Sudan University of Science and Technology

College of Graduate Studies

Manufacture of set yoghurt with probiotic Bifidobacteruim longum BB 536 and Bifidobacteruim infantis 20088

انتاج زبادى جامد بالبكتيريتين الصديقتين

B.longum BB536 و B. Infantis 20088

Dissertation Submitted to Sudan University of Science and Technology in Partial Fulfillment for the Requirements of the Degree of Master of Science in Food Science and Technology

By

Ibrahim Mohammed Saeed Mahmoud Osman

B.Sc in Food Engineering and Technology, Engineering and Technology College, University ofGezira (2008)

Supervisor:

Associate Professor Dr. Barka Mohammed Kabier Barka

Department of Food Science and Technology

College of Agricultural studies, SUST

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الآيـــــــة

قال تعالى:

بِسْمِ اللّهِ الرَّحْمَنِ الرَّحِيمِ

﴿ اقْرَأْ بِاسْمِ رَبِّكَ الَّذِي خَلَقَ ﴿ ١ ﴾ خَلَقَ الْإِنسَانَ مِنْ عَلَقٍ ﴿ ٢ ﴾ اقْرَأْ وَرَبُّكَ الْأَكْرَمُ ﴿ ٣ ﴾
الَّذِي عَلَّمَ بِالْقَلَمِ ﴿ ٤ ﴾ عَلَّمَ الْإِنسَانَ مَا لَمْ يَعْلَمْ ﴾

صدق الله العظيم

سور العلق الآية (1–5)

Dedication

To My

***Family** for their kind support and*

encouragements,

Teachers

And all my Friends.

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List of abbreviations

Abbreviation	Full Name
LAB	Lactic Acid Bacteria
FOS	Fructo Oligo Saccharides
FAO	Food and Agriculture Organization
WHO	World Health Organization
ECP	Extra Cellular Proteinases
NIDDK	National Institute Of Diabetes And Digestive And Kidney Diseases
DDF	Dal Dairy Factory
MCCs	Milk Collection Centers
SMP	Skimmed Milk Powder
WMP	Whole Milk Powder
RPM	Round Per Minute
UHT	Ultra Heat Treatment
WHC	Water Holding Capacity
TA	Treatable Acidity
TS	Total Solids
SG	Specific Gravity
SN	Solids Nonfat
TVC	Total Viable Count
LPC	Lab Pasteurization Count

Abstract

This study was carried out to evaluate the potential of producing probiotic set type yoghurt in Sudan. Yogurt culture (lactic acid bacteria (LAB), Bifidobacterium longum BB536, Bifidobacterium infantis 20088 and mix Bifidobacterium cultures were used for processing. Fresh milk was standardized; heat (at 95 °C for 5 minutes), inculcated (with yoghurt starter cultures at 43 ◦C and at 37 ◦C for Bifidobacterium strains) and then cooled after the pH reach 4.60. Different analysis were carried out including: physiochemical (TSS, TA, pH and water holding capacity), chemical (fat, protein, lactose, ash, moisture, and mineral), microbial (lactic acid bacteria, bifidobacteria, yeast &mould, coliform and viability of cultures) and sensory (taste, flavour, appearance, consistency and overall acceptabitity). The results obtained for the fresh milk used in this study did not deviate from standard values of each specific quality parameter for raw milk. During mixing process fat, solid nonfat, total solid content, titarable acidity and specific gravity were not significantly ($p< 0.05$) difference than the standard parameters of milk yoghurt. For the final product the pH, acidity and mineral were not significantly ($p<0.05$) difference between formulated yoghurt. The total viable count of different bacterial strains were ranged between 8.0 – 8.9 log CFU /ml between yoghurt products; exceeding the number required to presence in probiotic foods which at least 6 log CFU/ ml. However, during shelf life of different set yoghurt at refrigeration for 15 days, there were significant ($p<0.05$) decreases in viable number of strains. In general, the levels of the strains in set yoghurt were still maintained above 6 log CFU/ml. Sensory characteristics of set yoghurts types revealed no significant ($P<0.05$) differences in appearance, flavor, consistency and overall acceptability between different types of yogurt. Overall scalding manufacture of probiotic set yoghurt fulfilling probiotic products requirements was successful.

ملخص البحث

اجريت هذه الدراسة لتقييم امكانيه انتاج زبادي جامد بالباكتيريا الصديقة في السودان. تم استخدام بادىء الزبادي، الباكتيريا الصديقة BB536 ، الباكتيريا الصديقة ٢٠٠٨٨ وخليط من بادىء البكتيريتين الصديقتين للتصنيع. تم تقييس اللبن الطازج و تسخينه (عند درجة 95° لمدة خمس دقائق) و اضيف بادىء الزبادي (عند درجة 43 وبادءات الباكتيريا الصديقة عند درجة(37°) ومن ثم برد الزبادي بعد وصول الرقم الهيدروجيني لـ6.4. اجريت تحاليل مختلفة شملت فيزيوكيميائية (الجوامد الصلبة الذائبة، الحموضة ، الرقم الهيدروجيني والمقدرة على حمل الماء) و كيميائية (رطوبة، دهون، بروتين، رماد، لاكتوز ومعادن)، وميكروبيولوجية (باكتيريا حامض اللاكتيك، الباكتيريا الصديقة، خمائر واعفان وباكتيريا الكولوفورم) وحسية (التذوق، النكهة، المظهر، التماسك و القبول العام). النتائج التي تم الحصول عليها للبن الطازج المستخدم في هذه الدراسة لم تحيد عن القيم القياسية في أي من خصائص الجودة للبن الخام. اثناء عملية الخلط الدهون، الجوامد الصلبة الغير دهنية، محتوى الجوامد الكلية، الحموضة و الكثافة النوعية لم تختلف معنويا عن المواصفة القياسية للبن الزبادي. الرقم الهيدروجيني، الحموضة وال المعادن لا توجد اختلافات منوية بين خلطات الزبادي. العدد الكلى لسلالات الباكتيريا كانت في المدى بين 8 – 8. 9 بين منتجات الزبادي وتجاوز العدد المطلوب وجودة في اغذية الباكتيريا الصديقة والذى كحد ادنى 6 /مل. لكن اثناء العمر التخزينى لأنواع الزبادي المختلفة عند التخزين في الثلاجة لمدة 15 يوم هنالك انخفاض معنوي في العدد الحلى للسلالات المختلفة. بصورة عامة مستويات السلالات في الزبادي الجامد ما زالت اعلى من 6 /مل. الخصائص الحسية لأنواع الزبادي الجامد كشفت عدم وجود اختلافات معنوية في المظهر، النكهة، الثباتية والقبول العام بين انواع الزبادي. بصوره عامة الانتاج التجاري للزبادي الجامد بالباكتيريا الصديقة كان ناجحا وفى بمتطلبات منتجات الباكتيريا الصديقة.

CHAPTER ONE

INTRODUCTION

Fermentation is one of the oldest methods practiced by human beings for the transformation of milk into products with an extended shelf life. The exact origin(s) of making fermented milks is difficult to establish, but it could date from some 10000-15000 years ago as the way of life of human beings changed from being food gatherer to food producer (Pederson, 1979). This change also included the domestication of animals (i.e. cow, sheep, goat, buffalo and camel), and it is most likely that the transition occurred at different times in different parts of the world. Archaeological evidence shows that some civilizations (e.g. the Sumerians and Babylonians in Mesopotamia, the Pharos in north-east Africa and the Indians in Asia) were well advanced in agricultural and husbandry methods, and in the production of fermented milks such as yoghurt. Although there are no records available regarding the origin of yoghurt, the belief in its beneficial influence on human health and nutrition has existed in many civilizations over a long period of time. According to Persian tradition, Abraham owed his fecundity and longevity to yoghurt and, in more recent times. Emperor Francis was said to have been cured of a debilitating illness by consuming yoghurt made from goat's milk (Rosell, 1932). It is likely, however, that the Origin of yoghurt was the Middle East, and the evolution of this fermented product through the ages can be attributed to the culinary skills of the nomadic people living in that part of the world. Today, fermented milk products are manufactured in many countries (Kurmann *et al*, 1992), although few are of commercial significance.

In recent years' consumers are increasingly interested in incorporating healthy foods into their diet and in many circumstances are willing to pay more for food with functional properties. Fermented dairy products, also categorized in functional foods group are considered to have functional

properties because of its enhanced nutritional values and the presence of probiotics (friendly bacteria).A number of health benefits have been claimed for probiotic bacteria such as Lactobacillus acidophilus, Bifid bacterium spp. and Lactobacillus casei (Shah, 2000).

The key factor of manufacturing of yoghurt depend on the fermentation process which is mainly depend on the starter culture that acts through biochemical reactions and inductively causes the formation of the curd and the development of flavor components (Walstra, et al; *2006). For a fermented dairy product to be labeled as "yoghurt", it should contain* Lactobacillus acidophilus, *live bacterial strains of* Streptococcus salivarius *subsp.* th.ermophirus and Lactobacillus delbruecldr subsp. bulgaricus in abundance however, yoghurt starter culture lactobacillus casei, Lactobacillus jugurti, Lactobacillus helveticus, Bifidobacterium longum. Bifidobacterium bifidus and Bifidobacterium infantis. Streptococcus thermophiles subsp. Thermophilus were also used.

Over the past decade, considerable interest has developed in the use of probiotic organisms in food, pharmaceutical and feed products but till now it is lacking application as commercial in food sector (Crittenden *et al*, 2005). However, in Sudan no records on probiotics applications on yoghurt at industrial scale manufacture.

Main Objective

To produce set type yoghurt with probiotic *Bifidobacterium Longum BB536 and* Bifidobacterium infantis20088 *at industrial scale.*

Specific Objectives

1. To explore growth and survival of different bacteria in yoghurt during processing and shelf life of study.

2. To verify physicochemical, chemical, microbial and sensory Characteristics of different set yoghurt.

3. To asses quality of different set yoghurt during the shelf life at refrigeration.

CHAPTER TWO
LITERATURE REVIEW

2.1. Milk

Milk is the product of milking of dairy female in good health also nourished and not overworked. It must be collected properly and not contain colostrum (Bertrand, 2009)

Milk is a whitish food generally Produced by the mammary secretory cells of females in a process called lactation, it is one of the characteristics of mammals. The milk produced by the glands is contained in the udder. Milk secreted in the first days after parturition is called colostrum (Kebchaoui, 2012).

2.1.1. Composition of milk

The nutritional value of milk is particularly high due to the balance of the nutrients that compose it. The composition varies among animal species and breeds within the same species, and also from one dairy to the other, depending on the period of lactation and diet table - (1).

Milk contains several groups of nutrients. Organic substances are present in about equal quantity and are divided into elements builders. Proteins, and energy components, carbohydrates and lipids it also comprises. Functional elements such as traces of vitamins enzymes and dissolved salts, especially in the form of phosphates, nitrates and chlorides of calcium, magnesium, potassium and sodium. It also contains dissolved gases (5% by volume) mainly carbon dioxide (CO2), nitrogen (N) and oxygen O2) (Gautheron and lepouze 2012).

Table 1: The composition of milk from different mammals in g/100ml gg/l00gmilk.

Species	Water	Proteins	Fat	Lactose	Ash
Cow	*87.2*	3.5	*3.7*	4.9	0.72
Sheep	82.7	5.5	6.4	4.7	0.92
Goat.	86.5	3.6	4	5.1	0.82
Camel	*87.7*	3.5	3.4	4.7	0.71

Sourc source: (Konte, 1999).

2.3. **Milk fermentation**

The fermentation of dairy foods represents one of the oldest techniques for food preservation, it involves the breakdown of sugar and protein which results in the production of a large array of organic compounds that contribute to the flavor, preservation and outer appearance of the food product. Yoghurt is one of the most popular fermented dairy product which has wide acceptance worldwide whereas its nutritional and health benefits are well known for centuries (Dairy goodness, 2013)

2.2. **Fermented milk products**

Fermented food has a long history of safe usage and is found in diets throughout the world. Fermentation is broadly defined as a biochemical changes in 0rganic substances that are caused by the action of microorganisms or enzymes to produce 0rganic acid, alcohol, carbon dioxide and energy in the form of ATP (adenosine tri phosphate). Fermentation is applicable for many purposes, among others to extend the shelf life by protection and preservation of foods, producing desirable taste and flavor, enhancement of nutritional value, producing required physicochemical properties, improvement of food

safety and food security (Caplice & Fitzgerald, 1999)

2.4. Yoghurt

Yoghurt can be defined as food produced by culturing one or more of the optional dairy ingredients namely, cream milk partially skimmed milk, and skim milk, used alone or in combination with a characteristic bacterial culture that contains lactic acid producing bacteria *Lacto bacillus bulgaricus and Streptococcus thermophiles. (FDA, 2013)*

It is generally accepted that the fermented milk products including yoghurt have been discovered accidentally when they used to store milk in sheep-skin bags and has been evolved over centuries into commercial yoghurt making which have paved the pavement for different commercially available varieties with arrange of flavors and form sand textures (Dairy goodness, 2013)

Yoghurt is considered as healthy food due to its high digestibility and bioavailability of nutrients and also can be recommended to the people with lactose intolerance, gastro intestinal disorders such as inflammatory bowel disease, irritable, aids in immune function and weight control (Blance, 1981)

2.5. History of making yoghurt

It seems that it is dated back to the dawn to the civilization. It has been reported that the early civilizations such as the Samarians, Babylonians, Pharos and Indians were well advanced in agricultural and animal husbandry practices (Tammie, 1999).

