from 0.68% to 0.84% and were comparatively lower than the non dairy samples which ranged from 1.3% to 1.4%. Niamah, (2017) reported nitrogen content value of 0.7% for cow yoghurt. The observed increased in percentage of total nitrogen could be as a result of the treatment. Yeast cells contain higher nitrogen content (Czerucka, D., Piche, T. & Rampal P, 2007). The addition of *S. boulardii* could have an increasing effect on the total nitrogen content to the yoghurt samples.

4.2.2 PROXIMATE COMPOSITION OF YOGHURT SAMPLES

The proximate composition of the diary and non-diary yoghurt were evaluated by their moisture content, ash content, protein content, fat content and carbohydrate content.

The moisture content ranged from 78.13% to 80.17% in dairy samples which were fairly lower than the non-dairy samples which ranged from 80.32% to 83.42%. The moisture content observed in the present study was similar to the value of 80.60% reported by Obakeng, G., Eyassu, S. & Sekwatimonang, B (2018) for cow yoghurt. However, the result was lower than that of Santosh, K. K., Parimita, L. W., Smita. M. & Singh, V. (2018) and Akeen *et al.*, (2018) that reported values 86.43% and 86.75% respectively for cow milk yoghurt. Higher values of 85.53% and 88.32% for cow and soy yoghurt respectively were observed (Ukwo 2015). The values from the result corresponded with the standard specification of EAS (2006), which stated the maximum moisture content of yoghurt should be 84%. Lots of water in yoghurt makes it less viscous thereby affecting the texture of the yoghurt and moth feel (Igbabul *et al*., 2014). A spoonful of yoghurt should be able to maintain its form without displaying sharp edges (USDA, 2001). The addition of *S. boulardii,* besides at D7/3 and ND7/3 decreased the moisture content of the yoghurt samples.

Ash content of the diary samples ranged from 0.8% in D10/0 to 1.2% in D8/2 while non- diary samples ranged from 1.2% to 1.6%. The ash content of the non-dairy samples was superior to that of the dairy samples. The result obtained in the present study is similar to the report of Obakeng *et al.* (2018), Njoya and Mahbou (2016), Mohammed and Mohammed (2016), Ehirim and Onyeneke (2013), which had the value of 0.7%, 0.67%, 0.73%, 1.7% respectively for cow milk yoghurt. A higher value of 4.57 was reported by Tona (2016) for the same sample. In respect to the result obtained, Ukwo (2015) and Abou-Dobara *et al.* (2016) reported a lower value of 0.7% and 0.6% respectively for soy milk yoghurt. The ash content is an index of the mineral content of milk or yoghurt, which is needed for bone devilment, teeth formation and

body functions (Bibiana and Joseph, 2014). The ash content in the non-dairy samples is higher than dairy samples from Table 4.2. This result indicates that soy milk is a better source of mineral. The addition of *S. boulardii*, had an increasing effect on the ash content (minerals) of the yoghurts of the dairy samples while it increased ND9/1 and decreased ND7/3 in the non-dairy samples.

Protein content obtained from table 4.2 showed that diary sample ranged from 4.3% to 5.3% while non-dairy samples ranged from 7.9% to 9.2%. The differences in the protein content of the samples were observed to be significant ($P \leq 0.05$). The treatment increased statistically in percentage. The dairy samples were lower than the non-diary samples. The protein content from the table 4.1 is similar to the values, 5.73%, 4.6%, 4.58%, 4.42% and 4.17% obtained by Tona (2016), Ghalem and Benttouche (2013), Nahar, A., Al-Amin, M., Alan, S. M. K., Wadud, A. & Islam, M. N. (2007), Ekram and El-Zubir (2011) and Mohammed and Mohammed (2017) respectively for cow milk yoghurt. Amanze (2011), Santosh, *et al.* (2018) and Abou-Dobara *et* al. (2016) reported lower values of 3.4%, 3.56% and 3.6% respectively for the same sample. In relation to the result, Amanze (2011), Ukwo (2015), Ehirim and Onyeneke (2013), Tang (2013) and Abou-Dobara *et* al. (2016) reported lower values of 2.0%, 2.5%, 3.22%, 3.91% and 3.54% for soymilk yoghurt. The result obtained in table 4.2 show that the protein content of the treatments was within the CODEX (2003) specification which states that the protein content of yoghurt, Alternate culture yoghurt and Acidophilus milk should be at a minimum of 2.7%. The protein content is an index of amino acid content of the yoghurt which is essential for body building and serves as an important fuel sources for skeletal muscles (Tang, 2013). The increases in percentage of the treatment were as a result of increased percentage of *S. boulardii* used in the treatment. *S. boulardii* contains higher protein content (Czeruka *et al*., 2007). Addition *S. boulardii* to the yoghurt increased the protein content of diary and non-diary samples. This increase became statistically significant ($p<0.05$) at D7/3, ND8/2 and ND7/3 respectively for dairy and non dairy samples

Fat content from the result in table 4.2 shows that in the dairy sample, the fat content ranged from2.6% to 1.6% while non-dairy samples ranged from 1.4% to 1.2%. The result showed that the treatment decrease in fat content when compared with the control. The dairy samples had relatively higher fat content than the non-dairy samples. The result obtained in Table 4.2, was similar to the values, 2.6%, 2.3% and 2.7% reported by Ghalem and Benettouche (2013), Amanze (2011) and Igbabul *et al.* (2014) for cow milk yoghurt. The values obtained from the result were much higher than the values, 0.01% and 0.32% reported by Ehirm and Onyeneke (2013) and Akusa and Wordu (2017) for the same sample. Ukwo (2015), Ghoneem *et al.* (2017), Ekram and El-Zubeir (2011), Essa *et al.* (2010), Nahar, A., Al-Amin, M., Alan, S. M.

K., Wadud, A. & Islam, M. N. (2007), Santosh, K. K., Parimita, L. W., Smita. M. & Singh, V, (2018) & Abou-Dabara *et al.* (2018), reported values higher than results from table 4.2, for cow yoghurt. Relative to the result obtained, similar value, 1.3% for soymilk yoghurt was reported by Amanze (2011) while higher values, 3.4%, 7.4%, 2.6%, and 2.82%, were also reported by Jayalalitha, V., Manoharan, A. P., Balasundaran, B & Brand E. A. (2015), Ghoreem, *et al.* (2017), Abou-Dabara *et al.* (2016), and Ukwo (2015) respectively for soymilk yoghurt. According to the International Standard of fat content in yoghurt, USDA (2001) stated a minimum fat content of 3.2%. The CODEX (2003) standard reported that milk fat percentage should be less than 15%. The result observed had a lower fat content from the International Standard. Fat plays an important role in improving the consistency of yoghurt and also provide twice as much energy as same quantity of carbohydrate and protein (Ehirim and Onyeneke, 2013). Soymilk composition varies depending on processing condition and bean variety (Amanze, 2011). Fats serve as a vehicle for soluble vitamins Vit A, D and K and promote their absorption. (Jayalalitha *et al.*, 2015). The quantity and type of fat in the dairy diet influence the level of cholesterol and triglycerides in the blood. The inclusion of *S. boulardii*, asides from ND8/2 caused a significant decrease in the fat content of diary samples and non-dairy samples.

