



Sudan University of Science and Technology
College of Graduate Studies



**Yoghurt Partially Supplemented with Barley Flour fermented with
Probiotic *Bifidobacterium longum* BB536**

***Bifidobacterium* الزبادي المدعم جزئيا بدقيق الشعير المخمر بالبكتريا الصديقة
longum BB536**

Dissertation Submitted to Sudan University of Science and Technology in
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الآية

قال تعالى:

(وَإِنَّ لَكُمْ فِي الْأَنْعَامِ لَعِبْرَةً لِّئْتَسِفَى كُمْ مِمَّا فِي بُطُونِهِ مِنْ بَيْنِ فَرْثٍ وَدَمٍ لَبَنًا خَالِصًا سَائِعًا لِشَّارِبِينَ)

صدق الله العظيم
سورة النحل الآية (66)

Dedication

this dissertation is dedicated to soul of my late father Ahmed Mustafa

To dear my mother Nafisa Khalid

To my dear Grandmother

To my dear Aunts and Uncles

To my dear Brothers and sisters

To my all Relative for their kind helps and support

Acknowledgment

First, almost grateful thanks to Allah for giving me health, patience, and assistance to complete this work.

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Abstract

The study was carried out to produce yoghurt from cow milk partially supplemented with barley flour fermented with probiotic *Bifidobacterium longum BB536*. The yoghurt was formulated with different levels of barley flour 2%, 4%, and 6%. Yoghurt A without Barley flour fermented with commercial starter culture. Yoghurt B without Barley flour fermented with *B. longum BB536*. Yoghurt C contains 2% Barley flour fermented with strain *longum BB536*. Yoghurt D contains 4% Barley flour fermented with strain *BB536*. Finally yoghurt E contains 6% Barley flour fermented with strain *BB536*. Proximate composition was carried out for cow milk, barley and yoghurt. Physical, Physiochemical, microbial, and sensory analysis were also carried out for yoghurt products. The addition of barley lead to significant ($P<0.05$) increases in protein and fat of yoghurt partially supplemented with barley as compare to control yoghurt. The highest protein content obtained in Yoghurt E due to the highest level of supplement barley, while the lowest protein content obtained in control yoghurt without barley supplement. The results also indicated that there was no significant ($P>0.05$) difference in total carbohydrates and lactose between different types of yoghurt except in yoghurt E. Microbiological analysis showed there was significant ($P<0.05$) difference in commercial starter culture and strain *BB536* levels in all yoghurt products. The highest bacterial growth was obtained in yoghurt A. The sensory evaluation indicated significant ($P<0.05$) difference in color, flavor, taste, texture, and overall acceptability between different yoghurt products supplemented with barley as compare to control except yoghurt B. Therefore it possible to produce fermented yoghurt partially supplemented with 2% barley containing *Bifidobacterium longum BB536*.

المخلص

أجريت هذه الدراسة لإنتاج زبادي من لبن البقر مدعم جزئياً بدقيق الشعير تم تخميره بالبكتريا الصديقة *Bifidobacterium longum* BB536. تم تجهيز خلطات الزبادي بإستبدال جزئي للبن البقر بنسب مختلفة من الشعير وهي 2%، 4% و6%. الزبادي A خالي من إضافة الشعير ومخمّر بالباديء التجاري، الزبادي B خالي من إضافة الشعير ومخمّر ب *B. longum* BB536، الزبادي C يحتوي على 2% شعير ومخمّر بالسلالة BB536، الزبادي D يحتوي على 4% شعير ومخمّر بالسلالة BB536. واخيراً الزبادي E يحتوي على 6% شعير ومخمّر ب السلالة BB536. تم إجراء إختبارات التحليل التقريبي للبن والشعير الخام والزبادي. كما تم إجراء إختبارات فيزيائية، فيزيوكيميائية، ميكروبيولوجية، وحسية لمنتجات الزبادي. أدت إضافة الشعير لزيادة معنوية ($P < 0.05$) في نسبة البروتين والدهون في الزبادي المدعم بالشعير مقارنة بالزبادي الخالي من الشعير. أعلى نسبة بروتين تم الحصول عليها في الزبادي E وذلك نتيجة لمستوى التدعيم العالي بالشعير وأقل نسبة بروتين تم الحصول عليها في الزبادي الخالي من إضافة الشعير. نجد أنه ليس هنالك فرق معنوي ($P > 0.05$) في نسبة السكريات الكلية واللاكتوز بين الأنواع المختلفه من الزبادي ما عدا الزبادي E. وبيّنت الإختبارات الميكروبيولوجية أن هنالك فرق معنوي ($P < 0.05$) في مستويات نمو بكتريا الباديء التجاري والسلالة BB536. أعلى نمو باكتيري تم الحصول عليه في الزبادي A. وضّح التقييم الحسي وجود إختلاف معنوي ($P < 0.05$) بين منتجات الزبادي في اللون، الرائحة، الطعم، القوام والقبول العام عند المقارنة بالعينة القياسية بإستثناء الزبادي B. وبناءً على ذلك يمكن إنتاج زبادي مدعم جزئياً ب 2% شعير يحتوي على البكتريا الصديقة *Bifidobacterium longum* BB536.