This can be supported by the findings of Copley *etal*,2003 in which the dairy fat residues were found in pottery fragments from Neolitic Bronze-age and lr0n-age settlements, which suggests that the practice of dairying had existed in Britain approximately 6500 years ago (Copley,2003)

The first industrialized production of yoghurt was taken place in 1919

in Barcelona, Spain at accompany named DANONE(Dairy Goodness, 2013) Yoghurt was firstly introduced to the USA in the early 20th century in the form of tablets especially designed for those with digestive intolerance However, it became popular in the North America when Dannon, a small-scale yoghurt factory started manufacture of yoghurt in New York in 1940.Even though, yoghurt has been evolved for centuries, it was subjected to a significant and dynamitic evolution process in the20th Century to Originate a vast array of products. For instance, fruit yoghurts, with fruit on bottom and blended yoghurts were introduced in l937, 1947 and 1963 respectively (Danone, 2013)

2.6. Nutritional profile of yoghurt

Yoghurt is a highly nutritious and easily digestible dairy product which is a rich source of more than ten essential nutrients in particular, certain minerals and vitamins which is a rich source of more than ten essential nutrients.

The nutritional composition of yoghurt can be varied according to the strains of starter culture used in the fermentation, type of milk used (whole ,semi or skimmed milk) ,species that milk is obtained (bovine, goat, sheep), type of milk solids ,solid non-fat ,sweeteners and fermentation process However ,the general composition of yoghurt is more or less similar to that of milk. Therefore, yoghurt is a rich source of milk proteins, carbohydrate, minerals (Mckinley, 2005).

Table: 2 Nutritional profile of yoghurt

Components	Whole milk yoghurt	Low fat yoghurt	Nonfat yoghurt
Energy (kcal)	79		54
Protein %	*5.7*	4.8	5.4
Carbohydrate %	7.8	7.4	8.2
Pat %	3	1	0.2
Thiamin (mg)/\oc・	0.6	0.12	0.04
Riboflavin *(mq)A***	0.27	0.22	0.29
Niacin (mg/100gm)	0.2	0.1	0.1
Vitanrin B6 (mg)	0.1	0.01	0.07
VitaminB12(mg)	0.2	0.3	0.2
Polate (|ig)	18	18	8
Carotene (|ig)	21	trace	trace
Vitamin D	0	0.1	trace
Potassium	280	228	247
Calcium (mg/100gm)	200	162	160
Phosphorus(mg/I00gm	170	143	151

Source: Dairy council (2013)

2.7. Health benefits of yoghurt

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

Many people who cannot tolerate milk either because of protein allergy or lactose intolerance can enjoy yoghurt. The culturing process makes yoghurt more digestible than milk (Bertrand *et al*, 2003).

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

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

bacteria before the harmful ones take over (Macfarlane and Cummings, 1999).

Yoghurt can decrease yeast infection and it has prevention of growth of Pathogenic bacteria (Gillil, 1989).

Yoghurt is a rich source of calcium because the live-active cultures in yoghurt increase the absorption of calcium, serving of yoghurt get more calcium into the body than the same volume of milk. Daily intake of yoghurt may also either reduce the risk of osteoporosis because it increases calcium assimilation in body or help lactase deficient individuals take steps to prevent osteoporosis (Wynckel *et ah*, 1991).

Yoghurt is an excellent source of protein besides being a rich source of proteins؛ the limited proteolysis of the milk proteins during fermentation makes these proteins easier to digest. For this reason proteins in yoghurt are often called "predigested protein" and have beneficial uses for certain people who lack the digestive enzyme due to disease states (Savaiano and Levitt, 1984).

Fermented milk products are excellent dietary minerals, particularly calcium, phosphorus, magnesium and zinc.

Several LAB are capable of synthesizing B-vitamins and their concentration in fermented milk is generally high (Shahani & Chandan,

1979).

According to some studies, yoghurt can reduce the blood cholesterol this is because the live cultures in yoghurt can assimilate the cholesterol or because yoghurt binds bile acids (which has also been shown to lower cholesterol), or both (Liong & Shah, 2006).

Yoghurt and various dairy contain LAB are believed to confer a variety of important nutritional and therapeutically benefits to consumers including anti - mutagenic, anticancer- and anti- carcinogenic activity (Rao *et al*, 1986)

It is well known that whey proteins, especially ß-lactoglobulin (ß LG) and to a lesser extent a-lactalbumin (ALAC), are allergenic (Wal, 1998).

Hydrolysis of these proteins by lactic bacteria may decrease this allergenicity.

Certain whey peptides are known to have biological activity such as opioid and bactericidal activity (Schlimme & Meisel, 1995).

Several peptides arising from proteolysis of milk proteins have been cited as exerting biological activity and influence calcium absorption and have pharmacological effects on the central nervous system, cardiovascular system, and digestive system including immune-modulating properties (Schlirnme& Meisel, 1990).

2.8. Yoghurt culture bacteria

The thermophiles LAB, Streptococcus thermophiles & Lactobacillus

Delbrueckii subsp. bulgaricus are used together as important starter' culture for the production of yoghurt and some kind of cheeses. Because both bacteria are able to grow alone in milk, this indirect positive inter-action is called proto-cooperation, this positive relationship often has a beneficial effect on bacterial growth and on the production of lactic acid and aromatic compounds. Lactic acid production results in the lowering of pH and this makes it unsuitable for growth of spoilage or pathogenic microorganisms (Donkor *et al*, 2007).

The proteolytic activity of the two yoghurt bacteria is moderate but is very significant and leads to symbiotic growth of the two organisms, and production of flavor compounds. *L. bulgaricus* is known to be the more proteolytic of the two bacterial strains used for yoghurt production. *L bulgaricus* has the ability to hydrolyze caseins whereas *S. thermophiles* has only limited proteinase activity (Tamime and Deeth, 1980).

2.9. Changes in milk protein structure during fermentation

The central process in conversion of milk to yoghurt is agglomeration of casein micelles into a three dimensional network structure. Casein micelles disperse evenly in the milk and are separated from each other by a distance of

three micelle diameters in fresh milk, they are subject to Brownian motion and thus they do not settle at the bottom of the container. In fresh 0r boiled milk, the surfaces of the casein micelles are non- reactive. When the temperature of the milk reaches 85.^{o}C, one particular micelles protein (κ- casein) at the surface of the casein micelles reacts with one particular whey protein (B-lactoglobulin).This interaction produces minute ,bumps" on the casein micelle surfaces, when yoghurt bacteria metabolize lactose and produce lactic acid, the milk starts to coagulate and casein micelles are destabilized. However the B-Lactoglobulin – K-casein complex prevents other casein micelles from getting attached at these sites.

In the presence of *Lactobacillus delbrueckii subsp. bulgaricus,* and Streptococcus salivarius subsp. Thermophiles additional lactic produced and this increases the acidity of the milk and destabilizes the micelles. After certain acidity is reached, the micelles stick together and the milk can be observed by naked eye to coagulate or curdle. The surfaces of the heated casein micelles are partially blocked, so only a few micelles can interact. This leads to the formation of short branched micellar chains. The milk changed into a gel when the coagulation is complete. Under an electron microscope, the gel looks like a sponge with small pores filled with the whey. Milk that has not been heated consists of casein micelles with smooth surfaces, this milk is used to make cheese. Casein micelle surfaces interact with other casein micelles and form large micellar clusters from which whey separates easily. The casein micelles become compacted to form curd which is then processed into one of the cheeses varieties. Cheeses have markedly lower water content than yoghurt (Rapp, 1969).

2.10. Varieties and types of yoghurt depend on the culture

Yoghurt can be categorized into two different groups namely:

2.10.1 Standard culture yoghurt

Standard yoghurt refers to those made with *L.bulgaricus and thermophilus* these bacteria said to be not actually inhabit gut; however able to stimulate the friendly microflora already present in the gut to maintain the general intestinal health.

2.10.2 Bio- or probiotic yoghurt

Bio yoghurts are manufactured by culturing beneficial microorganism that claim to have Nemours health benefits once ingested, typically the probiotic strains of *Bifidobacteria and Acidophilus* .Unlike standard yoghurt culture, these probiotic strains are said to claim more specific health benefits and represent the type of friendly microorganism present in the gut (Dowden,2013).

2.11. Probiotics

Probiotics (derived from the Greek word meaning “for life”) are live microbes which influence the well-being of their host through their effect on the intestinal microflora (Guarner and Schaafsma, 1998). It was also called "a live microbial food ingredient that is beneficial to health. Probiotic improves intestinal microbial balance and reduction in these bacteria which are naturally found in the human small intestine and large intestine increases the presence of potentially pathogenic microbes.

Many probiotics are members of the genera of *Lactobacillus and Bifidobacteria* (Macfarlane and Cummings, 1999). At present approximately ten to fifteen bacterial strains have passed extensive investigations for some of the probiotic criteria. Some probiotic strain with scientific documentation include: Lactobacillus *rhamnosus, Lactobacillus acidophilus, Lactobacillus* paracasei, Lactobacillus plantarum, Lactobacillus *delbrueckii subsp.*

*bulgaricus, Bbifdobacterium lactis ,Bifidobacterium longum and Lactobacillus gasser*i.

Dairy products including yoghurt and cheese, due to the presence of lactose and peptides are preferred medium for probiotics or health promoting bacteria. They provide the ideal food system for the delivery of these beneficial bacteria to the human gut, given tire suitable environment to promote growth and support viability of these cultures (Pillai & Riclce, 1999).

In fact fermented dairy products are increasingly consumed as functional foods in recent years because of the probiotics as well as highly digestible fermentation products. Functional food contains a proper balance of nutrients and non-nutrients such as dietary fiber and various bioactive compounds as well as probiotics aid in the health of the human being. (Malagelad & Guarner, 2003).

2.11.1. Hearth benefits of probiotics

Several reported health benefits of probiotic bacteria are reduced duration of Diarrhea, .antagonistic effects against pathogenic microorganisms, improved lactose digestion, regulation of intestinal motility, reduced activities of cancer-related enzymes improved calcium re sorption and provision of water soluble vitamins (Crittenden *et al;* 2005). The action of probiotics on intestinal flora results in vital benefits, including protection against pathogens, development of the immune system and positive effects on colonic health and host nutrition ,There is also evidence to suggest that certain species/strain of probiotics are ant carcinogenic. Other important properties that have been attributed to probiotics include prevention and treatment of gastrointestinal disorders, reduction of food intolerance ,modulation of the host immune responses and prevention of cancer and cardiovascular diseases (reduction of serum cholesterol and lipids). Multiple species or high numbers of probiotic

organisms may be required to be administered simultaneously to achieve colonization (Wallowski *et al*, 1999).

2.11.2. Required Attributes of Probiotics

It is commonly stipulated that probiotics must adhere to intestinal cells. However, data that support adherence of probiotics are mostly derived from in vitr0 assays, which have limited predictability for the in vivo situation. And must be reconciled with the fact that, in general, probiotics persist only short term in the host after feeding has stopped. The nature of the association of probiotics with the epithelial cell surfaces or mucous layer Remains was determined. Although it often suggested that probiotics for human use must be of "human 0rigin," some strains that are not normally isolated from humans have been shown to be effective probiotics (e.g., strains of the species Bifidobacterium animalis), which negates this Requirement. (Sanders, 2006).

The statement that probiotics "improve the balance of microflora" is often made. However, it is not clear what this assertion means 0r how is it measured. Probiotics have been shown to alter populations or activities of colonizing microbes. Improved balance is often equated with increased fecal levels of lactobacilli or bifidobacteria. This is a measure not of balance but of fecal microbiota alteration. Since no scientific consensus exists on the composition of a "healthy microbiota," the health implications of such microbiota alterations remain unclear. Furthermore, it is difficult to measure intestinal microbiota, fecal microbiota is not equivalent to intestinal microbiota, and luminal microbiota is not equivalent to epithelial microbiota (Bernstein *et a*, 2005), Probiotics may, in fact, facilitate a return to normal status after a perturbation of the microbiota (e.g., because of the use of antibiotics or illness) or may reduce the degree of change invoked by such challenges. This function more closely supports the concept that probiotics

can improve the balance of microbiota. A few studies have measured a probiotic-enhanced return to baseline levels after antibiotic use in humans. The concept of probiotic-induced improved balance of microbiota would benefit from further study. When probiotic strains are selected, attributes important for efficacy and technological function must be assessed. Because the range of targets for in vivo function is broad, spanning Oral, stomach, respiratory, intestinal, vaginal, and immune functions, it would be a daunting task to develop a list of characteristics required for all probiotic functions. Basic initial characterization of strain identity and taxonomy should be conducted, followed by evaluation with validated assays both in studies of animal models and in controlled studies in the target host. In vitr0 assays are frequently conducted that have not been proved to be predictive of in vivo function. Technological robustness must also be determined, such as the strain's ability to be grown to high numbers, concentrated, stabilized, and incorporated into a final product with good sensory properties, if applicable, and to be stable, both physiologically and genetically, through the end of the shelf life of the product and at the active site in the host. Assessment of stability can also be a challenge, since factors such as chain length and injury may challenge the typical assessment of colony-forming units, as well as in vivo function (Von der, 2003).