The carbohydrate content in Table 4.2 for diary samples ranged from 14.26% to 11.78% while non-dairy samples ranged from 7.82% to 4.27%. The treatments were observed to be significantly ($P \leq 0.05$) different. The diary samples were superior to non- dairy samples. The carbohydrate content obtained from Table 4.2, were similar to that reported by Ukwo (2015) and Amanze (2011) for soymilk yoghurt. The carbohydrate content falls within the range obtained by Osundahuns *et al.* (2007) for soymilk yoghurt. Igbabul *et.al* (2014) reported a similar value (12.85%) for cow milk yoghurt. Ehirim and Onyeneke (2013) and Amanze (2011) reported lower values in cow milk yoghurt. A lower value of 4.67% was reported by Ukwo (2015) for cow milk yoghurt. The low carbohydrate value is attributed to possess of fermentation which converts carbohydrate basically to simple sugar as an energy source for the organisms (Ehirim and Ndimantang, 2004). *S. boulardii* converts carbohydrates in cow milk and soy milk into simple sugar which aids as a sugar source during the fermentation and storage period. *S. boulardii* showed no significant effect the diary samples (Hattingh and Vilijeon, 2001). The addition of *S. boulardii*, apart from D7/3 and ND7/3 had an increasing effect on the carbohydrate content of both dairy and non dairy samples.

4.2.3. MICRONUTRIENT EVALUATION OF YOGHURT

As shown in table 4.3, the samples contain various amounts of calcium, potassium, phosphorous, magnesium, manganese, sodium, iron and zinc. The highest values (556.11560.54 mg/l and 296-301.01 mg/l) were obtained for potassium in non-dairy and dairy samples respectively, followed by phosphorous (397-401 mg/l) in non dairy samples and calcium (221.34-224.65 mg/l) in dairy samples. The least value (0.004-0.005 mg/g) in iron for dairy samples. The observed values for the mineral concentrations, except iron in dairy samples, were all high. For both samples, while K and Mg were generally increased, Fe and Zn were reduced by the treatment. The treatment reduced Ca and Mn concentrations in the cow milk sample and generally increased them in the soy sample. The results obtained in this study are in line with the Recommended Daily Allowance (RDA) for minerals by the USDA food Composition Databases (USDA, 2019). Minerals perform important functions in the body. It is very essential in muscle contraction, oocyte activation, building strong bones and teeth, blood clothing, nerve impulse and regulating heart beat and fluid balance within cells (Pravina *et al.*, 2015). Potassium is a very significant body mineral, important to both cellular and electrical functions, regulates water and acid-base balance in blood and tissues (Elson, 2011). Phosphorus is a component of nucleotide molecules, its involved in the control of acid-alkaline state of the blood (Anyalogbu, E. A., Onyeike, E. N. and Monanu, M. O, 2014). Phosphorus maintains blood formation, bone, teeth and muscle growth (Anyalogbu*et al.*, 2014). Iron is required for function of the central nervous system. Zinc serves a major function in promoting testosterone and sperm production. With great insight to the functions of some minerals, and considering the USDA recommendation, it is essential that these samples could be valuable in human nutrition. The addition of *S. boulardii* improved the mineral contents of the samples.

The vitamin concentration of the samples is shown in Table 4.4. The samples contained various levels of Vit.A, B_1, B_2, B_3, B_6, B_9, B_{12}, and E as the major vitamins. The highest value (Mg/L) was observed in Vit.B_3 (131.9) non diary samples and B_{12} (36.1) in dairy, followed by Vit.A (90.9) and (32.14) in non-dairy and diary samples respectively. The least values were observed in Vit.B_{12} (4.27) and Vit.B_3 (2.15) in non- dairy and dairy samples respectively. These vitamins are important in function of the body, such as: Vit.A helps form and maintain healthy teeth, skeletal and soft tissue, mucus membranes, and skin, it is also known as a retinol because it produces the pigment in the retina of the eyes (Fennema, 2008). Vit.B_1 serves as a Coenzyme in the catabolism of sugar and amino acids (Whitney, 2011). Vit.B_{12} serves as a coenzyme involved in the metabolism of every cell of the human body, especially affecting DNA synthesis and regulation, but also fatty acid metabolism and amino acid metabolism (Whitney,

2011). B_2 and B_3 are coenzyme involved in energy metabolism (Anyalogbu, E. A., Nweje-Anyalowu, P. C., Nnoli, M. C., Ibeneme, C. S. & Chigbu, S. C, 2018). Vit.E which is an antioxidant protects the body tissue from damage caused by substances called free radicals; which can harm cells, tissue and organs. With understanding of the various vital functions of some vitamins, the incorporation of *S. boulardii* into the samples could have beneficial effects in humans by supplementing the functions of these vitamins.

4.2.5. SHELF LIFE EVALUATION OF YOGHURT

The shelf life of yoghurt was evaluated by pH, titratable acidity, water holding capacity (WHC) and syneresis. The shelf life evaluation determines the quality of the set yoghurt during the 21-day storage time.

The pH changes of the yoghurt samples treated with *S. boulardii* during the 21-day storage time are shown in Fig 4.1. The pH values for dairy samples after 1 day of storage ranged from 4.38 to 4.63 and 4.72 to 4.91 in non-dairy samples. The pH of the nondairy samples was relatively higher than the dairy samples. The pH values ranged from 3.82 to 4.20 and 4.42 to 4.74 for dairy and non-dairy samples respectively. The results obtained in this work are in line with the observation of Amanze (2011) for yoghurt produced with cow milk. LourensHattingh and Viljeon (2011) also observed a decrease in pH of cow milk yoghurt. The reduction in the pH can be due to the breakdown of lactose into lactic acid (Hassan and Amjad, 2010). Niamah (2017) observed a decrease a decrease in pH of cow milk yoghurt within a period of 21 days. The addition of *S. boulardii* decreased pH value of the yoghurt samples during the storage time. This might be attributed to the high metabolic activity of *S. boulardii* and yoghurt starter (*L. bulgaricus* and *S. thermophilus*) during the storage (Adhikari, K., Mustapha, A. & Greun, I. 2000).

The Titratable acidity of the dairy samples after day 1 of storage ranged from 9.27 to 10.98 (fig: 4.2). The non-dairy sample ranged from 7.93 to 8.55. The observed values gradually increased with storage with the dairy being consistently higher than the non-dairy giving the range 10.89 to 12.78 for dairy samples and 9.18 to 10.08 by the 21st day of storage.