CHAPTER ONE

INTRODUCTION

Milk is a whitish liquid produced by the mammary glands of all adult female mammals after childbirth and serves as food for their young. (Guetouache *et al.*, 2014) Milk for human consumption must come from well nourished healthy lactating animals. Not from infected animals (resulting from inflammation of the udder), and animal undergoing a veterinary treatment. Milk is rich in protein, carbohydrates, mineral, vitamin and calcium. (Guetouache *et al.*, 2014). Cow milk contains more protein than does human milk, but human milk contains more lactose, resulting in comparable energy contents. (Bettoni and Burlingame, 2013).

Fermented milk are made with various lactic acid bacteria , including *bifidobacteria, lactobacillus acidophilus, specific strain of Lb.casei* and *bifidobacterium spp.* These Are the most commonly used probiotic bacteria in the manufacture of fermented milks these and some other microorganisms are thought to confer health and nutritional benefits to consumer, through their activity in the intestinal tract. The traditional yoghurt starter culture are ,*S. thermophilus* and *Lb.delbrueckii spp . bulgaricus*. The number of types of fermented milks made with probiotic microorganism has increase markedly over past few decades. These product may contain aprobiotic microorganism in addition to *S.thermophilus* and *Lb. delbreckii spp.bulgaricus*. Alternatively, *S.thermophilus* can be combined with one or two probiotics. The concentration of probiotics does not generally reach the level of that of youghurt bacteria. The resulting product are commercialized under trade names like Bioyoghurt, Biogade, and culture (Walstra,*et al.*,2006)

Probiotics (derived from Latin and Greek) means “for life” is defined in many ways. The most recent and accepted definition of probiotics is “live microorganisms administered in adequate amounts which confer a beneficial

physiological effect on the host” (Soccol *et al.*,2010). Joint (FAO and WHO, 2010) experts consultation report defines probiotics as: Live microorganisms which when administered in adequate amounts confer a health benefit on the host .(Iqubale *et al.*,2014)

Probiotic is contribute to intestinal microbial balance and play a role in maintaining health. The probiotic microorganisms consist mostly of the strains of the genera *Lactobacillus* and *Bifidobacterium*. Regular consumption of food containing probiotic microorganisms is recommend to establish a positive balance of the population of useful or beneficial microbes in the intestinal flora , Probiotic *Bifidobacterium* resist gastric acid, bile salts and pancreatic enzymes, to adhere to colonic mucosa and readily colonize the intestinal tract (Soccol *et al.*, 2010)

Triticale, oats and barley belong to the group of crops with high energy and nutritional value arising from a high content of biologically valuable proteins, high portion of lipids compared to other cereals, favourable saccharide composition as well as significant levels of dietary fibre, vitamins and mineral substances (Senhofa *et al.*, 2015).

Probiotic *bifidobacterium* strain were successfully incorporated in deferent fermented food based on dairy ,cereal ,legumes ,fruits ,and vegetables ,. The main carrier of *Bifidobacterium* is dairy based, however the energy value of fermented dairy is low since lactose is fermented by bacteria during fermentation process . The addition of carbohydrates such as cereals grain at a level that does not affect the general acceptability of youghurt well improve the energy value of dairy base food and decrease partly cost at the same time.

Objectives of this study are:

1. To evaluate the growth of *bifidobacterium longum BB536* on yoghurt partly supplemented with different levels barley flour.
2. To determine the physiochemical properties and chemical composition of prepared youghurt.
3. To evaluate the organoliptic characteristics and general acceptability of different made youghurt.