2.11.3. Probiotics application challenges:

From a technological standpoint. Champagne has listed many challenges in the development of a probiotic food product including: strain selection, inoculation, growth and survival during processing, viability and functionality during storage, assessment the viable counts of the probiotic strains particularly when multiple probiotic strains are added and when there are also starter cultures added, and the effects on sensory properties. (Champagne, 2009)

Other challenges such as: maintaining of probiotics, diversity and origin of probiotics, probiotic survival and being active, dealing with endogenous microbiota, and proving health benefits have also been discussed, the viability and sensory acceptance are the most important challenges to ensure transferring the health benefits and the commercial success (Antoine, 2011)

2.11.4.1. **Viability and survival**

Probiotics have been proved to provide many health benefits. However, the claimed health benefits can't be achieved without high number of viable cells. Many probiotic bacteria have shown to die in the food products after exposure to low pH after fermentation, oxygen during refrigeration distribution and storage of products, and/or acid in the human stomach. Probiotic products need to be supplemented with additional ingredients to support the viability throughout processing, storage, distribution, and gastrointestinal tract to reach the colon. Several reports have shown that survival and viability of probiotic bacteria is often low in yoghurt. The efficiency of added probiotic bacteria depends on dose level and their viability must be maintained throughout storage, products shelf life and they must survive the gut environment (Chin, 2000)

Several studies have focused on the effect of adding certain compounds to enhance the probiotic viability. Many evidences have shown that inulin, oligosaccharides, and fructo oligo saccharides (FOS) have good impacts on the probiotics viability. Growth of probiotics in non-fermented foods is not desirable (due to possible off flavor formation), but their growth during the production of fermented foods can lower process costs and increase the adaptation of probiotics leading to enhanced viability. The starter microbes in fermented foods can sometimes inhibit probiotics but they can also enhance their survival by producing beneficial substances or by lowering the oxygen

pressure. (Sandholme, 1999)

The viability and survival of probiotics are strain specific. To maintain the viability of very sensitive strains, encapsulation is often the only option, especially micr0- capsulation that do not affect the sensory properties of the food produced. Microencapsulation technologies have been developed and successfully applied using various matrices to protect the bacterial cells from the damage caused by the external environment. (Deidda *et* a1 .2006)

The immobilization of probiotics using microencapsulation may improve the survival of these microorganisms in products, both during processing and storage, and during digestion. Some probiotic bacteria, such as the spore- forming bacteria, GanedenBC30 viability and stability, making it an ideal choice for product development, compared to other probiotic bacteria strains, such as *R acidophilus and bifidobacteria.* This spore safeguards the cell's genetic material from the heat and pressure of manufacturing provides better probiotic. (Rodrigue, 1993).

2.11.4.2. **Sensory acceptance**

Probiotic foods must show at least the same performance in any sensory test as conventional foods. In most probiotic foods sensory tests are aiming to determine acceptance of the products, without obtaining details concerning the addition of the probiotics to the food and their interaction with the consumer. Therefore, it is important to development sensory tests for probiotic foods that can be accompanied by specific sensory analyses. Sensory testing must cover all characteristics with regard to change over time during storage. Some studies have reported the possibility of obtaining similar, or even better, performance with probiotic products as compared to conventional products.

In most cases the developed products need to match similar commercial products in parallel In general, metabolism of the probiotic

culture can result in the production of components that may contribute negatively to the aroma and taste of the food product, probiotic off-flavor. For example, acetic acid produced by *Bifidobacterium spp.* can result in a vinegary flavor in the product, prejudicing the performance in sensory assessments. Masking is one technique that has been used to reduce the off flavors in foods and it has been performed successfully through the addition of new substances or flavors to reduce the negative sensory attributes contributed by probiotic cultures. The addition of tropical fruit juices, mainly pineapple, but also mango or passion fruit, might positively contribute to the aroma and flavor of the final product and might avoid the identification of probiotic off flavors by consumers (Tuckow, 2006)

2.11.5. The future of probiotics

Dairy based products containing live bacteria are the main vehicles of probiotics to human. Non-dairy beverages would be the next food category where the healthy bacteria will make their mark. Microencapsulation technologies have provided the necessary protection for probiotics and moved them outside the pharmaceutical and supplemental use to become food ingredients.

2.11.5.1. Nano -technology, encapsulation and probiotics

The basic of probiotic nanotechnology applications is currently in the development of Nano-encapsulated probiotics. The nanostructured food ingredients are being developed with the claims that they offer improved taste, texture and consistency. (Yen, 2007)

2.11.5.2. Biotechnology and probiotics

With the revolution in sequencing and bioinformatics technologies well under

way it is timely and realistic to launch genome sequencing projects for representative probiotic microorganisms. (Altermann, 2005) .Increasing knowledge of genes important for the technological functionality and rapid development of the toolboxes for the genetic manipulation of *Lactobacillus* and *Bifidobacterium* (Ahmed, 2003)

2.11.6. Selection Criteria of Probiotics

While selecting the probiotics strain, a safety entry must be kept in mind regarding production /manufacturing relating to the technological aspects, application, survival and colonization in the host and their health benefits. Characterization of the probiotics is important in concern with gain the knowledge of the strain and mechanism of the probiotic action. (Caselli *et al*, 2012)

General aspects of selection

2.11.6.1. Origen

The Origin of probiotics depends upon the application of probiotics. It should be originated from a targeted animal micr0 flora. The source can be from a human Origin like human large intestine, small intestine, 0r a breast milk. (Dash, 2009).

2.11.6.2. Genus, Species and Strain Identification

According to the WHO/FAO guidelines, probiotics are the strain species so it must be identified at genus, species and strain lever (Nemcova, 1997).

2.11.6.3. Biosafety

Selected strains should be non-pathogenic and non-toxic. Generally, Probiotics strains must be characterized at a minimum with the following tests:

1) Assessment of the side effects during previous human studies.
2) Assessment of certain metabolic activities (e.g. D-lactase production, bile salt de conjugation.)
3) Determination of antibiotic resistance pattern.
4) Post market surveillance of adverse incidents on consumer (Wright and Salminen, 1998)

2.11.6.4. Functional Aspects

1) Resistance to the gastric condition

Probiotic bacteria must be able to survive in the gastrointestinal tract. The survival of ingested probiotics in different parts of gastrointestinal tract varies with the strain. (Dunne, et al; 2001)

2) Resistance to the bile acid

Probiotics Organisms must be resistance to bile acids

3) Modulation of immune system

Strains of probiotics should be able to stimulate as well as regulate a several aspects of the natural and the acquired immune response. (Kosin, 2006)

2.11.6.5. Health Aspects

The selection of the probiotic organisms depends upon a health claims. Probiotic must be able to exert their benefits on the host through the growth and /or activity in the human body. Most proven probiotics strains are human origin, a strong case can be made that they are normal commensals and,

therefore, safe to use. To achieve the health benefits, probiotic bacteria must be viable and available at high concentration, typically 105 to 10.7 CPU/g of product (Mishra, 2000).

2.11.6.6. probiotic stability and viability

Probiotic must have the capabilities for its survival in the food, feed and dietary supplements. Manufacturer has given a great attention to probiotic stability. More importantly the probiotics strain should be stable enough to withstand a conventional industrial production process. Probiotic stability is affected by the high temperature, oxygen humidity and high water activity in the culture. (Wright and Salminen, 1998).

2.11.6.7.Quality Control Aspects

The quality control criteria are important in concern with the approval of the probiotic over the species, health claims. Thus functional food regulations should take into account strain properties and their stability during the industrial processing and use.

Consideration for the probiotic manufacturing includes quality control procedure such as:

- The criteria and procedures for quality control must be determined and implemented.
- Verification of genetic identity of selected species.
- Assuring the probiotic potency
- Ensuring the purity of probiotics.
- Ratifying the finished product through independent testing (Tuomola et al 1998).

2.11.7. Food applications of probiotics

An increase in knowledge of functional foods has led to develop foods

with health benefits beyond adequate nutrition. The last 20 years have shown an increased interest among consumers in functional food including those containing probiotics. The presence of probiotics in commercial food products has been claimed for certain health benefits. This has led to industries focusing on different applications of probiotics in food products and creating a new generation of 'probiotic health' foods. Below section will summarize the common applications of probiotics in food products.

2.11.7.1 Dairy-based probiotic foods

Milk and its products is good vehicle of probiotic strains due to its inherent properties and due to the fact that most milk and milk products are stored at refrigerated temperatures. Probiotics can be found in a wide variety of commercial dairy products including sour and fresh milk, yoghurt, cheese, etc. Dairy products play important role in delivering probiotic bacteria to human, as these products provide a suitable environment for probiotic bacteria that support their growth and viability (Philips, et al, 2006).

Several factors need to be addressed for applying probiotics in dairy products such as:

- Viability of probiotics in dairy. (Shah, 2000)
- The physical, chemical and organoleptic properties of final products (Kirmaci, 2007)
- The probiotic health effect. (Parvez, 2006)
- The regulations and labeling issues. (FAO/WHO, 2001)

2.11.7.1.1 Drinkable fresh milk and fermented milks

Among probiotics carrier food products, dairy drinks were the first commercialized products that are still consuetude in larger quantities than other probiotic beverages. Functional dairy beverages can be grouped into two categories:

Fortified dairy beverages and whey-based beverages. (Kirmaci, 2010). Several factors have been reported to affect the viability of probiotic cultures in fermented milks. Acidity, pH, dissolved oxygen content, **r**edox potential, hydrogen peroxide, starter microbes, potential presence of flavoring compounds and various additives (including preservatives) affect the viability of probiotic bacteria and have been identified as having an effect during the manufacture and storage of fermented milks .Probiotics such as *Lactobacillus* and *Bifidobacterium* strains grow weakly in milk due to their low proteolysis activity and inability to utilize lactose. These bacteria also need certain compounds for their growth which is missing in milk., There are some substances have been tested to improve growth and viability of probiotics in dairy beverages such as; presence of Citrus fiber in fermented milks was found to enhance bacterial growth and survival of probiotic bacteria in friended milks (Paquin , 2009)

2.11.7.1.2Yoghurt

Yoghurt is produced using a culture of L. delbrueckii subsp. bulgaricus and Streptococcus salivarius subsp. thermophilus *bacteria*. *In addition, other* Lactobacilli and bifidbacteria are also sometimes added during or after culturing yoghurt. The probiotic characteristics of these bacterial strains that form the yoghurt culture are still debatable. The viability of probiotics and their proteolytic activities in yoghurt must be considered. Numerous factors may affect the survival of Lactobacillus and Bifidobacterium spp. in yoghurt. These include strains of probiotic bacteria, pH, and presence of hydrogen peroxide and dissolved oxygen, concentration of metabolites such as lactic acid and acetic acids, buffering capacity of the media as well as tire storage temperature (Shah, 2006)

Viability of probiotics in yoghurt depends on the availability of

nutrients, growth promoters and inhibitors, concentration of solutes, inoculation level, incubation temperature, fermentation time and storage temperature. Survival and viability of probiotic in yoghurt was found to be strain dependent.

The addition of fruit in yoghurt may have negative effect on the viability of probiotics, since fruit and berries might have antimicrobial activities. Inoculation with very high level of probiotics with attempts to compensate the potential viability loss, might result in an inferior' quality of the product. The present of probiotic was found to affect some characteristics of yoghurt including: acidity, texture, flavor and appearance (McGrew, 2007)

2.11.7.1.3 Cheese

Alternative carriers such as cheese seem to be well suited. Cheeses have a number, of advantages over- yoghurt and fermented milks because they have:-

1- higher pH and buffering capacity, 2-highly nutritious, 3-high energy, more solid consistency, 4-relatively higher fat content, 5- longer' shelf life.

Several studies have demonstrated a high survival **r**ate of probiotics in cheese at the end of shelf life and high viable cells. Probiotics in cheese were found to survive the passage through the simulated human gastrointestinal tract and significantly increase the numbers of probiotic cells in the gut (Ouwehand, 2009)

2.11.7.2 Nondairy based probiotic products

With an increase in the consumer vegetarianism throughout the developed countries, there is also a demand for the vegetarian probiotic products. Nondairy probiotic products have shown a big interest among vegetarians and lactose intolerance customers. According to the National

Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) of the U.S. National Institutes of Health, about 75% of the world population is lactose intolerant. The development of new nondairy probiotic food products is very much challenging, as it has to meet the consumer's expectancy for healthy benefits. (Webb, 2002)

2.11.7.3 Vegetable-based probiotic products

Fermentation of vegetables has been known since ancient time. Fermented vegetables can offer a suitable media to deliver probiotics. However, it shows that the low incubation temperature of vegetable fermentation is a problem for the introduction of the traditional L.acidophilus and Bifidobacterium probiotic bacteria. Nevertheless, when the temperature is adjusted at 37^{o}C, probiotic bacteria grow quite rapidly in plant-based substrates. (Champagne, 2003)

The suitability of car**r**ot juice as a **r**aw material for the production of probiotic food with Bifidobacterium strains was investigated Kun and others have found that Bifidobacteria were capable of having biochemical activities in car**r**ot juice without any nutrient supplementation. (Hoschke,2008)

Soybean has received attention from the researchers due to its high protein and quality. Soymilk is suitable for the growth of LAB and bifid bacterial Several studies have focused on developing fermented soymilk with different strains of LAB and Bifidobacteria to produce a soymilk product with improved health benefits, (Chou, 2002)

2.11.7.4 Fruit-based probiotic products

Now days, there is increasing interest in the development of fruit-juice based probiotic products. The fruit juices contain beneficial nutrients that can be an ideal medium for probiotics. The fruits are rich in several nutrients such

as minerals, vitamins, dietary fibers, antioxidants, and do not contain any dairy allergens that might prevent usage by certain segments of the population. Those characteristics allow the selection of appropriate strains of probiotics to manufacture enjoyable healthy fruit juice. To develop probiotic fruits, many studies have been carried out. The suitability of noni juice as a raw material for the production of probiotics was studied by Wang and others found that B. longum and L.plantarum can be optimal probiotics for fermented noni juice (Shyu, 2009).