The values from this work were higher than values, 0.16 and 0.91 reported by Abou-Dobara *et al.,* (2010) and Igbabul, *et al.,* (2014) respectively for cow milk yoghurt. Ahmad, *et al.,* (2013) also reported a value of 1.18 for unbranded cow milk yoghurt. However, Ukwo (2015) and Abou-Dobara *et al.*, (2010) reported lower values of 1.02 and 0.17 respectively for soy milk

yoghurt. According to Afoakwa, E. O., Kwasikpodo, F. M., Amoa, B. B., Badu, A. S. & Saalia, F. K., (2014) cow milk contains lactose which is an ideal substrate for yoghurt starter culture. The micro-organism efficiently utilizes the lactose in cow milk to release more lactic acid which results in higher acidity in the yoghurt produced from cow milk than in soy milk (Ukwo, 2015). Donkor, O. N., Henriksson, A., Vasiljevic, T. & Shah N. P, (2007) observed that the starter culture produces lower amount of organic acids in soy milk than in cow milk even if they grow well. The addition of different percentage of *S. boulardii* increased the titratable acidity of the yoghurt during the storage period of 21 days.

One of the most important structural characteristics of set type yoghurt is the strength of coagulum and its stability during storage. The water holding capacity (WHC) values in the yoghurt samples supplemented with various percentage of *S. boulardii* are presented in Fig 4.3. After Day 1 of storage, the samples ranged from 59% to 68% for dairy samples and 49% to 57% for non-dairy samples. The highest WHC value was observed with D7/3, followed by

D8/2 while ND10/0 had the least value. These values declineduring the 21-day storage

The WHC of the dairy and non-dairy samples were affected by the increased percentage of *S. boulardii*. At 21 days of storage, D7/3 (61%) had the best WHC while ND10/0(46%) had the least WHC. The observed decline in WHC with storage time was agreed with the report by Niamah (2017) for cow milk yoghurt. Ukwo (2015) observed a decrease in the WHC of cow yoghurt but with an increase when substituted with soy milk. Akalin *et al.*, (2012) reported a decrease in WHC during storage period on probiotic yoghurt fortified with skim milk. Yang and Li (2010) and Kumari, A. G., Ranadheera, C. S, Prasanna, P. H., Senevirathne, N. D. & Vidanarachchi, J. K, (2015) also reported decrease in WHC during storage. The water holding capacity is an important parameter in yoghurt production since it is related to syneresis which is due to intrinsic instability in protein gel (Afoakwa *et al.*, 2014). Kumari *et al.*, (2015) reported that the reduction of WHC was due to the irresolute gel network of yoghurt production. The addition of *S. boulardii* increased the WHC of the samples. High values were observed in non-dairy than dairy yoghurt. Syneresis generally increased with storage time but decreased progressively with the addition of *S. burlardii*

Akalin, A. S., Unal, G., Dinkci, N. & Hayaloglu, A. A. (2012) also reported an increase in syneresis during the storage period. Obakeng *et al.*, (2018) reported higher values of syneresis in cow and camel milk yoghurt during storage period. Also higher values of syneresis in cow and soymilk yoghurt were reported by Ukwo (2015). Domagala (2009) and Hemamali (2016) observed a decrease in syneresis of cow milk yoghurt after 14 days of storage. Syneresis is a defect in yoghurt. It is defined as the separation of whey from the coagulum in yoghurt and is

related to shrinkage of the gel (Sahan *et al.*, 2008). This quality defect occurs in yoghurt due to low total solid, over acidification, mechanical shaking of the gel, insufficient denaturation of the whey protein, incompatibility of dairy and non dairy ingredients, too high incubation temperature or too low acidification (pH>4.6) (Chandan, 2013). Syneresis can limit the shelf life and acceptability of yoghurt because of the undesirable appearance it causes (Obakeng *et al.*, 2018). When high degree of syneresis occurs in yoghurt, it could reduce the gel matrix leading to a suspension of yoghurt materials in whey within a short period of time (Habtegebriel and Admassu, 2016). Yoghurt with high level of syneresis is not liked by consumers.

4.2.6. TOTAL COUNTS AND VIABILITY OF MICROORGANISMS IN YOGHURT

From fig 4.5, the viable count of *Lactobacillus delbruecki subsp. bulgaricus* though higher in dairy sample decreased with storage in both dairy and non-dairy yoghurt. The inclusion of *S. boulardii* increased the microbial count in a dose-dependent manner in both dairy and nondairy samples. Day 1 recorded the highest growth of 8.62 logcfu/ml while the least growth was 7.93logcfu/ml at day 21 for D10/0. D7/3 had the highest growth of 8.67 logcfu/ml and least growth of 8.31 logcfu/ml at day 1 and day 21 respectively. Non-dairy sample had the highest growth of 7.59 logcfu/ml at day 1 while least growth was at 6.86 logcfu/ml for day 21 for ND10/0 samples. ND7/3 samples had the highest growth at day 1 while least growth was recorded at day 21. The result obtained were similar to the result observed by Niamah (2017), that the viability counts of the *L. bulgaricus* decreased with increase in days on cow milk yoghurt. Falade *et al.,* (2015) also reported a decrease in *L. bulgaricus* after a period of 9 days. Arioui *et al..,* (2016) reported an increase in cell count of *L. bulgaricus* during 21 days of storage time in cow milk yoghurt with added pectin. Ahmad, *et al.,* (2013) recorded lower values (1.15 Log cfu/ml) in cow milk yoghurt. Supavititpatana, P., Wirjantoro, T. & Raviyam, P. (2010) reported that the cell count of *L. bulgaricus* decreased in corn milk but increased in cow milk yoghurt during storage. Rekha and vijayalakshmi (2010) reported an increase in *L. bulgaricus* cell count for soymilk yoghurt. The addition of *S. boulardii* showed synergistic effect by enhancing the growth and viability of *L. bulgaricus.*

The cell count of *S. thermophilus* is presented in Fig 4.6. Though higher in dairy sample decreased with storage in both dairy and non-dairy yoghurt. The inclusion of *S. boulardii* increased the microbia count in a dose-dependent manner in both dairy and non-dairy samples. Day 1 recoreded the highest growth of 8.47 logcfu/ml while the least growth was 8.00 logcfu/ml at day 21 for D10/0. D7/3 had the highest growth of 8.79 logcfu/ml and least growth