CHAPTER TWO

LITERATURE REVIEW

2.1 Definition of milk

In France, human milk consumption was defined in 1909 by the International Congress of Food by the following formula: "milk is the product of the total, full and uninterrupted milking of a dairy female in good health, also nourished and not overworked. It must be collected properly and not contain colostrums (Guetouache *et al.*,2014).

Milk is a whitish food generally produced by the mammary secretory cells of females in a process called lactation; it is one of the defining characteristics of mammals. The milk produced by the glands is contained in the udder. Milk secreted in the first days after parturition is called colostrums. The quality of milk is paramount; therefore, it must be properly stored and transported in optimal conditions. This vital product consists of four physical phases: A gas phase, which essentially comprises CO₂ at milking time. (Guetouache *et al.*,2014)

A fatty phase composed of cells, fat (2 to 5 μm of diameter) which contain lipids and fat-soluble elements, the fatty globules are surrounded by phospholipids and protein membrane.

A colloid phase comprising casein micelles associated with phosphates and citrates of calcium and magnesium.

An aqueous phase consisting of the soluble proteins (whey protein), lactose and minerals (electrolytes). There is an inverse relationship between the content of lactose and minerals, in order to keep the milk in relation with the isotonic blood plasma. .(Guetouache *et .al*, 2014).

2.2 The role of milk as a source of macronutrients

Milk is a major source of dietary energy, protein and fat, contributing on average 134 kcal of energy/capita per day, 8 g of protein/capita per day and 7.3 g of fat/capita per day (FAOSTAT, 2012). Water is the main component in all milks, ranging from an average of 68 percent in reindeer milk to 91 percent in donkey milk. The main carbohydrate is lactose, which is involved in the intestinal absorption of calcium, magnesium and phosphorus, and the utilization of vitamin D. (FAOSTAT, 2012).

2.3 Factors affecting milk composition

Milk composition is affected by various factors, including stage of lactation, breed differences, number of calvings (parity), seasonal variations, age and health of animal, feed and management effects including number of milkings per day and herd size (Bettoni and Burlingame, 2013).

2.4 Cow milk

Cow milk accounted for 83 percent of global milk production in 2010 (FAOSTAT, 2012). Cow milk contains more protein and minerals, especially calcium and phosphorus, than human milk. The protein in cow milk is of high-quality (defined as protein that supports maximal growth), containing a good balance of all the essential amino acids, including lysine. Many human diets are deficient in certain essential amino acids. For example, wheat and maize-based diets contain only 57 percent and 58 percent of required levels of lysine, and cassava-based diets are deficient in leucine, valine and isoleucine, containing only 79 percent of required levels, (WHO, FAO and UNU, 2007). more than 600 million people depend on cassava in Africa, Asia and Latin America for food security (FAO, 2002). Including milk (and dairy products) in staple-based diets increases availability of these limiting amino acids,

improving overall dietary quality (Bettoni and Burlingame, 2013). Cow milk and human milk differ in the amounts of various proteins they contain. Human milk does not contain β -lactoglobulin, one of the main proteins associated with cow milk allergy. Caseins comprise nearly 80 percent of the protein in cow milk but less than 40 percent in human milk. Caseins can form leathery curds in the stomach and be difficult to digest. In addition, the type of caseins that predominate in the two milks also differs, human milk containing more β -casein, which is more susceptible to peptic hydrolysis than α S-casein, particularly α S1-casein, which predominates in cow milk (Bettoni and Burlingame, 2013).

Cow milk generally contains between 3 and 4 g of fat/100 g, although values as high as 5.5 g/100 g have been reported in raw milk. Most milks consumed now contain a standardized fat content of around 3.5 g/100 g. The conjugated linoleic acid (CLA) content in cow milk is generally reported to vary from 0.1 to 2.2 g/100 g total FA depending on season, region, farming system and feeding, and animal and breed (Bettoni and Burlingame 2013). For example, milk from the Mafriwal cow breed was shown to contain a significantly higher ($P < 0.05$) percentage of CLA than Jersey cow milk (0.35 g/100 g total FA vs 0.23 g/100g total FA) (Yassir *et al.*, 2010).