2.11.7.5 Cereal-based probiotic products

Cereal-based probiotic products have health-benefiting microbes and potentially prebiotic fibers. The development of new functional foods which combine the beneficial effects of cereals and health promoting bacteria is a challenging issue. Nevertheless, cereal-based products offer many possibilities. Indeed, numerous cereal-based products in the world require a lactic fermentation, often in association with yeast or molds. Cereals are good substrates for the growth of probiotic strains and due to the presence of non-digestible components of the cereal matrix may also serve as prebiotics (Webb, 2002)

CHAPTER THREE
MATERIALS AND METHODS

3.1. The study area

The study was conducted in DAL Daily Factory (DDF) Capo. The first name was Blue Nile Dairy's company which was constructed in 1996 in Soba area with capacity of 80 tons per day. As the business grew, it was expanded to meet the demands of the market for delicious tasting, natural dairy products. The old factory reached its full capacity so it was very necessary to establish another factory that have much larger manufacturing facility to meet the demand of the market with capacity of 500 tons per day.

The new plant (CAPO 2) was opened in September 2010. It is situated in Bahri Industrial Area (North Khartoum). CAPO 2 now considered the first and largest dairy factory in Sudan.

One of the most important sections in capo is quality and innovation section, in this section they invent their new product and develop the exciting.

3.2. Sources of materials

Fresh cow milk obtained from DAL farm, powdered skim milk powdered whole milk from Fonterra dairy and yoghurt starter culture (***Streptococcus themophilus and Lactobacillus delbrueckir subsp. Bulgaricus***)from ***Hansen*** were obtained by local DAL dairy factory, Khartoum. Sudan.

Two strains of probiotics starter culture **(*Bifidobacterium infantis 20088 and Bifidobacterium longum BB536***) were obtained from university of Khartoum biochemistry and food science section (Micr**0** lab).

3.3. Manufacturing procedures

Base mix of set yoghurt was prepared using؛ fresh cow milk 80% and 3.60 skim milk powder and 1.6% whole milk powder and 14.8%water.

The base was prepared by gradually adding 0.72kg of skimmed milk powder (SMP) and 0.31kg of whole milk powder (WMP) to 16 liters' fresh milk and 2.921iters water in 30 liters' stainless steel can with continues agitation by using IKA T 50 digital mixer at 8000 RPM. The can was covered and lift for 30 minutes to ensure complete hydration of the mix.

3.4. Preparation of culture

Bifidobacterium longum and infantis were obtained from the stock culture of microbiology laboratory the strains were maintained at -20 ^{o}**C** in 20 % glycerol solution.

Stock culture were prepared by activation of the strains in skim milk incubated an aerobically at 37^{o}C for 24 hours. The obtained cultures were re-activated again under the same condition to prepare enough stock for the experimental. The working cultures were prepared by twice successive transformation in I0 **%** sterilized skim milk (121° C for **15** minutes) and incubation at 37^{o} **C** for 24hours.

Processing of yoghurt mix

To deactivate the enzymes and destroy microorganisms in the milk and to denature whey protein pasteurization was accomplished in ASEPTO UHT + S1 pilot plant made in Germany, heated by circulating water through tubular heat exchanger to 95 ^{o}C for 5 minutes after pre-heating to 55°C and homogenization 160 bar (120 first stage and 30 second stage) with outlet temperature of 43°**C.** Collected pasteurized milk was divided into four equal quantity as follow:

1- The first portion considered as control which was inoculated with 2% of

lactic acid culture,

2- the second portion is inoculated with *bifidobacteruim longuin* BB536

3- third portion is inoculated with *Bifidobacterium infantis* 20088

4- The fourth portion is inoculated with 50% B. longuin and 50% *B.infantis* then each quantity poured into small plastic cups and incubated at 43 ^{o} C for control sample (C1) in controlled incubator (memmert UN 55. Germany) while probiotic yoghurt (C2,C3,C4) was incubated anaerobically at 37°C until the pH reached 4.5 then cooled to 5 ^{o}C .

3.5. Physiochemical and chemical analysis

3.5.1. Determination of pH

The pH of samples was determined using electronic pH meter (JENWAY 3510 pH Meter, designed and manufactured in the UK by Bibby Scientific Stone LTD, model 3510, serial no. 51030). The pH meter was calibrated by using pH 7.0 and 4.0 standard buffer solutions. The electrode was cleaned and rinsed with distilled water before and after each reading. Duplicate measurement was determined for each treatment.

3.5.2. Water holding capacity

The water-holding properties of the yoghurts were examined by centrifugation as described by Li and Guo (2006). A portion of each of the formulations of yoghurt (Y) were prepared and were weighed before incubation. The yoghurt was then centrifuged at 4.^{o}C for 10 minutes at 2500 RPMs, The supernatant (S) layer was poured off and weighed. Water holding capacity was determined by using the following formulation:

WHC (%) = (S/Y) X 100%

3.5.3. Determination of ash

Clean empty crucibles were placed in a muffle furnace at 600.C for an hour, cooled in desiccators and then weight of empty crucible was noted (W1). Two gram of each samples was taken into crucibles (W2). The sample was ignited over a burner with the help of blowpipe, until it is was charred. The crucibles were placed in muffle furnace at 550°C for 6 hours. The appearances of gray white ash indicate complete oxidation of all organic matter in the sample. The crucible was cooled and weighed (W3). The percentage ash was calculated as follows: (AOAC, 2003).

ASH % = different in weight of ash * 100/ weight of the sample

3.5.4. Determination of Mineral Contents

The analyses of minerals were performed directly on fresh yoghurts without any previous treatment of the samples according to J.M Pauwels *et al (1992)* each sample (10ml) was poured in a crucible and 2.5ml concentrated HCI and 7.5ml of concentrated HNO solutions were added. The resulting solution was gently digested on an electric plate until the volume was reduced to about half in 30 minutes. The digest was filtered using a What man paper into a 50ml volumetric flask and the volume of the content was made to 50ml with distilled water to obtain solution A. Aliquots of this solution were used for the estimation of K, Ca, p, Na.

p was estimated by spectrophotometry ؛Na and K by flame photometry, while Ca content was determined by complexometry.

3.5.5. Determination of fat content

Fat content was determined by Gerber method according to AOAC (1990) as mentioned: In a clean dry Gerber tube, 10 ml of sulphuric acid

(density 1.815gm/ ml at 20.^{o}C) were poured and then 11 gm. of a well-mixed sample was gently added. One ml of amyl alcohol (density 0.815 gm. /ml at 20.^{o}C) was added to the mixture, the contents were then thoroughly mixed till no white particles could be seen. Gerber tubes were centrifuged at 1100 revolutions per minutes (rpm) for 4 minutes and the tubes were then transferred to a water bath at 65^{o}C for 3 minutes. The fat percent was then read out directly from the fat column.

3.5.6. Determination of treatable acidity

The acidity of the samples was determined according to the method described by the AOAC (1990).

Ten ml of each sample was placed into white porcelain dish and five drops of phenolphthalein indicator were added. Titration was carried out using 0.1N NaOH until a faint pink color appeared. The titration figure was divided by ten to get the percentage of lactic acid (1 ml of 0.1 N sodium hydroxide (NaOH) 0.009 grams of lactic acid).

Total treatable acidity was calculated from this equation.

$$\mathbf{M*V = M*V}$$

Where:

M= molarity, v= volume

3.5.7. Determination of total solids content:

Total solids content was determined according to AOAC (1990). Three grams of the sample were weighed into dry clean flat bottomed aluminum dish, and heated on a steam bath for 10 min. The dish was then cooled in a, desiccator weighed and heated, cooled and weighed were repeated until the difference between two readings was <0.1 mg. The total solids content was calculated from the successive equation

$$TS = \frac{w1}{w} \times 100$$

Where:

W1 = Weight of sample after drying.

W = Weight of original sample

3.5.8.Determination of protein. Lactose

Proteins and lactose measured by Milk scan, FOSS Analytical A/S.69, Slangeruggade, and DK3400 Hillerod Denmark.

The devise works with wave length of the component , when sample put into the devise trough cuvate it took more than 20 reading then give means

3.5.9. Determination of moisture content

Two grams of each of the sample was weighed into dried weighed moisture dish. The samples were put into a moisture extraction oven at 105^{o}C and heated for 3hours. The dried samples were put into desiccators, allowed to cool and Reweighed. The process was repeated until constant weight was obtained. The difference in weight was calculated as a percentage of the Original sample (AOAC, 2003).

Moisture % = W1-W2/W1 * 100

Where

W1= Weight of the sample before drying.

W2 = Weight of sample after drying.

3.5.10. Temperature of fresh milk

Temperature of fresh milk was measured directly by using digital calibrated thermometer

3.5.10 Specific gravity of fresh milk‹

Specific gravity was measured by using digital device called Anton par which gave direct reading for specific gravity of the milk‹.

3.5.11. Antibiotic test

Trisensor was used to completely rapid test in a dipstick ‹format for multi-antibiotic detection in one single operation. It is a receptor assay allowing the detection of every B-lactam, Tetracycline and Sulfonamide in milk.it toke 6 minutes to get the result at 40°C ± 3 °C.

3.6. Microbiological analysis

3.6.1. Preparation of media

All media were obtained in a dehydrated form stored in a hygroscopic environment in a cool dry place, away from light and prepared according to the manufactures instructions.

3.6.2. Types of culture media used for microbiological examination of set yoghurt.

3.6.2.1. Violet red bile agar (Merck)

This medium was used to determine the total coliform counts (Harrigan and McCance, 1976). It was obtained in dehydrated form, each dehydrated liter of the medium composed of lactose (10.0 grams). Neutral red (0.03 grams), sodium chloride (13.0 grams), Crystal violet (0.002 grams) and agar agar (13.0 grams).

According to the manufacturer's instructions 39.5 grams were suspended in 1000 ml distilled water, it was boiled to dissolve completely and sterilized by water bath at 100° C for 30 minutes, cooled to 45°C ± 2.C and immediately poured into sterile Petri dishes containing the dilution.

3.6.2.2. Yeast extracts glucose. Chloramphenicol agar (YGC. agar, Merck)

This medium was used to determine the total yeast and mould counts ISO 6611(E) (2004). It was obtained in a dehydrate form. Each rehydrated liter of the medium composed of yeast extract (5 grams), glucose (20.0 grams), chloramphenicol (0.1 gram) and agar (14.9 grams). According to the manufacturer's instructions, 20 grams were suspended in 1000 ml distilled water, boiled to dissolve completely and sterilized by autoclaving at 15 bar pressure 121.^{o} C for 15 minutes, cooled to 45 ± 2.^{o}C and immediately poured into sterile Petri dishes containing the dilution.

3.6.2.3. Standard Plate Count Agar (Merck)

This medium was used to determine the total bacterial count (Houghtby *et al*, 1992). It was obtained in dehydrated form (Biomarker, B 298). Each rehydrated liter of the medium composed of casein enzymic hydrolysate (5.0 grams), yeasts extract (2.5 grams), dextrose (1.0 gram) and agar (15.0 grams). According to the manufactures instructions, 23.5 grams were suspended in 1000'ml distilled water, it was boiled to dissolve completely and sterilized by autoclaving at 121.^{o}C for 15 minutes.

Serial dilution of samples

One ml of each milk sample and 1gm of yoghurt sample was weighed aseptically and added to test tube containing 9ml of sterile diluents and well mixed to give 101-, using sterile pipette 1ml of the last dilution was transferred to test tube containing 9ml of sterile diluents and well mixed to give 10-2 in the same way continued to the prepare other serial dilution (Harrigan, 1998).

3.6.3. Preparation for plating

3.6.4.1. Sterilization of equipment

Glass wares such as test tubes, pipettes, Petri-dishes, flasks and bottles were sterilized in a hot oven at 180 ^{o}C for one hour, whereas ringer solution and tips were sterilized by autoclaving for 15 minutes at 121^{o}C (Marshall, 1992).

3.6.4.2. Plating method

One ml of the yoghurt sample was transferred aseptically by sterile pipette to 9ml sterile ringer's solutions. This procedure was repeated to make tenfold dilutions from 10-1 – 10-3 according to Richardson (1985).

Culturing method from each dilution, 1 ml was transferred to duplicate Petri- dishes and the culture medium was poured aseptically into each Petri-dish using pour plate technique, mixed gently, left to solidify and incubated in an inverted position. The cultured Petri-dishes for the coliform count were incubated at 37 °C for 24 hours, 32 °C for (48 hours) for the total bacterial count and 28°C for 5 days was estimated for the yeasts and moulds count. The typical colonies in each Petri-dish were counted using a colony counter (Houghtby *et al*, 1992).