of 8.54 logcfu/ml at day 1 and day 21 respectively. Non-dairy sample had the highest growth of 8.4 logcfu/ml at day 1 while least growth was at 7.93 logcfu/ml for day 21 for ND10/0 samples. ND7/3 samples had the highest growth at day 1 while least growth was recorded at day 21. The results obtained, were similar to observations of Harackova, S., Slukova, M., Muhlhansova, A., Schulzova, V. & Plockova, M. (2015) that reported values (8.20 Log cfu/ml) in cow milk yoghurt after fermentation. Ahmad, *et al.,* (2013) reported a low value of 6.06 Log cfu/ml in cow milk yoghurt. Arrioui, F., Saada, D. A & Cheriguene, A, (2016) and Niamah (2017) observed a decrease in cell count of *S. thermophilus* after 21 days of storage. Hemamali, K. K., Jayamanne, S. V & Amarathunge, S. M. (2016) observed an increase in cell count in cow milk yoghurt during storage. Horackova *et al.,* (2015) that reported similar value of 8.08Log cfu/ml in soymilk yoghurt after fermentation. Ghoneem *et al.,* (2017) reported a decrease in cell count of *S. thermophilus* in cow and soymilk yoghurt during storage time. The result obtained were satisfactory with the standards of USDA (2001), EAS (2006), CODEX (2003) and Kenya Standard (2011) regulations of yoghurt having a minimum of 10^7cfu/ml cell count per organism. The slow rate of growth of *S. thermophilus* may be partly due to the presence of sorbic acid; these salts are known to offer antimicrobial activity (Ifediba and Ozoh, 2018). Niamah (2017) reported that decrease in lactic acid bacteria during the storage period was as an increase of pH and acidity. *S. thermophilus* is essential in yoghurt due to its curdling of milk properties. The addition of *S. boulardii* increased the shelf life of *S. thermophilus* with its increase in percentage.

The cell count for *S. boulardii* as presented in fig 4.7 followed the same trend as D9/1 had the highest growth (6.10 logcfu/ml) while the least growth (5.84 logcfu/ml) was recorded at day 21. D7/3 had the highest growth of 6.61 logfu/ml at day 1 while least growth (6.09) was recorded at day 21. Non-day sample ND9/1 had the highest growth of 6.05 logcfu/ml at day 1 while day 21 had the lowest growth (5.88 logcfu/ml). ND7/3 had lowest growth (6.03 logcfu/ml) at day 21 while highest growth (6.33 logcfu/ml) at day 1. The results were similar to report of (Hattingh and Vilijeon, 2001), that observed the decrease of *S. boulardii* in cow yoghurt but increased. Niamah (2017) observed an increase in *S. boulardii* in cow milk yoghurt during the storage period. Rekha and Vijayalakshmi (2010) recorded an increase in *S. boulardii* in soy milk yoghurt during the storage period. The results obtained in this work are in accordance with the Codex (2003) standard of minimum. As stated by Hatting and Vijeon (2001) *S. boulardii* does not ferment or assimilate lactose which is the major sugar in milk, however, *S. boulardii* produced small amount of lactic acid and alcohol. According to Fleet and Mian (1987), other milk components such as amounts of galactose any glucose present in milk where utilized by *S. boulardii*. Harackova, *et al*., (2015) explained that the growth of

S.boulardii in soy milk yoghurt was as a result of its ability to utilize sucrose, the main sugar in soya milk.The incorporation of *S.boulardi* showed a synergistic effect by enhanced growth and cells viability of lactic acid bacteria in yoghurt.

It is worth noting that pathogenic bacteria such as *Salmonella*, Coliforms, *E. coli* and *Feacal Enterococci* were not detected. Eissa *et al.*, (2010) stated that the presence of coliform 5 in yoghurts suggests unsanitary condition during processing. This is in agreement with study of Obakeng *et al.*, (2018) that observed the absence of coliforms in yoghurts from cow and carmel milk and also the study of Sengupta, S., Bhowal, J. and Bhattacharyya, D. K. (2013), where absence of coliform, *Escherichia coli* and *Salmonella spp* were reported in fresh and fortified soy yoghurt during the 7 days of storage. The absence of *enterobacteria* signifies the degree of safety of the yoghurt samples as the presence of coliform in food is an indication of fecal pollution, which is of public health concern (Farinde, E. O., Adesetan, T. O., Obatolu, V. A. and Oladapo, M. O. 2009). Yoghurt produced under good manufacturing practices should have a shelf-life of 3-4 weeks at 5^{o}C (Ledenbach and Marshall, 2009).

4.2.7 SENSORY EVALUATION OF YOGHURT

The sensory evaluation of yoghurt was evaluated by the odour/aroma, texture, color/appearance, taste/sourness and general acceptance.

The result of Aroma/Odour evaluation in Table 4.6 showed that dairy samples ranged from 6.5 to 8.4 while non-dairy samples ranged from 5.9 to 7.2. The dairy samples were fairly higher than the non-dairy samples. The result revealed that D7/3 (8.4) had the best result from the panelist because of its strong creamy aroma while ND8/2 (5.9) was least favoured because of its strong beany and less creamy aroma. The result obtained in this work was comparable to the value of 8.5 reported unanimously by Ukwo (2015) and Abou-Doubara *et al.*, (2016) for cow milk yoghurt. Lower odour values for cow milk yoghurt were also observed (Ehirim and Onyeneke, 2013; Mohammed and Mohammed, 2017). Abou-Doubara *et al.*, (2016) and Ukwo (2015) reported the values of 7.5 and 5.4 for soy milk yoghurt respectively. The addition of sucrose to soy milk before fermentation could remove the usual beany aroma detected in the non-dairy product (Favaro-Trinidade, 2001). The addition of *S. boulardii*, except D7/3, decreased the aroma of both dairy and nondairy samples.

From the result in Table 4.6, the texture of the dairy samples which ranged from 6.3 to 7.8 was similar to that of the nondairy samples which ranged from 6.1 to 6.6. These values were higher than the values (2.88) and (2.20) reported by Mohammed and Mohammed (2017) and Ehirim

and Onyeneke (2013) for cow milk yoghurt. Ukwo (2015) reported a higher value of 8.10 for cow milk yoghurt and lower value of 5.6 for soy milk yoghurt. The firmness of fermented milk is highly dependent on the culture composition, total solid and protein content of the product and also on the type of protein (Oliverira *et al.*, `2001). USDA (2001) stated that, whole milk yoghurt should possess a firm, custard –like body with smooth and homogenous texture. The observed result shows that that the treatments increased in the sensory parameter which proves that *S. boudardii* had a positive effect on both dairy and non dairy samples.

The colour/appearance of the samples ranged from 7.1 (ND10/0) to 8.2 (D7/3). The dairy samples were comparatively higher than the non-dairy samples. Igbabul *et al.,* (2014) reported similar values for cow milk yoghurt. Favaro Trindade, C. S., Terzi, S.C., Trugo, R. C. & Della, M. S. (2001) reported similar values of colour/appearance for soy milk yoghurt. The values obtained were higher than the value of 5.2 reported by Ehirim and Onyeneke (2013) for cow yoghurt. The appearance of dairy samples was observed to be creamy to white while non-dairy samples appeared brownish to deep brown. USDA (2001) stated that yoghurt should present a clean and smooth appearance. *S. boulardii* had relatively little effect on the colour/appearance of the yoghurt samples. The addition of adjunct culture of *S. boulardii* increased the colour of the dairy samples while the non dairy samples had slight change in colour.