2.5 History of fermentations

Fermentation is considered the second oldest method for preserving food in the world, after drying (Pallin, 2015). It is the process of transforming simple raw materials into different products with added value by exploiting the growth and activity of microorganisms on different substrates. People soon found other advantages of fermentation, *e.g.* not only could they store their food for a longer time, but they could also change the taste, texture and overall sensory sensation of that food. Other advantages regarding health and nutritional benefits also emerged (Pallin, 2015).

Fermentation is believed to have been used as soon as people started domesticating cows, sheep, goats *etc.* Even though they did not know exactly what happened, people realised that they could keep milk for longer if they stored it in animal stomachs. Being stored in stomachs curdled the milk and exposed it to lactic acid bacteria and other microbes present in the environment, which formed the first primitive cheeses. An advantage of fermented milk and other food items was that they did not have to be further processed, *e.g.* by heating or cooking. As soon as they had been fermented, they were ready to be consumed. For many thousands of years, people used the art of fermenting to produce different varieties of food items in order to extend the time for which they could store them or to achieve specific aromas or textures, without really knowing anything about the science behind this. (Pallin, 2015).

2.6 Fermented milk

The CODEX standard for fermented milks defines fermented milk as “a milk product obtained by fermentation of milk, which milk may have been manufactured from products obtained from milk with or without compositional modification by the action of suitable micro-organisms and resulting in reduction of pH with or without coagulation (Isoelectric precipitation). These starter micro-organisms shall be viable, active and abundant in the product to the date of minimum durability. If the product is heat-treated after fermentation the requirement for viable micro-organisms does not apply.” The standard specifies a minimum milk protein content of 2.7 percent m/m, and a milk fat content of less than 10 percent m/m. The CODEX standard also includes yoghurt and alternate culture yoghurt. (Bettoni and Burlingame, 2013). Although about 400 generic names are applied to fermented milks around the world, the real number of distinct products is much smaller (Khurana and Kanawjia, 2007). proposed a classification scheme that classifies fermented

milks according to the type of fermentation: a) lactic fermentations (with mesophilic-, thermophilic-, therapeutic- or probiotic-type fermentations); b) yeast–lactic fermentations; and c) mould–lactic fermentations).

2.6.5 Other fermented milk

Other traditional fermented milk products include *lassi* (buffalo, cow) and *shrikhand* or *chakka* (Afghanistan and India, from cow, sheep and goat milk); *taette* or Lapp's milk (Scandinavia, cow); *roub* and *mish* (Sudan, cow); *kule naoto* (Kenya, cow); *suusac* (Kenya, camel); acidophilus milk (Australia, various milks); cultured buttermilk (Scandinavian and European countries, from cow milk), *laban*, *leben* and *labneh* (Lebanon, Arab countries, from cow, sheep and or goat milk), *xynogalo* (Greece, sheep); *ymer* (Denmark, cow) and *shubat* (Kazakhstan, camel) (Zhang *et al.*, 2005).

2.7 History of Yoghurt

The history of yogurt goes back over six thousand years. It is believed that the word yogurt evolved from the Turkish word “*jugurt*” (Tesfaye,2013) Today, yogurt is known by different names in different regions in the world. In Finland it is called “*fili*” It is assumed that limited availability of milk due to dry desert surroundings in Middle East led to development of a yogurt like product. In Turkey, it was thought to be consumed as a preserved milk product (Tesfaye, 2013) Traditionally, Greek yogurt is prepared from ewe's milk, yet cow milk is used commercially. In South Asia the yogurt is called “*dahi*” and it exhibits soft coagulum, lumpy texture and mild acidic flavor. In India, “*raita*” is made from “*dahi*” with addition of grated cucumber or grated bottle gourd, black pepper, cumin seeds and coriander. Bulgarian yogurt has container surrounded with banana leaves. “*Taratur*” is a variety of yogurt made in Albania and a unique flavor and taste due to different

microbial strains in the yogurt preparation. In Indonesia different varieties of yogurt called “*dadiah*” are prepared by fermenting milk in a bamboo Republic of Macedonia by mixing yogurt with vegetables, walnuts, garlic, oil, and water. “*Rahm joghurt*”, yogurt with higher milk fat content (10%), is produced in Germany and other European countries. “*Matsoni*” is another variety of yogurt product made by using *Lactococcus lactis* which gives it a distinctive viscous texture. In Middle Eastern countries, such as Jordan and Palestine, yogurt named “*Jameed*” is combined with salt and dried for preservation (Tesfaye, 2013).