3.6.4. Microbiological Analysis for culture viability

3.6.5.I. Microbiological Analysis for viability of thermophiles

The culture growth and viability were evaluated taking 1 ml of each sample, decimally diluting it and plating on the media, M17-Agar was used for S. thermophiles incubated aerobically in 37^{o}C for 72 hrs. ,Dave and Shah (1996) for L.***bulgaricus*** MRS agar were used incubated Anaerobically in 43^{o}C for 72 hrs (Tharmaraj and Shah, 2003).

3.6.5.2 Microbiological Analysis for viability of *B.longum BB536, B. infantis 20088*

For Bifidobacterium ssp. (infantis& longum), LP-MR agar was used, incubated anaerobically in 37.^{o}C for 72 hrs (Vinderola and Reinheimer, 1999)

3.6.5. Enumeration of yeast/moulds, coliform and probiotic

Ten gram of each cultured yoghurt samples was diluted with 90 ml of 0.15%

Sterile peptone water Ten-fold serial dilutions (10-2 – 10-8) was prepared in 9 ml of 0.15% sterile peptone water (Arjmand, 2011).The bacterial counts of each treatment were carried out in quadruplicate after intervals of 0,3,6,9,12,15days. Enumeration was done using the pour plate technique. Plates were gently mixed clockwise and anticlockwise to disturb the samples uniformly and allowed to set. Plates were then incubated under anaerobic condition (using Gas-pack system. An aero Gen - 1.3) at 37^{o}C for 72 hrs, according to Arjmand, 2011.The numbers of Colony Forming Units (CFU) on plates containing 15 to 300 colonies (Aijmond , 2011) were calculated per gram of samples as shown below:

CFU-1 = Number of colonies * volume of dilute sample / dilution factor

3.7 Sensory evaluation method

Sensory evaluation test determined according to 5 point hedonic scale designed by Ihekoronye and Ngoddy, (1985) ٩

A hedonic rating is a technique to measure the degree of liking for a product by untrained assessors. A 5-point hedonic scale designed was employed to elucidate panelists' acceptance of appearance, flavor, texture, taste and overall acceptability of the control and the three experiments. Every

panelist received the four types of formulation to be judged side-by-side and water for rinsing. Before tasting the products, panelists were asked to evaluate the samples' appearance using a 5-point hedonic scale ranging from "5-Excellent; 4-Very good, 3-Good; 2-Acceptable; 1-Poor. After judging appearance, the panelists were then allowed to taste the samples and evaluate their flavor, texture taste and overall acceptability using a 5-point hedonic scale, once again ranging from 5-Excellent; 4-Very good; 3-Good; 2-Acceptable; 1-Poor

3.8. Statistical analysis

One way ANOVA test was performed to examine significant difference between normally distributed data. Tukey's -test was used to perform multiple comparisons between mean within each specific parameter. Probability level of less than 0.05 was considered significant ($p<0.05$).All data were analyzed using MINITAB 17 statistical software for windows (2013).

CHAPTER FOUR
RESULTS AND DISCUSSION

4.1. Physicochemical properties and chemical content of fresh milk

4.1.1 Temperature of fresh milk

Temperature of fresh milk must be kept in range between 5 to 8° C to avoid the growth of pathogens and presence of heat resistance toxins, the temperature of used milk was 5.3°C as shown in table (3) this result in line with Tamime and Ropenson (1999) who stated that. The temperature of milk ، in storage should be between 0- 10°C ± 1°C temperature tolerances.

4.1.2. The pH of fresh milk:

The pH of fresh milk used was 6.72.as shown in table (3) the result is similar to the value obtained by Hoolasi (2005) who stated that the pH of raw milk was 6.73 Walstra, et al (1999) and FAO (1999) were stated that the pH of fresh milk was between 6.6 to 6.8 therefore our result table (3) was within the range.

4.1.3. Titratable acidity (TA) of fresh milk

The Titratable acidity of fresh milk was 0.142 % (table 3) which was in range, stated by O, Connor (1995) who stated that the TA of fresh milk **r**ange between 0.14 to 0.16 ،% as lactic acid, while Heineiuan, (2001)reported that taste of milk becomes sensible. At 0.4% acidity. Milk ،is clearly sour, at 0.6% it precipitates at normal temperature. At acidity over 0.9%., Campbell and Robert (1995) stated that the off-flavor and odors" of milk and milk products can be placed in categories based on their causative factors.

4.1.4. Specific gravity (SG) of fresh milk

As shown in table (3) the Specific gravity (SG) of fresh milk was 1.030 the value is in line with Tamime (2009) who reported specific gravity of normal milk within 1.027 -1.035 g per ml with a mean value of 1.032 g per ml. O'Connor, (1994), Morris, (1999) had also reported normally milk has a specific gravity between 1.027 and 1.035 with an average value of 1.032 at I6^{o}C which is similar to our finding in table 3.

4.1.5. Fat content of fresh milk

The fat content of the fresh milk was 3.8% (table 3) which is in agreement with Tamime (2009) he stated fat of unprocessed fresh milk should not be less than 3.5% according to the European Union quality standard.

4.1.6. Solids nonfat (SNF)

As shown in table (3)The sold nonfat was 9.10% , this result is in line with that stated in European Union quality standards for unprocessed whole milk, Solid-not fat content should not be less than 8.5% (Tamime, 2009).

4.1.7. Total Solids (TS)

Total solid of raw milk was 12.75 % ,this result is approximately similar to that for European Union established quality standards for total solids content of cow milk is not less than 12.50/0 (FAO/WHO, 2007).

4.1.8. Antibiotic test

Antibiotic test show negative result for milk sample.

Table 3: Properties and composition of fresh cow milk

parameters	Value N= 3±SD
Temperature ° C	5.33 ± 0.22
Hydrogen ion concentration	6.72±0.9
Acidity%	0.142± 0.002
Fat%	3.8±0.12
Solid non -fat %	9.1±0.98
Protein %	3.3±0.7
Lactose %	4.47±0.43
Specific gravity%	1.030±0.024
Total solid %	12.75±0.984

* Values are means and standard deviations for triplicate independent analysis

4.2 Microbiological Analysis for fresh milk ،and during yoghurt Manufacturing stage.

Microbial counts were presented as colony forming units per ml (log CFU/ml) of milk samples.

4.2.1 Coliform count

Coliform count for the fresh milk gave positive reading of 3.7 log10 cfu/m1 which is in agreement with that reported by Fekadu (1994) who found coliform counts of 3.8-4 log 10 cfu/m1 while Zelalem and Bernard (2006) obtained higher coliform count of 6.57 log 10 cfu/ml for cow milk collected from different producer. Both Felcadu and Zelalem and what we obtained in this Study was in accordance with Shojaei and Yadollahi (2008). That stated the acceptable limits of Coliform counts in fresh milk should be less than 100

cell/ml (21og/ml).coliform count of milk yogurt was 3.8 as we added dry ingredient to standardize the total solid, while it was NIL after pasteurization.

4.2.2 Total bacterial count

The total bacterial count of fresh milk was 4.8 log 10 cfu/ml which is less than that reported by Fecadu (1994) that the maximum (TVC) of raw cow milk must be.6 to 8.81ogl0 cfu/m1 also the result is in agreement with the Regulative EU (Regulation853, 2004) which state the average to all number of microorganism should not exceed 100000 per ml (5 log 10 cfu/ml) of raw cow milk from primary production. The total bacterial count also increased at mixing stage then finished after pasteurization stage table 4.

4.2.3. Mesophilic bacteria lab pasteurization count

The acceptable amount of total Mesophilic bacteria count in pasteurized milk is less than 4.699 log cfu/ml (Berry ,2004).The Mesophilic bacteria result of raw milk samples used in this study was 2.3 log10 cfu/ml) which is less than that reported by Berry (2004).

4.2.4 Thermophilic bacteria lab pasteurization count

Thermophilic bacteria lab pasteurization count was 1 log 10 cfu / m1 which is similar to finding by Siddig (2015) he found that the lab thermophilic pasteurization count was 1.07 log 10 cfu/m1.

Table 4: Microbial analysis for fresh cow milk and milk yoghurt during manufacturing steps.

Samples	Total bacterial	Coliform	Yeast and Mould	LPC mesophilic	LPC Thermophilic
		Cfu/ml			
Raw milk	**$4.8*10^5$± 0.35**	**$3.7*10^4$**±0.3	**Nil**	2.3±0.0195	1±0.002
Mixing step	**$5.1*10^2$**±0.5	**$3.8*10^2$**±0.2	**Nil**	**-**	**-**
After pasteurization	**43**±01.9	**Nil**	**Nil**	**-**	**-**

* Values are means and standard deviations for triplicate independent analysis.

LPC= lap pasteurization count.

4.3. Physicochemical properties and chemical content during mixing step

4.3.1. Titratable acidity (TA) of milk during mixing process

Titratable acidity of milk yoghurt Table (5) during mixing stage was 0.215% as compared to 0.142% for fresh milk due to addition of the skimmed and whole milk.

4.3.2 Total solid (TS) content of milk during mixing stage and after pasteurization

The total solid of mixed milk yoghurt table (5) during mixing stage was 14.50%. The result was similar to Tamime and Robinson (1999) who stated that milk solids contents of many commercial yoghurt products ranged between l4-15%.this high value came from the addition of skimmed and whole milk powder. While after pasteurization process was less than before pasteurization as it effected by heat treatment.

4.3.3 Solids non -fat (SNF) content of milk during mixing stage after pasteurization

SNF during mixing throughout this study ranged between 11.5 to 12 % which was higher than that of fresh milk 9.1% and little bit less after pasteurization process.

4.3.4 Fat content of milk during mixing stage

Table (5) showed the fat content of milk yoghurt during mixing process. The fat content of milk yoghurt was 3% as shown in the table which is lower than its level in fresh milk (4) % due to addition of water.

4.3.5 Specific gravity (SG) of milk during mixing stage

Specific gravity of milk yoghurt was 1.043 which is accordance with the result of Tamime and Robinson (1999) during mixing step for yoghurt.

Generally as presented in (table 4) the fat, SNF, SG, protein slightly decreased after pasteurization as compared to their level before pasteurization except in moisture content ($p<0.o5$) these change was not significant in fat, SNF, and protein due to pasteurization.

Table5: Physiochemical properties and chemical content of milk ،yoghurt during the mixing step before and after pasteurization:

Parameters	Mixing before pasteurization	Mixing after pasteurization
Fat	3 ± 0.057^{A}	2.99 ± 0.057^{A}
SNF	11.5 ± 0.100^{B}	11.4 ± 0.057^{B}
TS	14.5 ± 0.153^{C}	14.4 ± 0.005^{D}
SG	1.043 ± 0.00015^{E}	1.0426 ± 0.00006^{F}
Protein	4.2 ± 0.057^{G}	4.13 ± 0.115^{G}
Moisture	85.5 ± 0.153^{H}	85.6 ± 0.003^{1}
AT	0.215 ± 0.2	0.2 ± 0.13

Values are mean ± SD for replicate independent runs

Values that bearing same subscript letter in the same rows are not significantly different at $p>0.05$

4.4. Physiochemical properties and chemical content of different set yoghurt in the first day of shelf life

Table 6 gives the results of chemical analysis of yogurt samples on the 1st Storage day. It is seen that ash, fat and protein contents change between 0.877 and 0.887%, 2.98 and 3.10اه and 3.76 and 4.09%, respectively. C1 yoghurt gave the highest fat, protein rate while C3 was highest in ash content. Dry matter content of C4 yoghurt was found to be significantly lower than that of C1, C2, C3 ($p<0.01$). Acidity was the highest in C1 yoghurt the pH value of the C1 sample was found lower than the other yoghurt (C2, C3, and C4). Generally, higher pH values was found in C4 yogurt than other yoghurt samples. Kailasapathy et al (2008) stated that as the buffering capacity of yogurt increases then pH changes decrease depending on the changes in acid content of the food system. These findings are convenient with the findings in the present study. Water holding capacity is among the quality parameters of yogurt, high rate of which implies high quality (Mahmoud et al 2008). C3 yoghurt reflected higher water holding capacity rate (69%) on the 1st storage day than the others. Due to the contracting effect of low pH on casein particles and so increased resistance of yogurt to syneresis (Lucey & Singh 1997).

The changes in the mineral contents of the yogurt samples. K and Na contents decreased significantly in the C1, C2, C3 yoghurt compared to control (C1) ($P<0.05$). And also (C1) yoghurt showed the highest rates of Ca, p values. Lactose content (%) of C1 yoghurt was found to be significantly lower than that of the C2, C3, and C4.