The taste of the dairy samples ranged from 5.7 to 7.6 while non-dairy samples ranged from 5.7 to 6.3. The dairy samples were fairly higher (except D8/2) than the non dairy samples. The obtained taste values were higher than the value (3.7) reported by Ehirim and Onyeneke for cow milk yoghurt on a 5-point hedonic scale. Amanze (2011) and Ukwo (2015) reported 4.45 and 5.35 for soy milk yoghurt respectively on a 9-point hedonic scale. Jayalialitha *et al.* (2015) reported value (8.52) for soy milk yoghurt on 9-point hedonic scale. The USDA (2001) stated that whole milk yoghurt should possess a pleasant, clean acid flavour, it should be free from undesirable flavours such as; bitter, rancid, oxidized, stale, yeast and unclean. Polyphenotics for example, phytates in soy milk interact with mucoprotein in the mouth and could contribute to its astringent taste (Ukwo, 2015). The non-dairy samples possessed a strong sour taste which is associated with the raw material it was produced from. The addition of adjunct culture of *S. boulardii,* except at D8/2 had an increasing effect on the taste of dairy and non-dairy samples.

As also indicated in table 4.3, the panelist generally accepted D7/3 (7.50), followed by D10/0 (7.2) and least accepted ND8/2 (5.7). The non-diary samples had least acceptance because of its strong beamy aroma and taste when compared to the diary sample. Trindade, *et.al* (2001) also reported the same reason.

CHAPTER FIVE

5.0 CONCUSION AND RECOMMENDATION

5.1 CONCLUSION

The results obtained from the present study show that incorporation of probiotic yeast *Saccharomyces boulardii* improved the proximate, sensory, nutritional and shelf life of the yoghurt. *Saccharomyces boulardii* showed a synergistic effect by the enhanced growth and cell viability of lactic acid bacteria (*L. bulgaricus and S. thermophilus*) in fermentation which increases its probiotic effectiveness.

5.2 RECOMMENDATION

The probiotic yeast *Saccharomyces boulardii* can be incorporated into dairy foods to develop therapeutic and functional foods.

Soymilk should be fortified with thickeners and sucrose to enable microbial growth of organisms and better texture for lactose intolerant individuals.

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APPENDIX I

Morphological and Biochemical Characteristics of Organisms

PARAMETERS	ISOLATES		
	YS(M17)	YS(MRS)	FN(PDA)
Colony Shape	Circular	Circular	Circular
Elevation	Flat	Raised	Flat
Optical Characteristics	Opaque	Opaque	Opaque
Pigmentation	Creamy White	White	Cream
Colony Surface	Dry	Smooth	Smooth
Gram Stain	+	+	+
Cellular Morphology	Single rod with round	Cocci in short chains	Ellipsoid
Catalase	_	_	_
Indole	_	_	+
Triple Sugar Ion	+	+	+
Methyl Red	_	_	+
Voges Proskauer	_	_	NO
Nitrate Reduction	_	+	_
Citrate Utilization	_	+	+
Urease Production	+	+	_
Starch Hydrolysis	_	+	+
Motility	_	_	+
Oxidase	_	_	+
Acid production	+	+	+
Oxygen Relationship	FA	FA	FA
Lactose	+	+	+
Maltose	+	+	+
Mannitol	+	+	No
Sucrose	+	+	+
Glucose	+	+	+
Fructose	+	+	+

Probable Organism	*S. thermophilus*	*L. bulgaricus*	*S. boulardii*

Key: + = positive; - = negative; FA = facultative anaerobe; No = not observed; YS(M17) =Yoghurt Starter inoculated in M17; YS(MRS)= Yoghurt starter inoculated in MRS; FN(PDA)= Flora norm inoculated in PDA.

APPENDIX II

Physicochemical evaluation

	SAMPLES							
PARAMETER	D10/0	D9/1	D8/2	D7/3	ND10/0	ND9/1	ND8/2	ND7/3
Total Solid	19.84	19.96	21.88	19.82	18.45	18.93	19.68	19.83
Total Solid Non-Fat	17.24	17.96	20.28	17.83	17.05	17.73	18.28	18.63
Total Nitrogen	0.68	0.7	0.77	0.84	1.3	1.4	1.40	1.4

Anova: Two-Factor Without Replication

SUMMARY	*Count*	*Sum*	*Average*	*Variance*
Total Solid	8	158.39	19.79875	0.99266964
Total Solid Non-Fat	8	145	18.125	1.01888571
Total Nitrogen	8	8.49	1.06125	0.11584107
D10/0	3	37.76	12.5866667	108.016533
D9/1	3	38.62	12.8733333	112.142533
D8/2	3	42.93	14.31	138.1387
D7/3	3	38.49	12.83	108.8101
ND10/0	3	36.8	12.2666667	90.6908333
ND9/1	3	38.06	12.6866667	95.9016333
ND8/2	3	39.36	13.12	103.5088
ND7/3	3	39.86	13.2866667	106.329633

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*
Rows	1720.17843	2	860.089213	1745.33409
Columns	7.99266667	7	1.14180952	2.3170144
Error	6.89910833	14	0.49279345	
Total	1735.0702	23		

If P-values ≤ the alpha levels selected (0.05), we REJECT the NULL hypothesis
If P-values ≥ the alpha levels selected (0.05), we ACCEPT the NULL hypothesis.

APPENDIX III

Proximate Evaluation

% OF SAMPLES

PARAMETER	D10/0	D9/1	D8/2	D7/3	ND10/0	ND9/1	ND8/2	ND7/3
Moisture	80.16	80.04	78.13		80.17	82.55	81.07	80.32 83.42
Ash	0.8	1	1.2		0.9	1.4	1.6	1.4 1.2
Protein	4.3	4.4	4.8		5.3	7.9	8.8	9.1 9.2
Fat	2.6	2	1.6		2	1.4	1.2	1.4 1.2
Carbohydrate	12.14	12.56	14.26		11.78	6.75	7.38	7.82 4.27

Anova: Two-Factor Without Replication

SUMMARY	*Count*	*Sum*	*Average*	*Variance*	
Moisture	8	645.86	80.7325	2.672392857	
Ash	8	9.5	1.1875	0.075535714	
Protein	8	53.8	6.725	4.925	
Fat	8	13.4	1.675	0.239285714	
Carbohydrate	8	76.96	9.62	12.32945714	
D10/0	5	100	20	1149.7238	
D9/1	5	100	20	1147.1288	
D8/2	5	99.99	19.998	1083.77112	
D7/3	5	100.15	20.03	1148.2232	
ND10/0	5	100	20	1231.59875	
ND9/1	5	100.05	20.01	1176.5622	
ND8/2	5	100.04	20.008	1149.39612	
ND7/3	5	99.29	19.858	1273.23712	

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Rows	37296.9731	4	9324.24329	1843.890211	1.8306E-33	2.7140758
Columns	0.1004	7	0.01434286	0.002836332	0.99999999	2.35925985
Error	141.5913	28	5.05683214			
Total	37438.6648	39				