2.8 Definition of Yoghurt:

According to the Code of Federal Regulations of the United States Food & Drug Administration (FDA), yogurt can be defined as a 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, *Lactobacillus bulgaricus* and *Streptococcus thermophilus*. (FDA, 2013).

2.8.1 Type of Yoghurt:

Yogurt can be categorized into two different groups namely, standard culture yogurt and bio- or Prebiotic yogurt. Standard yogurt refers to those made with *L. bulgaricus* and *S. thermophilus*. These bacteria said to be not actually inhabit gut; however able to stimulate the friendly micro flora already present in the gut helping to maintain the general intestinal health. Bio yogurts are manufactured by culturing beneficial microorganisms that claim to have numerous health benefits once ingested, typically the probiotic strains of *bifodbacteria* and *L. acidophilus*. Unlike standard yogurt cultures, these probiotic strains are said to claim more specific health benefits and represent the types of friendly micro flora present in the gut.

According to the National Yogurt Association's guidelines, the refrigerated products should contain at least 100 million live cultures per gram and the frozen products should contain at least 10 million live cultures per gram at the time of manufacture in order to obtain the *live and active culture seal* (National Yogurt Association, 2013c.). Differentiation of yoghurt into diverse types according to legal standards, technique of production, flavor and post incubation processing have been suggested, depending on method of production, the industries recognize two main types of yoghurt that is set and stirred. This classification is based on the system of manufacturing and physical structure of the coagulum (Abdelkarim, 2010). Yoghurt is produced by lowering the pH of milk proteins to their isoelectric points (about pH 4.6) by the fermentation of lactose to lactic acid using starter bacteria . Yoghurts can be differentiated according to the fat content of the milk used to produce the yoghurt (non-fat, low-fat or whole, fat milk), the milk source (e.g. cow, buffalo, goat or sheep milks; for example, traditional Greek yoghurt is produced with full fat sheep milk) and processing of UHT-treated yoghurt, fruit-flavoured yoghurt, yoghurt drinks, smoothies and whipped or aerated yoghurt (Bettoni and Burlingame, 2013) . The milk used for yoghurt production varies, including milk concentrated by evaporation or filtration, by supplementing milk with milk powders or by reconstituting milk powders directly to the desired concentration . The milk is homogenized and heat-treated, with typical heat treatments being 85 °C for 30 minutes or 95 °C for 5 minutes. The milk is then cooled to 42 °C, inoculated with cultures and incubated at 42 °C for about 4.5 h, until the pH decreases (Bettoni and Burlingame, 2013)

The heating step leads to denaturation of whey proteins. These proteins, together with the caseins, precipitate at low pH, leading to the properties associated with yoghurt. (FAO and WHO, 2010)

2.8.2 Yoghurt as a Functional Food

Fermented dairy products, having the tradition as healthy foods, are a natural choice for their makeover as functional foods. A vast array of yogurts is now available in the market to suit all palates and meal occasions. Yogurts are available in a variety of textures (e.g. liquid, set, and smooth), fat contents (luxury, low-fat, virtually fat-free) and flavors (natural, fruit, cereal). The low-fat varieties of yogurt provide an array of important nutrients in significant amounts in relation to their energy and fat content, therefore making them a nutrient-dense food (Tesfaye, 2013)