Table 6: Physiochemical properties and chemical content of different set yoghurt

- **Values are mean** ± SD **for replicate independent runs**

Sample Name	Fat %	SNF %	TS %	Protein %	Moisture %	Lactose %	pH %	Acidity %	Ash %	WHC %	Calcium %	Sodium %	Potassium %	Phasphour %
C1	3.10333	11.0833	14.32	4.09667	85.7133	4.25	4.44	0.9717	0.87733	66	209.333	81.000	15161	139.000
	±0.00577^{A}	±0.0153^{A}	± 0.0115	± 0.00577^{A}	± 0.0115^{B}	± 0.01000^{A}	± 0.010^{D}	± 0.0189^{A}	±0.0202^{A}	± 2.42 ^{A}	± 1.155^{A}	± 1.000^{A}	± 2.52^{A}	± 1.000 ^{A}
C2	3.07333	11.1533	14.2267	3.9333	4.35	4.35	4.46333	0.9533	0.874633	65	201.00	79.333	250.333	134.333
	±0.00577^{B}	± 0.0115^{A}	± 0.0058^{A}	± 0.0289^{B}	± 0.0058^{AB}	±0.0208^{B}	± 0.00577^{C}	± 0.0208^{AB}	±0.01415^{AB}	± 3.13^{A}	±1.73^{B}	± 0.577^{A}	± 0.577^{AB}	± 0.577^{B}
C3	3.02667	11.243	14.230	3.8433	85.730	4.38	4.48667	0.91333	0.887150	69	196.333	77.000	248.667	131.333
	±0.00577^{C}	± 0.196^{A}	± 0.199^{A}	± 0.0208^{C}	± 0.199^{AB}	±0.0200^{B}	± 0.00577^{B}	± 0.01155^{BC}	±0.00100^{B}	± 2.05^{A}	± 1.528^{BC}	± 1.000^{B}	± 0.577^{B}	± 1.155 ^{C}
C4	2.98000	11.0367	14.12	3.76333	85.9833	4.5067	4.51667	0.89667	0.883100	67	192.67	75.333 ±	244.000	128.667
	±0.01000^{D}	± 0.0058^{A}	± 0.0058^{D}	±0.01528^{D}	± 0.0058 ^{A}	±0.0379^{C}	± 0.00577^{A}	± 0.00577^{C}	± 0.00500^{B}	± 3.15^{A}	± 2.52^{C}	± 0.577^{B}	± 1.000^{C}	± 1.155^{D}

Values that bearing different superscript letters in the same column are significantly different at $p<0.05$.

- C1 ≡ set yoghurt produced with lactic acid culture. C2 ≡ set yoghurt produced with *B.infantis* 20088
- C3 E set yoghurt produced with *B. longum* BB536. C4Set yoghurt produced with the mixer of strain BB536and strain 20088

4.5. Growth of different bacterial culture in yoghurt and its survival during the storage

The changes in the viable counts of yoghurt and probiotic bacteria during the storage as shown in table 7 were affected by culture type. The highest viable count in all yoghurt was at the beginning of the storage.

L.bulgaricus counts decreased throughout the storage, the highest counts at initial was (8.32 log CFU /ml) whereas the lowest value on the day 15 was 7.25 log 10 CFU/m1.

The highest count for *bifidobacteruim infantis20088* at initial was 8.89 log CFU ml, whilst the lowest value was at the end of storage (7.10 log CFU/ml).

The highest *B. longum* BB536 count was (8.1 log CFU/ml) was at the beginning of shelf life study, the lowest count at the end of storage (as 7.13 log CFU/m1

For all bio-yoghurts the probiotic populations was more than 7 log CFU/ml at the end of 15-day-storage. This level in yoghurt can exert health-promoting effects on human. In general, the food industry applies the recommended level of 106 cfu at the time of consumption for probiotic product to have the expected health effects (Gomes, 1995) According to the results of present study, all probiotic strains grew well and exhibited satisfactory viability levels a storage. The major factors affecting the viability of probiotic microorganisms during manufacture and storage of fermented products include: strains selected, acidity, storage temperature, oxygen content, pH and hydrogen peroxide due to bacterial metabolism, inoculation level, fermentation time, post-acidification and food matrix.

However in the study by Vinderola *et al;* (2000) they observed that initial counts of probiotic bacteria in yoghurt ranged from 6-7 log CFU/ml, while the final counts were lower than 4 log CFU/m1

Carr and Ibrahim (2005) investigated the level of bifidobacteria in commercial yoghurts and concluded that 76% of products contain viable probiotics but their populations were at or Below 6 log CFU/m1 While This high level of viable count of probiotic is beneficial to utilize these products on industrial scale to manufacture functional products such as dairy and non-dairy products (Gajarbeygi, 2001)

4.6. Physicochemical analysis of yoghurt during storage

4.6.1 Titratable acidity (%)

Table (8) shows the effect of culture type and storage on titratable acidity (%) of set yoghurt.

The acidity of samples showed significant increase ($P<0.05$) during the storage due to acid production during shelf life in all type of yoghurt. Bulgaricus is the main bacteria responsible for acid production in control yoghurt (C1) which had the highest acidity at the beginning (0.97%) and at the end (1.21%) of the storage. The lowest acidity was observed in the yoghurt (C4) at the end of the storage (1.07°/o). The level of acidity in probiotic yoghurt (C2, C3, and C4) was found to be lower than control yoghurt as it shown in table 7. These results were in agreement with Singh *et al,* (2011) Vahicic and Hruskar (2000), Guler-Akin and Akin (2007), and Ozer *et al*, (2005) they stated the acidity of set yoghurt is 1 to 1.2%. It appears that the composition of starter culture, fermentation temperature and storage could influence the overall level of acidity and pH of stored yoghurt samples (Singh, *et al;* (2011).

The titratable acidity increased gradually till the end of the storage. This could be attributed to the changed of 0rganic acids content in yoghurt during cold storage, in addition to decrease in pH of yoghurt during storage (Fernandez-Garcia *et al;* 1994). The result was in accordance with that of Kavas *et al* (2003) who reported that the acidity increase in yoghurt during the storage was also to be significant.

Our result also are in line with finding by tarakci and erdogan (2003) in which acidity increase over storage. Guler and Mutlu (2005) also observed an increase in TTA during the storage. But these result are not in line with Kroger (1976) who reported that in yoghurt the probiotic culture tend to produce acid so ultimately the acidity of probiotic yoghurt increase. But in case of natural yoghurt there is no bio-live culture, so a decrease of acidity is expected.

Development of acidity during shelf life of yoghurt is due to the conversion of lactose to lactic acid which was higher in control yoghurt as compared to probiotic yoghurts. (Singh *et al*, 2011).

4.6.2 pH Value

Tables (9) Show the effect of culture type and storage on pH level of set yoghurt. The highest pH value (4.50) was obtained in yoghurt (C4) yoghurt. The lowest pH was showed (4.4) in yoghurt of LAB (C1)

Storage significantly ($P \leq .05$) affected the pH value. The pH value decreased as the storage progressed. The highest pH value obtained at the beginning of the storage while the lowest at the end ($P<0.05$). Fernandez-Garcia et al, (1994) found that the content of 0rganic acids in yoghurt during fermentation and cooled storage of yoghurt continuously changed, and this

affect pH of yoghurt during storage. The pH-values were decreased progressively due to excessive sugar fermentation and presence of lactic acid (Abdel Razig *et al*, 2014).

As shown in table 8 pH did not fall below pH 4.0, which is generally considered detrimental to the survival of probiotic bacteria. (Dave and. Shah, 1997).

4.6.3 Water holding capacity WHC./o

Table: 10 Shows the effect of culture type and storage on water holding capacity of set yoghurt. The highest value of WHC % (67%) was obtained in set yoghurt inoculated with the mixture of *B.infantis and B.longumf (C*4), Samples show significant difference during the shelf life ($p< 0.05$)

The percentage of WHC of yoghurt through the storage at 4°C were decreased, these changes are significantly different ($p< 0.05$) between probiotic (C2, C3, C4), and non-probiotic (C1).

WHC was minimum in yoghurt (C 1) and was maximum in probiotic yoghurt. The obtained results are in accordance with the results of the previous works where they showed that the percentage of WHO was directly related to the TA and inversely to pH value changes (Amigo, 1999).

Table 7: The viability of culture (cfu/m1) of different set yoghurt during storage

Storage (days)	Bacterial growth of starter culture			
	C_1 cfu/ml	C_2 cfu/ml	C_3 cfu/ml	C_4 cfu/ml
0	8.32 ± 0.01^{A}	8.9 ± 0.00577^{A}	8.1 ± 0.004^{A}	8.0 ± 0.002^{A}
3	8.05 ± 0.05^{A}	8.75 ± 0.002^{A}	8.15 ± 0.01^{A}	7.6 ± 0.01^{A}
6	7.9 ± 0.01^{A}	8.6 ± 0.023^{A}	7.9 ± 0.01^{B}	7.6 ± 0.01^{A}
9	7.4 ± 0.02^{B}	7.91 ± 0.001^{B}	7.51 ± 0.002^{AB}	7.6 ± 0.002^{B}
12	7.3 ± 0.01^{AB}	7.9 ± 0.002^{B}	7.5 ± 0.0021^{AB}	7.6 ± 0.003^{B}
15	7.25 ± 0.02^{AB}	7.4 ± 0.0024^{AB}	7.13 ± 0.03^{AB}	7.4 ± 0.5^{AB}

- Values are mean ± SD for replicate independent runs.
- Values that bearing different superscript letters in the same column are significantly different at $p<0.05$.
- C1 ≡ set yoghurt produced with lactic acid culture
- C2 ≡ set yoghurt produced with *B.infantis* 20088 , C3 E set yoghurt produced with *B. longum* BB536
- C4 ≡ Set yoghurt produced with the mixer of strain BB536and strain 20088.

Table 8: Acidity (%) of different yoghurt during storage

Yoghurt	Bacterial growth of starter culture					
type	0	3	6	9	12	15
C_1	0.97 ± 0.02^{A}	1.0 ± 0.005^{A}	1.06 ± 0.001^{A}	1.3 ± 0.0100^{A}	1.12 ± 0.0057^{C}	1.21 ± 0.010^{A}
C_2	0.95 ± 0.21^{AB}	0.9 ± 0.004^{C}	1.01 ± 0.012^{B}	1.2 ± 0.006^{B}	1.14 ± 0.0115^{B}	1.13 ± 0.0052^{B}
C_3	0.91 ± 0.012^{BC}	0.97 ± 0.002^{B}	0.99 ± 0.001^{C}	1.24 ± 0.002^{C}	1.15 ± 0.0011^{AB}	1.16 ± 0.004^{C}
C_4	0.9 ± 0.006^{C}	0.95 ± 0.001^{C}	0.98 ± 0.004^{C}	1.23 ± 0.003^{C}	1.16 ± 0.0026^{A}	1.07 ± 0.002^{C}

- Values are mean ± SD for replicate independent runs.
- Values that bearing different superscript letters in the same column are significantly different at $p<0.05$.
- C1≡ set yoghurt produced with lactic acid culture
- C2 ≡set yoghurt produced with *B.infantis* 20088 , C3 ≡set yoghurt produced with *B. longum* BB536
- C4≡Set yoghurt produced with the mixer of strain BB536and strain 20088.

Table 9: The pH of different yoghurt during storage

Yoghurt	Bacterial growth of starter culture					
type	0	3	6	9	12	15
C_1	4.4 ± 0.01^{D}	4.38 ± 0.002^{D}	4.3 ± 0.0022^{A}	4.25 ± 0.005^{A}	4.18 ± 0.005^{A}	4.12 ± 0.010^{C}
C_2	4.5 ± 0.001^{C}	4.4 ± 0.021^{A}	4.33 ± 0.0057^{B}	4.3 ± 0.00577^{B}	4.22 ± 0.0152^{AB}	4.15 ± 0.0051^{B}
C_3	4.49 ± 0.002^{B}	4.4 ± 0.003^{A}	4.4 ± 0.0152^{C}	4.28 ± 0.00577^{C}	4.2 ± 0.0115^{BC}	4.19 ± 0.011^{A}
C_4	4.50 ± 0.001^{A}	4.42 ± 0.02^{A}	4.3 ± 0.0057^{D}	4.35 ± 0.00577^{D}	4.28 ± 0.010^{C}	4.19 ± 0.0057^{A}

- Values are mean ± SD for replicate independent runs.
- Values that bearing different superscript letters in the same column are significantly different at $p<0.05$.
- C1 ≡ set yoghurt produced with lactic acid culture
- C2≡ set yoghurt produced with *B.infantis* 20088 , ≡C3 Set yoghurt produced with *B. longum* BB536
- C4≡Set yoghurt produced with the mixer of strain BB536and strain 20088

Table 10: Water holding capacity (%) of different yoghurt during storage:

Yoghurt	Bacterial growth of starter culture					
type	0	3	6	9	12	15
C_1	36 ± 2.42^{A}	58 ± 3.85^{B}	56 ± 2.95^{A}	53 ± 3.33^{B}	50 ± 2.85^{B}	49 ± 2.1^{A}
C_2	64 ± 3.13^{A}	62 ± 1.90^{A}	59 ± 3.86^{A}	57 ± 2.11^{AB}	53 ± 2.50^{AB}	52 ± 3.2^{B}
C_3	65 ± 2.05^{A}	64 ± 3.60^{A}	65 ± 2.10^{A}	63 ± 2.15^{A}	60 ± 3.01^{A}	57 ± 2.4^{C}
C_4	67 ± 3.15^{A}	67 ± 3.60^{A}	64 ± 3.63^{A}	63 ± 4.01^{AB}	61 ± 3.13^{AB}	60 ± 2.8^{B}

- Values are mean ± SD for replicate independent runs.
- Values that bearing different superscript letters in the same column are significantly different at $p<0.05$.
- C1 ≡ set yoghurt produced with lactic acid culture
- C2 ≡ set yoghurt produced with *B.infantis* 20088 , C3 ≡ set yoghurt produced with *B. longum* BB536
- C4 ≡ Set yoghurt produced with the mixer of strain BB536and strain 20088

4.6.4 Total solid (TS)

Table (11) shows the effect of culture type and storage on total solid (%) of set yoghurt the highest value (14.42°/o) was obtained in control yoghurt (C1) at the beginning of shelf life study. The lowest TS value (14.30%) was showed by set yoghurt inoculated with the mixture of *B. infant and B.longum* (sample C4) while the other samples ranked in an intermediate in its TS content.It was found that yoghurt inoculated with probiotic cultures (C2, C3, C4) not vary too much from that inoculated with lactic acid bacteria (C1) in the total solid content .These results are in line with the findings of Younus *et al,* (2002). They found not too much vary between probiotic and non-probiotic yoghurt.