If P-values ≤ the alpha levels selected (0.05), we REJECT the NULL hypothesis

If P-values ≥ the alpha levels selected (0.05), we ACCEPT the NULL hypothesis

APENDIX IV

ANOVA
SENSORY EVALUATION

SAMPLES

PARAMETER	D10/0	D9/1	D8/2	D7/3	ND10/0	ND9/1	ND8/2	ND7/3
Odour	7.9	7.3	6.5	8.4	7.2	6.7	5.9	6.9
Texture	6.3	7.1	6.8	7.8	6.1	6.5	6.6	6.4
Color/Appearance	7.7	7.9	7.7	8.2	7.1	7.3	7	7.1
Taste 6.1	6.6		5.7	7.6	5.7	6.1	5.9	6.3
General Acceptability	7.2	6.8	6.9	7.5	6.2	5.9	5.7	6.1

Anova: Two-Factor Without Replication

SUMMARY	*Count*	*Sum*	*Average*	*Variance*	*S.Deviation*
Odour	8	56.8	7.1	0.625714286	0.79102104
Texture	8	53.6	6.7	0.291428571	0.539841247
Color/Appearance	8	60	7.5	0.191428571	0.437525509
Taste	8	50	6.25	0.388571429	0.623354978
General Acceptability	8	52.3	6.5375	0.425535714	0.652330985
D10/0	5	35.2	7.04	0.658	0.811171992
D9/1	5	35.7	7.14	0.253	0.502991054
D8/2	5	33.6	6.72	0.522	0.722495675
D7/3	5	39.5	7.9	0.15	0.387298335
ND10/0	5	32.3	6.46	0.433	0.658027355
ND9/1	5	32.5	6.5	0.3	0.547722558
ND8/2	5	31.1	6.22	0.307	0.554075807
ND7/3	5	32.8	6.56	0.178	0.421900462

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*

Rows	7.679	4	1.91975	15.24907801	9.86549E-07	2.714075804
Columns	9.93375	7	1.419107143	11.27234043	1.04922E-06	2.359259855
Error	3.525	28	0.125892857			
Total	21.13775	39				

If P-values ≤ the alpha levels selected (0.05), we REJECT the NULL hypothesis

If P-values ≥ the alpha levels selected (0.05), we ACCEPT the NULL hypothesis

APPENDIX V

MINERAL EVALUATION

ANOVA

PARAMETERS (mg/100g)	SAMPLE							
	D10/0	D9/1	D8/2	D7/3	ND10/0	ND9/1	ND8/2	ND7/3
Calcium	224.65	218.71	224.05	221.34	186.95	192.04	190.84	187.98
Potassium	296.76	301.01	300.78	299.23	556.11	560.54	557.32	559.43
Phosphorous	194.1	192.59	193.05	193.76	397.67	401.06	392.16	398.77
Magnesium	7.89	8.14	7.92	7.79	154.44	162.98	157.81	154.98
Manganese	0.27	0.21	0.22	0.25	3.12	3.97	3.54	3.71
Sodium	125.34	122.52	126.53	124.23	7.41	7.25	7.82	7.35
Iron	0.005	0.004	0.005	0.005	18.15	17.02	17.76	17.63
Zinc	0.42	0.34	0.39	0.32	2.16	2.07	2.11	2.04

Anova: Two-Factor Without Replication

SUMMARY	*Count*	*Sum*	*Average*	*Variance*
Calcium	8	1646.56	205.82	311.7937
Potassium	8	3431.18	428.8975	19155.3
Phosphorous	8	2363.16	295.395	11901.26
Magnesium	8	661.95	82.74375	6402.384
Manganese	8	15.29	1.91125	3.256555
Sodium	8	528.45	66.05625	3925.631
Iron	8	70.579	8.822375	88.95187
Zinc	8	9.85	1.23125	0.854698
D10/0	8	849.435	106.179375	14541.3
D9/1	8	843.524	105.4405	14524
D8/2	8	852.945	106.618125	14724.1
D7/3	8	846.925	105.865625	14558.09
ND10/0	8	1326.01	165.75125	43830.38
ND9/1	8	1346.93	168.36625	44582.97
ND8/2	8	1329.36	166.17	43601.82
ND7/3	8	1331.89	166.48625	44282.3

Source of Variation	*SS*	*Df*	*MS*	*F*	*P-value*	*F crit*
Rows	1408915.38	7	201273.6257	42.21933	1.3001E-18	2.203231588

Columns	58926.75436	7	8418.107765	1.76579	0.11576464	2.203231588
Error	233599.3174	49	4767.333009			
Total	1701441.452	63				

If P-values ≤ the alpha levels selected (0.05), we REJECT the NULL hypothesis

If P-values ≥ the alpha levels selected (0.05), we ACCEPT the NULL hypothesis

APPENDIX VI

VITAMIN EVALUATION

ANOVA

PARAMETERS (mg/L)	SAMPLES							
	D10/0	D9/1	D8/2	D7/3	ND10/0	ND9/1	ND8/2	ND7/3
Vitamin A	30.126	28.38	29.45	32.143	90.917	82.31	85.941	89.707
Vitamin B1	2.439	2.21	2.317	2.118	8.763	8.125	8.328	9.061
Vitamin B2	19.07	19.85	18.752	19.51	36.851	39.116	37.239	36.913
Vitamin B3	2.15	3.6	3.05	2.45	126.94	129.55	128.95	131.9
Vitamin B6	12.012	12.81	12.982	12.489	17.3	17.3	17.3	23.3
Vitamin B9	12.68	11.616	11.819	13.027	24.032	24.396	25.357	27.117
Vitamin B12	35.91	36.117	35.23	35.124	4.27	4.59	4.916	5.82
Vitamin E	3.869	4.276	3.244	4.177	12.74	14.15	10.989	12.74

Anova: Two-Factor Without Replication

SUMMARY	*Count*	*Sum*	*Average*	*Variance*	*S.Deviation*
Vitamin A	8	468.974	58.62175	942.2053	30.695363
Vitamin B1	8	43.361	5.420125	11.41833	3.3791016
Vitamin B2	8	227.301	28.41263	95.58818	9.7769209
Vitamin B3	8	528.596	66.0745	4575.768	67.64442
Vitamin B6	8	125.493	15.68663	15.01287	3.8746439
Vitamin B9	8	150.044	18.7555	48.8523	6.9894419
Vitamin B12	8	161.977	20.24713	269.5123	16.416829
Vitamin E	8	66.185	8.273125	22.75189	4.7698944
D10/0	8	118.256	14.782	163.3314	12.780116
D9/1	8	118.859	14.85738	153.3011	12.381482
D8/2	8	116.844	14.6055	155.2776	12.461045
D7/3	8	121.038	15.12975	167.121	12.927528

ND10/0	8	321.819	40.22738	1989.63	44.605266
ND9/1	8	319.537	39.94213	1929.568	43.926846
ND8/2	8	319.02	39.8775	1976.504	44.457892
ND7/3	8	336.558	42.06975	2029.303	45.047781