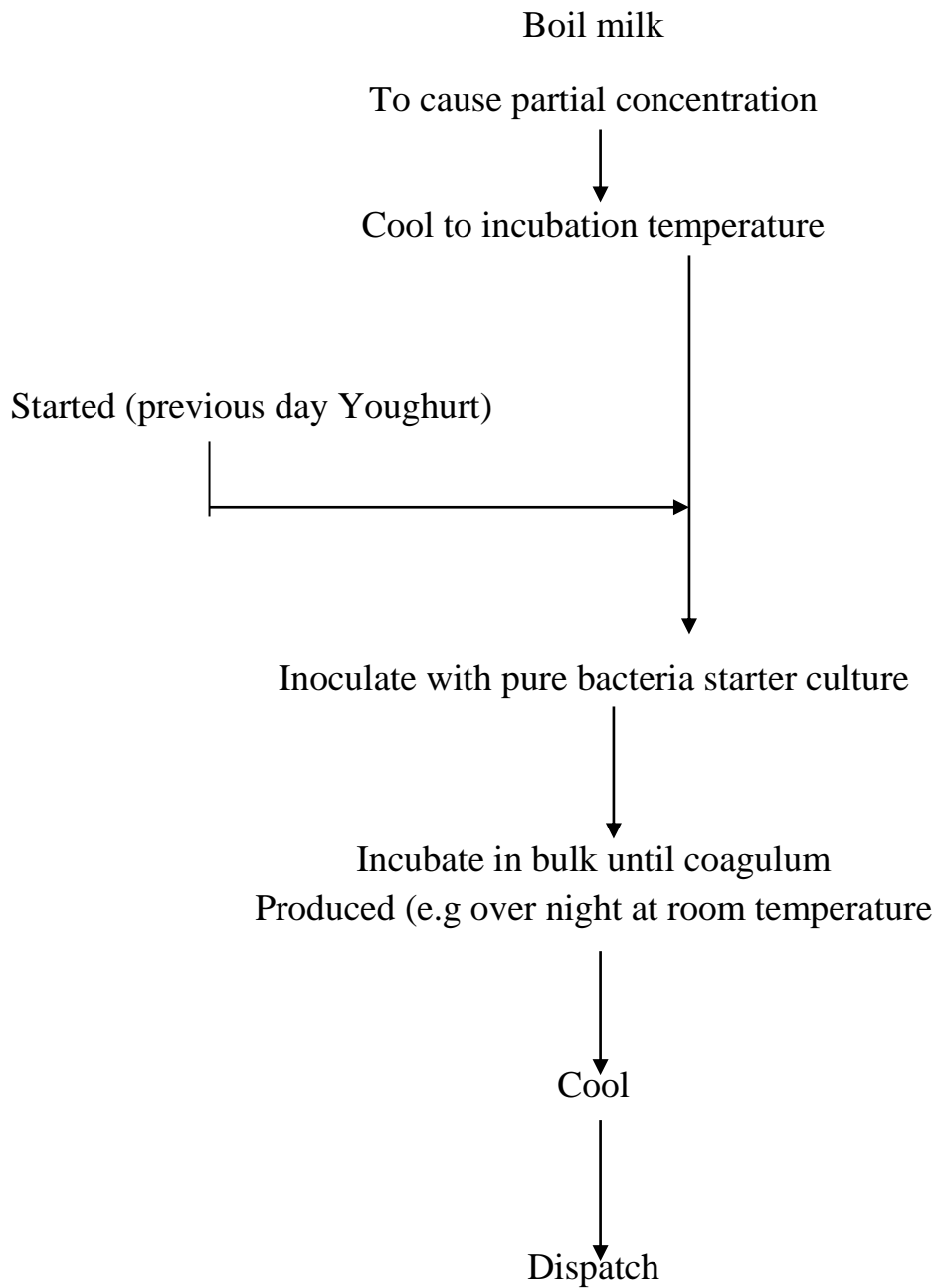
The healthy image of yogurt is further endorsed by the addition of various fruit preparations in yogurt to include the health benefits of fruits such as providing fiber and antioxidants . In recent years soymilk ,corn milk and peanut milk (Isanga and Zhang, 2009) yogurts are being developed as a vegetarian alternate to bovine milk yogurt that can also overcome the problem of milk protein allergenicity. The Australian standards define low-fat yogurt as ‘the yogurt prepared by culturing skim or low fat cow’s milk, resulting in a thickened, tangy yogurt and does not contain fruit or flavoring. It contains on an average 6.6% protein and 0.3% fat’. A starter culture can be defined as ‘a microbial preparation of large number of cells of at least a strain to be added to a raw material to produce a fermented food by accelerating and steering its fermentation process’ During fermentation, lactic acid is produced from lactose by the yogurt bacteria, *S. thermophilus* and *L. delbrueckii ssp. bulgaricus*. These LAB also produce acetic acid, ethanol, aroma compounds, bacteriocins, exopolysaccharides, and several enzymes. In this way they enhance shelf life and microbial safety, improve texture and contribute to the pleasant sensory profile of the yoghurt (Tesfaye, 2013)

2.8.3 Nutritional and health benefit of yoghurt

Milk and milk products such as yoghurt are good sources of some minerals. They are the best dietary source of calcium and have a calcium-to-phosphorus ratio that is conducive for optimal skeletal growth. The presence and amount of vitamin D in these products give them excellent calcium bioavailability .Yoghurt is also nutritionally rich in protein and the B-vitamins (riboflavin, vitamin B₆ and vitamin B₁₂). People who are moderately lactose-intolerant can enjoy yoghurt without ill effects due to the conversion of lactose to lactic acid during the fermentation of the product (Shah, 2007).