It was observed that the total solid increased as the storage progressed, this result was in agreement with Anjum *et al*, (2007) who reported that treatment and storage had significant effect on the total solids of yoghurt samples prepared by locally isolated starter culture and commercial starter culture. Kavas *et al,* (2003) reported that it is accepted that the increase during 14 days on total solids content was not significant and attributed to the evaporation, this supported by Akalin (1993) who reported that the TS increase determined during the storage is normal.

However the result disagreed with Vasiljevic and elen (2002) that started reduction in TS could be due to the utilization of sugar by the starter cultures. It is evident from the result that reduction in total solids throughout storage might be due to change of lactose into lactic acid by lactose fermenting bacteria in yoghurt. These results were confirmed Tamime and Robinson, (1985).

4.7 Chemical content of different yoghurt during storage:

4.7.1 Protein content (%)

Table (12) Shows the protein content (%) of set yoghurt. The highest value was obtained by the control yoghurt (C1) as 4.09%. The lowest protein content (%) by the set yoghurt inoculated with mixture of (*B.infantis and B.longum)* as 3.76%. While the other yoghurt ranked in an intermediate protein level this result is in line with finding of janhog *et al,* (2006) who reported the protein of yoghurt ranged from 3.4-6%.

Storage significantly ($P \leq 0.05$) affected the protein content of different set yoghurt. The protein content decreased as the storage progressed. The highest value obtained at the beginning of the storage while the lowest at the end of the storage.

These results are similar to Shanley (1973) who found that the protein and ash contents of yoghurt decreased with the progress of storage. Also Galal *et al,* (2004) Reported that the protein content during storage decreased in all samples refer to decrease in total solids content during storage and breakdown of amino acids by starter culture. Serra *et al,* (2009) reported that in all treatments studied, caseins were hydrolyzed and hydrophobic peptides were increased during storage, as reflected by the increase in soluble nitrogen at the end of the storage. The result disagree with Koestanti and Romziah (2008) who reported that during the fermentation process, the *Lactobacillus bulgaricus* and *Streptococcus thermophilus* microbe biomass were increased, thus the sum of microbe protein was increase, that automatically increasing protein.

Table 11: Total solid content (%) of different set yoghurt during storage

Yoghurt type	Bacterial growth of starter culture					
	0	**3**	**6**	**9**	**12**	**15**
C_1	14.42 ± 0.0115^{A}	14.4267 ± 0.005^{A}	14.5 ± 0.058^{A}	14.6 ± 0.0058^{A}	14.7 ± 0.02^{A}	14.5 ± 0.001^{A}
C_2	14.4 ± 0.0058^{AB}	14.3367 ± 0.005^{A}	14.4 ± 0.01^{B}	14.4 ± 0.0058^{B}	14.39 ± 0.02^{B}	14.4200 ± 0.0100^{B}
C_3	14.35 ± 0.199^{AB}	14.36 ± 0.0118^{B}	14.37 ± 0.0100^{C}	14.36 ± 0.01^{C}	14.38 ± 0.01^{B}	14.3933 ± 0.0058^{C}
C_4	14.3 ± 80.00^{B}	14.32 ± 0.020^{C}	14.34 ± 0.0100^{D}	$14.35 \pm 0.00.58^{D}$	14.35 ± 0.01^{B}	14.36 ± 0.0042^{D}

- Values are mean ± SD for replicate independent runs.
- Values that bearing different superscript letters in the same column are significantly different at $p<0.05$.
- C1 ≡ set yoghurt produced with lactic acid culture
- C2 ≡ set yoghurt produced with *B.infantis* 20088 , C3 ≡ set yoghurt produced with *B. longum* BB536
- C4 ≡ Set yoghurt produced with the mixer of strain BB536and strain 20088

Table 12: Protein content (%) of different set yoghurt during storage

Yoghurt type	Bacterial growth of starter culture					
	0	**3**	**6**	**9**	**12**	**15**
C_1	3.2 ± 0.005^{A}	3.86 ± 0.0057^{A}	3.14 ± 0.03^{A}	3.70 ± 0.002^{A}	3.59 ± 0.01^{A}	3.54 ± 0.010^{A}
C_2	3.07 ± 0.006^{B}	3.0466 ± 0.003^{B}	3.02 ± 0.006^{B}	3.65 ± 0.015^{B}	3.51 ± 0.10^{B}	3.50 ± 0.077^{B}
C_3	3.03 ± 0.005^{C}	2.96 ± 0.003^{C}	2.95 ± 0.004^{C}	3.50 ± 0.006^{C}	3.50 ± 0.005^{B}	3.4 ± 0.0058^{C}
C_4	2.98 ± 0.01^{D}	2.9 ± 0.032^{D}	2.89 ± 0.01^{D}	3.51 ± 0.01^{D}	3.51 ± 0.01^{B}	3.4 ± 0.0054^{D}

- Values are mean ± SD for replicate independent runs.
- Values that bearing different superscript letters in the same column are significantly different at $p<0.05$.
- C1 ≡ set yoghurt produced with lactic acid culture
- C2 ≡ set yoghurt produced with *B.infantis* 20088 , C3 ≡ set yoghurt produced with *B. longum* BB536
- C4 ≡ Set yoghurt produced with the mixer of strain BB536and strain 20088

4.7.2 Fat content (%)

Table (13) shows the fat content (%) of different set yoghurt.

Storage significantly ($P<0.05$) affected the fat content. The fat content decreased as the storage progressed. The highest fat level was obtained at the beginning of the storage .Abdel-Salam *et al* (1996) found that, the fat content slightly decreased due to fat hydrolysis and liberation of free acids that escape determination by Gerber method. Tamime and Deeth (1980) reported a decrease in fat content of yoghurt during storage due to lipolysis in yoghurt. The decreased level of fat is probably a result of the decrease of whey (Kosikowski and Mistry, 1997). On the other hand this result was disagreed with Anjum *et al*, (2007) who reported that the fat content of yoghurt, displayed statistically not significant difference for reduction in fat content at the end of storage that might be due to production of volatile fatty acids by yoghurt organism.

4.7.3 Moisture content

As shown in table (14) Storage period significantly ($P\leq0.05$) affected the moisture content. The moisture content decreased as the storage progressed. The highest moisture content was at the beginning of the storage, while the lowest was at the end. The decrease might be due to breakdown of macro component and release of water.

Haq (1974) reported a decrease in moisture content in yoghurt during storage to be 86.03 to 83.340ه‍ا which is similar to our finding.

4.7.4. Ash content

As shown in table (15) slight increases were observed in ash content of all set yoghurt during the 15 day-storage. The slight increase in ash contents was because of the loss of Co2 and water. The results are in agreement with the findings of Guler (2005) who found that the ash value of probiotic yoghurt as 0.95%.

4.7.5 Lactose

As shown in table (16) Storage significantly ($P \leq 0.05$) affected the lactose content of yoghurt. The lactose value decreased as the storage progressed. Due to fermentation of the lactose with different starter.

The average of lactose content in the present study was 4.19% for yoghurt of (C1) in the end of storage, yogurt of *B.longum and B. Infantis* was higher in lactose content compared to that of LAB yoghurt (C1). Lactose content of different yoghurt was higher than that estimated by Aisha (2009) which was (3.28%) and similar to that reported by Elamin and Wilcox (1992) which was (4.2%). This variation might be attributed to the culture type and the acid formation during the storage.

Table 13: Fat content (%) of different set yoghurt during storage

Yoghurt	Bacterial growth of starter culture					
type	0	3	6	9	12	15
C_1	3.2 ± 0.005^{A}	3.15 ± 0.0057^{A}	3.14 ± 0.03^{A}	3.06 ± 0.0462^{A}	2.99 ± 0.010^{A}	2.95 ± 0.005^{A}
C_2	3.07 ± 0.006^{B}	3.0466 ± 0.003^{B}	3.02 ± 0.006^{B}	2.99 ± 0.010^{B}	2.89 ± 0.005^{B}	2.82 ± 0.01^{B}
C_3	3.03 ± 0.005^{C}	2.96 ± 0.003^{C}	2.95 ± 0.004^{C}	2.9 ± 0.0152^{C}	2.86 ± 0.015^{B}	2.84 ± 0.010^{BC}
C_4	2.98 ± 0.01^{D}	2.9 ± 0.032^{D}	2.89 ± 0.01^{D}	2.85 ± 0.00577^{D}	2.83 ± 0.005^{B}	2.81 ± 0.01^{D}

- Values are mean ± SD for replicate independent runs.
- Values that bearing different superscript letters in the same column are significantly different at $p<0.05$.
- C1 ≡ set yoghurt produced with lactic acid culture
- C2 ≡ set yoghurt produced with *B.infantis* 20088 , C3 ≡ set yoghurt produced with *B. longum* BB536
- C4 ≡ Set yoghurt produced with the mixer of strain BB536and strain 20088

Table 14: Moisture content of different set yoghurt during storage (%)

Yoghurt type	Bacterial growth of starter culture					
	0	**3**	**6**	**9**	**12**	**15**
C_1	85.6 ± 0.0115^{B}	85.5 ± 0.005^{B}	85.5 ± 0.0014^{d}	85.5 ± 0.0115^{A}	85.5 ± 0.0208^{B}	85.48 ± 0.0058^{A}
C_2	85.68 ± 0.0058^{AB}	85.6 ± 0.005^{AB}	85.65 ± 0.15^{C}	85.6 ± 0.0058^{B}	85.6 ± 0.0252^{A}	85.58 ± 0.0100^{B}
C_3	85.72 ± 0.199^{AB}	85.7 ± 0.012^{AB}	85.70 ± 0.01^{B}	85.6 ± 0.199^{C}	85.65 ± 0.01^{A}	85.6 ± 0.0058^{C}
C_4	85.76 ± 0.0058^{A}	85.7 ± 0.020^{A}	85.728 ± 0.012^{A}	85.7033 ± 0.006^{D}	85.68 ± 0.01^{A}	85.6 ± 0.012^{D}

- Values are mean ± SD for replicate independent runs.
- Values that bearing different superscript letters in the same column are significantly different at $p<0.05$.
- C1 ≡ set yoghurt produced with lactic acid culture
- C2 ≡ set yoghurt produced with *B.infantis* 20088 , C3 ≡ set yoghurt produced with *B. longum* BB536
- C4 ≡ Set yoghurt produced with the mixer of strain BB536and strain 20088

Table 15: Ash content of different set yoghurt during storage (%)

Yoghurt	Bacterial growth of starter culture					
type	0	3	6	9	12	15
C_1	0.97 ± 0.02^{A}	0.95 ± 0.002^{A}	0.953 ± 0.006^{A}	0.954 ± 0.02^{A}	0.96 ± 0.00577^{A}	0.96 ± 0.043^{A}
C_2	0.94 ± 0.01^{AB}	0.945 ± 0.01^{B}	0.95 ± 0.057^{B}	0.96 ± 0.01^{AB}	0.961 ± 0.0100^{AB}	0.97 ± 0.015^{A}
C_3	0.93 ± 0.001^{B}	0.94 ± 0.002^{B}	$0.945\ 0.0059^{B}$	$0.95\ 0.001^{B}$	$0.96\ 0.0095^{B}$	$0.96\ 0.002^{B}$
C_4	0.92 ± 0.005^{B}	0.93 ± 0.010^{B}	0.94 ± 0.005^{B}	0.945 ± 0.001^{AB}	0.955 ± 0.057^{AB}	0.96 ± 0.001^{B}

- Values are mean ± SD for replicate independent runs.
- Values that bearing different superscript letters in the same column are significantly different at $p<0.05$.
- C1 ≡ set yoghurt produced with lactic acid culture
- C2 ≡ set yoghurt produced with *B.infantis* 20088 , C3 ≡ set yoghurt produced with *B. longum* BB536
- C4 ≡ Set yoghurt produced with the mixer of strain BB536and strain 20088

Table 16: Lactose content (%) of different set yoghurt during storage

Yoghurt type	Bacterial growth of starter culture					
	0	3	6	9	12	15
C_1	4.3 ± 0.01^{A}	4.28 ± 0.0643^{A}	4.27 ± 0.001^{A}	4.25 ± 0.005^{A}	4.22 ± 0.005^{C}	4.19 ± 0.0347^{C}
C_2	4.28 ± 0.0208^{A}	4.27 ± 0.0723^{A}	4.26 ± 0.02^{B}	4.24 ± 0.003^{AB}	4.22 ± 0.003^{B}	4.17 ± 0.025^{A}
C_3	4.27 ± 0.02^{A}	4.25 ± 0.01^{A}	4.24 ± 0.012^{B}	4.21 ± 0.002^{B}	4.20 ± 0.0057^{AB}	4.15 ± 0.015^{B}
C_4	4.27 ± 0.04^{A}	4.24 ± 0.02^{A}	4.21 ± 0.014^{C}	4.19 ± 0.01^{C}	4.18 ± 0.01	4.13 ± 0.004^{C}

- Values are mean ± SD for replicate independent runs.
- Values that bearing different superscript letters in the same column are significantly different at $p<0.05$.
- C1 ≡ set yoghurt produced with lactic acid culture
- C2 ≡ set yoghurt produced with *B.infantis* 20088 , C3 ≡ set yoghurt produced with *B. longum* BB536
- C4 ≡ Set yoghurt produced with the mixer of strain BB536and strain 20088

4.8 Minerals content (mg/100gm) of different yoghurt during storage

Mineral analysis of different yoghurt (mg/100g) including calcium. Phosphorus, sodium and potassium were shown on the Tables l5, 16.17and 18 during the shelf life of the yoghurt. The results justifies the accretion of Ihemeje *et al* (2015) who stated that yoghurt is a very good source of essential minerals needed for human metabolism or functionality of cells.