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Rows	28663.44	7	4094.777	6.413468	2.248E-05	2.203232
Columns	10582.95	7	1511.851	2.367944	0.0362828	2.203232
Error	31284.81	49	638.4654			
Total	70531.2	63				

If P-values ≤ the alpha levels selected (0.05), we REJECT the NULL hypothesis

If P-values ≥ the alpha levels selected (0.05), we ACCEPT the NULL hypothesis **APPENDIX VII**

pH EVALUATION

DAY1	4.63	4.5	4.45	4.38	4.91	4.87	4.81	4.72
DAY7	4.47	4.31	4.24	4.18	4.83	4.77	4.72	4.68
DAY14	4.27	4.21	4.11	3.97	4.79	4.7	4.64	4.5
DAY21	4.2	4.16	4.04	3.82	4.74	4.63	4.58	4.42

Anova: Two-Factor Without Replic ation

SUMMARY	*Count*	*Sum*	*Ave rage*	*Va riance*	
SAMPLES					
Days	D10/0	D9/1	D8/2	D7/3	ND10/0 ND9/1 ND8/2 ND7/3
					S. Deviation
	1	8	37.27	4.65875	0.040241071 0.200601773
	7	8	36.2	4.525	0.066371429 0.257626529
	14	8	35.19	4.39875	0.090241071 0.300401517
	21	8	34.59	4.32375	0.102626786 0.320354157
D10/0		4	17.57	4.3925	0.038158333 0.195341581
D9/1		4	17.18	4.295	0.022566667 0.150222058
D8/2		4	16.84	4.21	0.032466667 0.18018509
D7/3		4	16.35	4.0875	0.059825 0.244591496

ND10/0	4	19.27	4.8175	0.005158333	0.071821538
ND9/1	4	18.97	4.7425	0.010491667	0.102428837
ND8/2	4	18.75	4.6875	0.009958333	0.099791449
ND7/3	4	18.32	4.58	0.020533333	0.143294568

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Rows	0.519559375	3	0.173186458	46.67761601	1.82494E-09	3.072467001
Columns	2.018446875	7	0.288349554	77.7166406	1.40076E-13	2.487577704
Error	0.077915625	21	0.003710268			
Total	2.615921875	31				

If P-values ≤ the alpha levels selected (0.05), we REJECT the NULL hypothesis

If P-values ≥ the alpha levels selected (0.05), we ACCEPT the NULL hypothesis

APPENDIX VIII

TITRATABLE ACIDITY EVALUATION

Days	D10/0	D9/1	D8/2	D7/3	ND10/0	ND9/1	ND8/2	ND7/3
DAY1	9.27	9.81	10.26	10.98	7.92	8.1	8.45	8.55
DAY7	9.99	10.62	11.25	11.61	8.1	8.46	8.92	8.91
DAY14	10.44	11.07	11.7	12.24	8.64	8.91	9.27	9.63
DAY21	10.89	11.61	12.33	12.78	9.18	9.54	9.72	10.08

Anova: Two-Factor Without Replication

SUMMARY	*Count*	*Sum*	*Average*	*Variance*	
1	8	73.34	9.1675	1.213421429	
7	8	77.86	9.7325	1.757535714	
14	8	81.9	10.2375	1.78605	
21	8	86.13	10.76625	1.830455357	
D10/0	4	40.59	10.1475	0.477225	
D9/1	4	43.11	10.7775	0.579825	
D8/2	4	45.54	11.385	0.7587	
D7/3	4	47.61	11.9025	0.606825	
ND10/0	4	33.84	8.46	0.324	
ND9/1	4	35.01	8.7525	0.385425	
ND8/2	4	36.36	9.09	0.289266667	
ND7/3	4	37.17	9.2925	0.477225	

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Rows	11.24673438	3	3.748911458	175.4401903	5.03622E-15	3.072467001
Columns	45.66349688	7	6.523356696	305.277666	1.1571E-19	2.487577704
Error	0.448740625	21	0.021368601			
Total	57.35897188	31				

If P-values ≤ the alpha levels selected (0.05), we REJECT the NULL hypothesis

If P-values ≥ the alpha levels selected (0.05), we ACCEPT the NULL hypothesis

APPENDIX XI

WATER HOLDING CAPACITY EVALUATION

Days	D10/0	D9/1	D8/2	D7/3	ND10/0	ND9/1	ND8/2	ND7/3
DAY1	59	63	65	68	49	54	56	57
DAY7	57	60	62	66	48	53	55	57
DAY14	56	57	61	63	47	51	54	56
DAY21	50	52	57	61	46	51	53	54

Anova: Two-Factor Without Replication

SUMMARY	*Count*	*Sum*	*Average*	*Variance*
DAY1	8	471	58.875	38.69642857
DAY7	8	458	57.25	30.78571429
DAY14	8	445	55.625	26.26785714
DAY21	8	424	53	20.57142857
D10/0	4	222	55.5	15
D9/1	4	232	58	22
D8/2	4	245	61.25	10.91666667
D7/3	4	258	64.5	9.666666667
ND10/0	4	190	47.5	1.666666667
ND9/1	4	209	52.25	2.25
ND8/2	4	218	54.5	1.666666667
ND7/3	4	224	56	2

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Rows	150.625	3	50.20833333	23.49582173	6.54623E-07	3.072467001
Columns	769.375	7	109.9107143	51.43454039	8.39904E-12	2.487577704
Error	44.875	21	2.136904762			
Total	964.875	31				

If P-values ≤ the alpha levels selected (0.05), we REJECT the NULL hypothesis

If P-values ≥ the alpha levels selected (0.05), we ACCEPT the NULL hypothesis

APPENDIX X

SYNERESIS EVALUATION

Days	D10/0	D9/1	D8/2	D7/3	ND10/0	ND9/1	ND8/2	ND7/3
DAY1	11	8	7	4	34	32	30	27
DAY7	13	9	8	5	35	33	30	29
DAY14	14	10	10	7	35	34	32	30
DAY21	14	12	11	8	36	35	32	30

Anova: Two-Factor Without Replication

SUMMARY	*Count*	*Sum*	*Average*	*Variance*
DAY1	8	153	19.125	161.8392857
DAY7	8	162	20.25	159.0714286
DAY14	8	172	21.5	150.2857143
DAY21	8	178	22.25	144.2142857
D10/0	4	52	13	2
D9/1	4	39	9.75	2.916666667
D8/2	4	36	9	3.333333333
D7/3	4	24	6	3.333333333
ND10/0	4	140	35	0.666666667
ND9/1	4	134	33.5	1.666666667
ND8/2	4	124	31	1.333333333
ND7/3	4	116	29	2