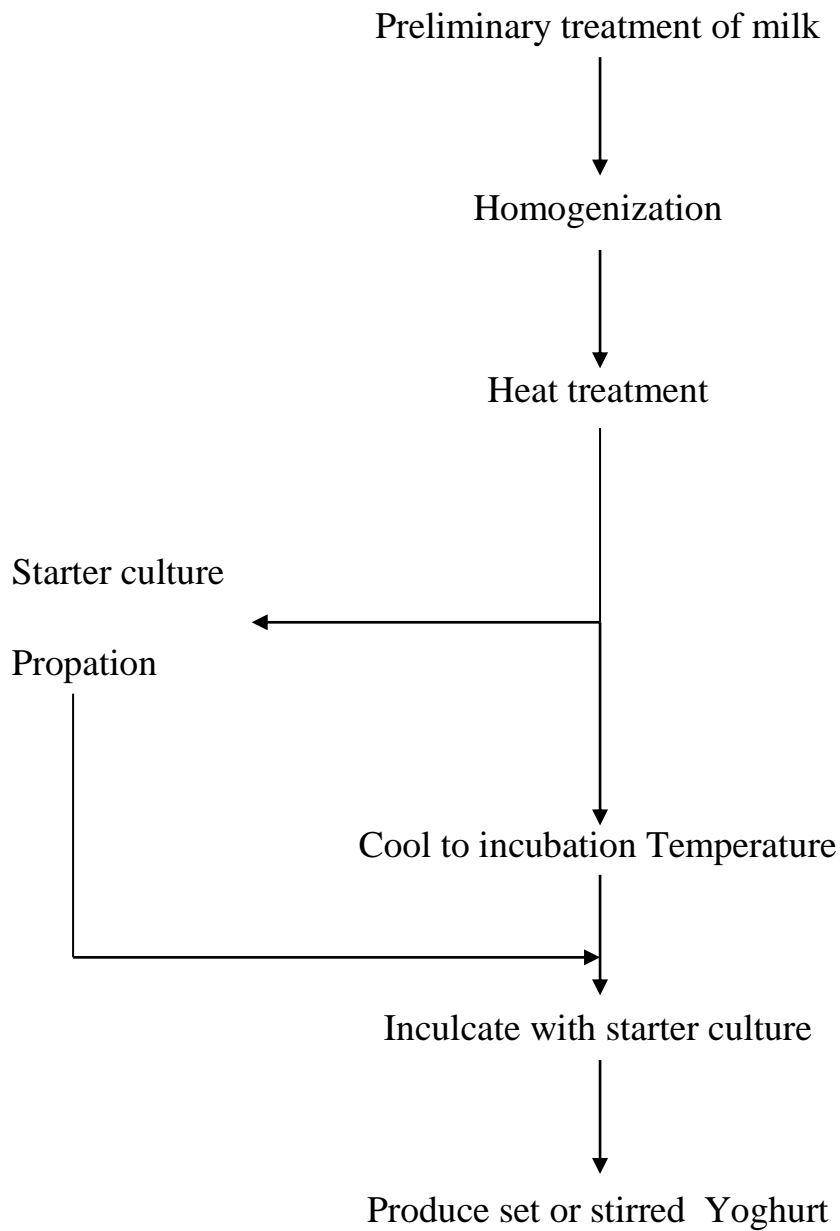
2.8.4 Flow diagram for yoghurt production

2.8.4.1 Traditional method



(Tamime and Robinson, 2000)

2.8.4.2 Improved process



(Tamime and Robinson, 2000)