4.8.1The calcium (Ca) contents of different yoghurt as shown in table (17) is ranged from 192 to 209 (mg/100gm) at initial storage there is significant decreases ($p>0.05$) in calcium between different yoghurt product the highest calcium content as compared to different yoghurt product was in the control yoghurt.

4.8.2The sodium (Na) contents of different yoghurt as shown in table (18) ranged from 77 to 82 (Mg/100gm) in yoghurt inoculated with LAB (C1) during the study period, and from 77 to 79 mg/100gm for yoghurt inoculated with 5. *infantis,* and ranged from 75 to 78mg/ 100gm for the yoghurt with *B. longiim.* While it Range from 74 to 76 for yoghurt of the mix of *B. longum* and *B. infantis* during the storage. Over all there is significant decreases in sodium content of each specific yoghurt during the storage.

4.8.3The potassium (K) contents of different yoghurt as shown in table 19 ranged from 232 to 254 (mg/100gm). With LAB yoghurt, and *B. infantis* sample ranging from 234. To 239(mg/100gm) 235 To 240 and 234. To 237.0 (mg/100gm) respectively. There is no significant decreases ($p>0.05$) between the yoghurt at the beginning of fermentation.

Results obtained for calcium, was higher than that obtained by De la

Fuente *et al,* (2003). While those of sodium, potassium and phosphorus were lower. There are numerous factors which affect yoghurts chemical composition. Mainly the methods of fortification used to increase the solid content, which is a common practice during yoghurt manufacture. A wide range of total solids and other minerals (sodium and potassium) was also found in the yoghurts studied by De la Fuente *et al,* (2003) indicating the possible addition of different dairy fractions or products. However, this supply can represent an advantage from a nutritional point of view as a source of essential nutrients in diet in comparison with other dairy products.

4.8.4 The Phosphorus (P) contents as shown in table (20) ranged from 127 to 131. Mg/100 gm in yoghurt inoculated with LAB during the study period, and from 130 to 134 mg/100 gm for sample inoculated with *B. infantis,* and ranged from 129 to 131mg/ 100 gm for the yoghurt of B. *Longum.* While it range from 128 to 129 for yoghurt of the mix of *B, longum* and *B. infantis* during the storage.

Generally, all yoghurt were significantly different ($p<0.05$) in minerals with yoghurt of *B. infantis* having the highest phosphorus contents, followed by LAB yoghurt, and the least was in yoghurt with mix cultures.

Table 17: Calcium content (mg/100 gm.) Of different set yoghurt during storage

Yoghurt type	Bacterial growth of starter culture					
	0	3	6	9	12	15
C_1	209.333±1.155^{A}	201.000 ±1.0^{A}	198.333±0.55^{A}	198.000±1.000^{A}	198.000±1.000^{A}	197.000±1.1^{A}
C_2	201.00±1.73^{B}	198.333±0.56^{B}	195.667 ± 0.51^{B}	194.333 ± 0.577^{AB}	194.333±0.577^{B}	192.000±1.2^{B}
C_3	196.333±1.52^{AB}	197.000 ±1.4^{B}	191.333 ± 0.57^{B}	191.000 ± 1.000^{C}	191.000±1.000^{C}	187.000±1.4^{C}
C_4	192.67±2.52^{C}	196.0 ±1. 2^{B}	185.000 ± 1.0^{C}	186.333 ±0.577^{D}	186.333±0.577^{D}	187.000±1.05^{C}

- Values are mean ± SD for replicate independent runs.
- Values that bearing different superscript letters in the same column are significantly different at $p<0.05$.
- C1 ≡ set yoghurt produced with lactic acid culture
- C2 ≡ set yoghurt produced with *B.infantis* 20088 , C3 ≡ set yoghurt produced with *B. longum* BB536
- C4 ≡ Set yoghurt produced with the mixer of strain BB536and strain 20088

Table 18: Sodium content (mg/100 gm.) Of different set yoghurt during storage

Yoghurt	Bacterial growth of starter culture					
type	0	3	6	9	12	15
C_1	81.000 ± 1.000^{A}	80.00 ± 1.2^{A}	79.433 ± 0.51^{A}	82.00 ± 6.08^{A}	82.00 ± 4.08^{A}	77.667 ± 0.577^{A}
C_2	79.333 ± 0.577^{A}	87.667 ± 1.1^{AB}	77.333 ± 0.47^{B}	77.400 ± 1.217^{AB}	77.400 ± 1.217^{AB}	77.733 ± 0.643^{A}
C_3	77.000 ± 1.000^{B}	77.667 ± 1.528^{AB}	75.667 ± 0.6^{C}	76.00 ± 0.004^{AB}	67.00 ± 0.00^{AB}	75.667 ± 1.155^{AB}
C_4	75.333 ± 0.577^{B}	76.500 ± 0.500^{B}	72.333 ± 0.5^{D}	74.667 ± 0.577^{B}	735.667 ± 0.577^{B}	74.000 ± 1.000^{B}

- Values are mean ± SD for replicate independent runs.
- Values that bearing different superscript letters in the same column are significantly different at $p<0.05$.
- C1 ≡ set yoghurt produced with lactic acid culture
- C2 ≡ set yoghurt produced with *B.infantis* 20088 , C3 ≡ set yoghurt produced with *B. longum* BB536
- C4 ≡ Set yoghurt produced with the mixer of strain BB536and strain 20088

Table 19: Potassium content (mg/100 gm.) of different set yoghurt during storage

Yoghurt type	Bacterial growth of starter culture					
	0	3	6	9	12	15
C_1	252.67 ± 2.52^{A}	245.667 ± 1.2^{A}	239.667 ± 0.56^{A}	238.333 ± 0.577^{A}	238.333 ± 0.577^{A}	234.333 ± 0.577^{A}
C_2	250.333 ± 0.577^{AB}	244.000 ± 1.1^{A}	239.667 ± 1.16^{A}	239.000 ± 1.000^{A}	239.000 ± 1.000^{A}	230.333 ± 0.577^{B}
C_3	248.667 ± 0.577^{B}	240.000 ± 1^{A}	240.000 ± 1^{A}	238.33 ± 2.8^{A}	238.33 ± 2.08^{A}	229.333 ± 0.517^{B}
C_4	244.000 ± 1.000^{C}	239.667 ± 1.6^{B}	237.333 ± 0.58^{B}	236.667 ± 0.577^{A}	236.667 ± 0.577^{A}	224.667 ± 0.57^{C}

- Values are mean ± SD for replicate independent runs.
- Values that bearing different superscript letters in the same column are significantly different at $p<0.05$.
- C1 ≡ set yoghurt produced with lactic acid culture
- C2 ≡ set yoghurt produced with *B.infantis* 20088 , C3 ≡ set yoghurt produced with *B. longum* BB536
- C4 ≡ Set yoghurt produced with the mixer of strain BB536and strain 20088

Table 20: Phosphor content (mg/100 gm.) of different set yoghurt during storage (mg/100 gm.)

Yoghurt	Bacterial growth of starter culture					
type	0	3	6	9	12	15
C_1	139± 1.000^{A}	139±1.0^{A}	132±0.115^{A}	131±0.577^{A}	131±0.5^{A}	129.7±0.208^{A}
C_2	134±0.577^{B}	131±1.0^{B}	131.2±0.551^{B}	130±0.265^{B}	127.7±0.265^{B}	128±0.58^{B}
C_3	133±1.155^{C}	129±1.0^{B}	129.4±0.462^{C}	129.6±0.12^{B}	129.6±0.12^{B}	128±0.058^{C}
C_4	128±1.155^{D}	128±0.577^{B}	217.9±0.100^{D}	127±0.351^{C}	127±0.351^{C}	127.6±0.100^{D}

- Values are mean ± SD for replicate independent runs.
- Values that bearing different superscript letters in the same column are significantly different at $p<0.05$.
- C1 ≡ set yoghurt produced with lactic acid culture
- C2 ≡ set yoghurt produced with *B.infantis* 20088 , C3 ≡ set yoghurt produced with *B. longum* BB536
- C4 ≡ Set yoghurt produced with the mixer of strain BB536and strain 20088

4.9 Microbial properties of different yoghurt during shelf life

4.9.1. Yeast and mould counts in different yogurt

Absence of Yeast and mould counts are considered indicative of the quality and the shelf life of set yoghurt. In this regard, yeasts and moulds were not detected in all type of set yoghurt throughout the storage, table (21). These results are in agreement with those reported by Schelz *et al*, (2006).

4.9.2. Coliform counts in different yogurt

In general, as shown in table (19) yoghurt products thought to be safe on microbial contamination because of its low pH, dominant lactic acid bacteria, and prebiotics effect. Ham *et al*, (2009).

4.10 Sensory evaluation

As shown in table (22) no significant ($P \leq 0.05$) differences in appearance of different set yoghurt except in yoghurt with mixer of *B.infantis* and *B. longum* (C4).

The results also Indicated no significant ($P \leq 0.05$) different in the flavor and consistency of different type of yoghurt (C1, C2,C3, C4). Whilst there was significant ($P < 0.05$) different in body and taste and over all acceptability between different type of yoghurt (C1, C2,C3, C4).

Table 21: Sensory Evaluation results of different set yoghurt

Yoghurt type	Bacterial growth of starter culture					
	Appearance	Flavor	Body / Texture	Taste	Consistency	Over all
C_1	4.200 ± 0.913^{A}	4.080 ± 0.802^{A}	4.240 ± 0.831^{A}	4.120 ± 0.833^{A}	4.080 ± 0.812^{A}	4.160 ± 0.850^{AB}
C_2	4.02 ± 0.812^{A}	3.840 ± 0.554^{A}	4.05 ± 0.781^{B}	4.080 ± 0.812^{AB}	3.840 ± 0.688^{A}	4.280 ± 0.843^{A}
C_3	3.840 ± 0.898^{A}	3.920 ± 0.997^{A}	3.8 ± 0.935^{AB}	3.840 ± 0.624^{AB}	3.840 ± 0.624^{A}	3.800 ± 0.645^{AB}
C_4	3.200 ± 0.0707^{A}	3.86 ± 0.879^{A}	3.60 ± 0.726^{AB}	3.560 ± 0.583^{B}	3.680 ± 0.690^{A}	3.6402 ± 0.638^{B}

- Values are mean ± SD for replicate independent runs.
- Values that bearing different superscript letters in the same column are significantly different at $p<0.05$.
- C1 ≡ set yoghurt produced with lactic acid culture
- C2 ≡ set yoghurt produced with *B.infantis* 20088 , C3 ≡ set yoghurt produced with *B. longum* BB536
- C4 ≡ Set yoghurt produced with the mixer of strain BB536and strain 20088

CHAPTER FIVE

CONCLUSION AND RECOMMENDATION

5.1 Conclusion

The findings of the present study evaluated the physicochemical and microbial properties of set type's yoghurt which were inoculated with different *Bifidobacterium* (B.*infantis* and *B.longitm)* as compared with standard yoghurt inoculated with LAB culture. Strains growths were assessed at set yoghurt and for survival during the shelf life for 15 days.

Generally, yoghurt inoculated with *B.infantis* 20088 alone had the highest viable count among other types of yoghurts, followed by control yoghurt and *B.Longum 20088* yoghurt and finally yoghurt inoculated with mix *Bifidobacterium* cultures **.**In addition, it was more acceptable as it has the highest score compared to other type of yoghurt.

All results obtained during the process and shelf life study did not deviate from standard values of each specific quality parameters.

All type of yoghurt showed negative result for yeast, mould and coliform count thus they are safe. During the shelf life for 15 day all types of yoghurt contained viable count more than 7 log CFU/ml, therefore fulfill probiotic foods requirement.

5.2. Recommendation:

1- Encourage using probiotic strains *Bifidobacterium longiun* BB 536 and *Bifidobacterium Infants* 20088 for production of set yoghurt in Sudan.

2- Further studies are needed to scale up the production of the formulated set yoghurts and do consumer bases study.

3- Improve the quality characteristic of set yoghurt with probiotics to suit sensory preference of consumers in Sudan.

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