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Rows	45.59375	3	15.19791667	51.84263959	6.99637E-10	3.07246700
Columns	4301.71875	7	614.53125	2096.269036	2.10775E-28	2.48757770
Error	6.15625	21	0.293154762			
Total	4353.46875	31				

values ≤ the alpha levels selected (0.05), we REJECT the NULL hypothesis

If P-

If P-values ≥ the alpha levels selected (0.05), we ACCEPT the NULL hypothesis

APPENDIX XI

L. bulgaricus VIABILITY COUNT (Log cfu/ml)

% OF SAMPLES

SUMMARY	*Count*	*Sum*	*Average*	*Variance*			
DAYS D10/0	D9/1	D8/2	D7/3	ND10/0	ND9/1	ND8/2	ND7/3
DAY1 8.6215	8.67	8.7577	8.8333	7.5924	7.6252	7.7158	7.7987
DAY7 8.3036	8.6086	8.71	8.7912	7.4461	7.5462	7.6311	7.7073
DAY14 8.1007		8.6543	8.75	7.0534	7.4951	7.5207	7.5812
8.5576							
DAY21 7.938	8.3141	8.5753	8.6246	6.8624	7.2817	7.3614	7.4586

Anova: Two-Factor Without Replication

			S. Deviation		
DAY1	8	65.6146	8.201825	0.3151	0.5613374
DAY7	8	64.7441	8.093013	0.322565	0.5679479
DAY14	8	63.713	7.964125	0.40821	0.6389129
DAY21	8	62.4161	7.802013	0.431574	0.6569429
		32.9638			
D10/0	4	8.24095		0.086731	0.2945008
D9/1	4	34.1503	8.537575	0.024308	0.1559092
D8/2	4	34.6973	8.674325	0.006144	0.0783816
D7/3	4	34.9991	8.749775	0.00812	0.0901134
ND10/0	4	28.9543	7.238575	0.114685	0.3386523
ND9/1	4	29.9482		0.021606	0.1469893
		7.48705			
ND8/2	4		30.229	0.023428	0.1530632
			7.55725		
ND7/3	4	30.5458		0.022009	0.1483554
		7.63645			

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*

Rows	0.71153	3	0.237177	23.76697	5.972E-07	3.072467
Columns	10.13257	7	1.447511	145.052	2.496E-16	2.487578
Error	0.209564	21	0.009979			
Total	11.05367	31				

If P-values ≤ the alpha levels selected (0.05), we REJECT the NULL hypothesis

If P-values ≥ the alpha levels selected (0.05), we ACCEPT the NULL hypothesis

APPENDIX XII

S, thermophilus VIABILITY COUNT (Log cfu/ml)

% OF SAMPLES

DAYS D10/0 D9/1 D8/2 D7/3 ND10/0 ND9/1 ND8/2 ND7/3

SUMMARY	*Count*	*Sum*	*Average*				
DAY1	8.4789	8.6034	8.7993	8.4033	8.5889	8.6432	8.6947
	8.7158						
DAY7	8.3758	8.5659	8.7057	8.2307	8.5042	8.5425	8.6097
	8.598						
DAY14	8.2098	8.4661	8.6033	8.0004	8.4342	8.4768	8.5189
	8.4901						
DAY21	8.0098	8.3247	8.5407	7.936	8.2146	8.3038	8.3803
	8.4223						

Anova: Two-Factor Without Replication

				Variance	*S. Deviation*
DAY1	8	68.9275	8.615938	0.016489	0.12841
DAY7	8	68.1325	8.516563	0.022267	0.149222
DAY14	8	67.1996		0.038697	0.196716
			8.39995		
DAY21	8	66.1322	8.266525	0.042144	0.20529
D10/0	4	33.0743	8.268575	0.042051	0.205063
D9/1	4	33.9601	8.490025	0.015505	0.12452
D8/2	4	34.2262		0.016506	0.128475
			8.55655		
D7/3	4	34.649		0.012973	0.113901
			8.66225		
ND10/0	4	32.5704		0.04621	0.214966
			8.1426		
ND9/1	4	33.7419	8.435475	0.025683	0.16026
ND8/2	4	33.9663	8.491575	0.020354	0.142667

ND7/3	4	34.2036	8.5509	0.018088	0.134492

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Rows	0.545069	3	0.18169	81.10705	1.03E-11	3.072467
Columns	0.790138	7	0.112877	50.38871	1.03E-11	2.487578
Error	0.047043	21	0.00224			
Total	1.38225		31			

If P-values ≤ the alpha levels selected (0.05), we REJECT the NULL hypothesis

If P-values ≥ the alpha levels selected (0.05), we ACCEPT the NULL hypothesis

APENDIX XIII

S. bulgaricus VIABILITY COUNT (Log cfu/ml)

% OF SAMPLES

SUMMARY	*Count*	*Sum*	*Average*	*Variance*	
DAYS D9/1	D8/2	D7/3	ND9/1	ND8/2	ND7/3
DAY1 6.1007		6.5017	6.058	6.4764	6.3351
6.6132					
DAY7 6.0402		6.4168	6.0013	6.3487	6.2847
6.5176					
DAY14	5.921	6.3233	5.9537	6.2433	6.079
6.3215					
DAY21	5.84	6.1209	5.8853	6.0745	6.0338
6.0903					

Anova: Two-Factor Without Replication

				S. Deviation
DAY1	6	38.0851	6.347517	0.051173 0.22621388
DAY7	6	37.6093	6.268217	0.042851 0.20700455
DAY14	6	36.8418	6.1403	0.03272 0.18088736
DAY21	6	36.0448	6.007467	0.013577 0.11652053
D9/1	4	23.9019	5.975475	0.013731 0.1171774
D8/2	4	25.3627	6.340675	0.026776 0.16363273
D7/3	4	25.5426	6.38565	0.053512 0.23132708
ND9/1	4	23.8983	5.974575	0.00536 0.07321145
ND8/2	4	25.1429	6.285725	0.028913 0.17003792
ND7/3	4	24.7326	6.18315	0.022185 0.14894501

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*

Rows	0.400289	3	0.13343	39.13747	2.4781E-07	3.287382
Columns	0.650466	5	0.130093	38.15882	5.1949E-08	2.901295
Error	0.051139	15	0.003409			
Total	1.101893	23				

If P-values ≤ the alpha levels selected (0.05), we REJECT the NULL hypothesis

If P-values ≥ the alpha levels selected (0.05), we ACCEPT the NULL hypothesis

APPENDIX XIV

SENSORY SCORE CARD FOR

SENSORY EVALUATION OF PROBIOTIC YOGHURT

NAME: OKAFOR SONIA

PRODUCT PARTICULARS: MILK, WATER, ACTIVE LAB DATE: 12TH SEPT. 2018

Kindly evaluate the given samples for attributes like flavor and taste, body and texture, colour, Odour/Aroma

and appearance using the following 9- point hedonic scale and enter the scores in the space provided in the table below.

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

	DAIRY				NON- DAIRY			
	D10/0	D9/1	D8/2	D7/3	ND10/0	ND9/1	ND8/2	ND7/3
ODOUR/AROMA								
FLAVOUR AND TASTE								
BODY AND TEXTURE								
COLOUR AND APPEARANCE								
OVERALL ACCEPTIBILITY								

Remarks (if any):

Signature:

Name:

APPENDIX XV