2.8.4.1 Definition of Probiotics:

The name probiotic comes from the Greek 'pro bios' which means 'for life'. The history of probiotics began with the history of man; cheese and fermented milk were well known to the Greeks and Romans, who recommended their consumption, especially for children and convalescents.(Soccol *et al.*, 2010). Probiotics are defined as the living microorganisms administered in a sufficient number to survive in the intestinal ecosystem. They must have a positive effect on the host . The term 'probiotic' describe the 'substances secreted by one microorganism that stimulate the growth of another. probiotics are 'organisms and substances which contribute to intestinal microbial balance' .(Soccol *et al.*, 2010) In more modern definitions, the concept of an action on the gut microflora, and even that of live microorganisms disappeared. probiotics are the 'food which contains live bacteria beneficial to health'. Best exemplifies the breadth and scope of probiotics as they are known to- day: 'live microorganisms which, when administered in adequate amounts, confer a health benefit on the host .This definition retains historical elements of the use of living organisms for health purposes but does not restrict the application of the term only to oral probiotics with intestinal outcomes (Reid, 2006).

The probiotics in use today have not been selected on the basis of all these criteria, but the most commonly used probiotics are the strains of lactic acid bacteria such as *Lactobacillus*, *Bifidobacterium* and *Streptococcus* (*S. thermophilus*); the first two are known to resist gastric acid, bile salts and pancreatic enzymes, to adhere to colonic mucosa and readily colonize the intestinal tract (Soccol *et al.*, 2010).

2.8.4.2 The history of probiotics

The origin of cultured dairy products dates back to the dawn of civilization; they are mentioned in the Bible and the sacred books of Hinduism. Climatic conditions for sure favoured the development of many of the traditional soured milk or cultured dairy products such as kefir, koumiss, leben and dahi. (Soccol *et al.*, 2010). These products, many of which are still widely consumed, had often been used therapeutically before the existence of bacteria was recognized. At the beginning of the 20th century the main functions of gut flora were completely unknown. Ilya Ilyich Metchnikoff, the Nobel prize winner in Medicine in 1908, at the Pasteur Institute linked the health and longevity to ingestion of bacteria present in yoghurt. He believed that the constitution of the human body presented several disharmonies inherited from primitive mammals, such as body hair, wisdom teeth, stomach, vermiform appendix, caecum, and large intestine. (Soccol *et al.*, 2010)

In 1907, he postulated that the bacteria involved in yoghurt fermentation, *Lactobacillus bulgaricus* and *Streptococcus thermophilus*, suppress the putrefactive-type fermentations of the intestinal flora and that consumption of these yoghurts played a role in maintaining health. Indeed, he attributed the long life of Bulgarian peasants to their intake of yoghurt containing *Lactobacillus* species (Metchnikoff, 2004). In particular, he reported that the large intestine, useful to mammals in managing rough food composed of bulky vegetables, is useless in humans. Moreover, it is the site of dangerous intestinal putrefaction processes which can be opposed by introducing lactobacilli into the body, displacing toxin-producing bacteria, promoting health, and prolonging life (Piano, 2006). Tissier's discovery of *bifidobacteria* in breast-fed infants also played a key role in establishing the concept that specific bacteria take part in maintaining health.

In 1906, Tissier reported clinical benefits from modulating the flora in infants with intestinal infections. At the time, many others were sceptical about the concept of bacterial therapy and questioned in particular whether the yoghurt bacteria (*L. bulgaricus*) were able to survive intestinal transit, colonize and convey benefits. (Socol *et al.*, 2010). In the early 1920s, *L. acidophilus* milk was documented to have therapeutic effects, in particular, a settling effect on digestion. It was believed that colonization and growth of these microorganisms in the gut were essential for their efficacy, and therefore, the use of intestinal isolates was advocated. In Japan in the early 1930s, Shirota focused his research on selecting the strains of intestinal bacteria that could survive passage through the gut and on the use of such strains to develop fermented milk for distribution in his clinic. His first product containing *L. acidophilus* Shirota (subsequently named *L. casei* Shirota) was the basis for the establishment of the Yakult Honsha company. Only at the end of the century, it became clear that intestinal microflora had several functions, including metabolic, trophic and protective ones. Metabolic functions are primarily characterized by the fermentation of non-digestible dietary residue and endogenous mucus, savings of energy as short-chain fatty acids, production of vitamin K, and absorption of ions. Trophic functions are based on the control of epithelial cell proliferation and differentiation, and development and homeostasis of the immune system. Finally, protective functions are connected with the barrier effect and protection against pathogens. (Socol *et al.*, 2010)

The health benefits derived from the consumption of foods containing *Lactobacillus acidophilus*, *Bifidobacterium* and *L. casei* are now well documented. *Streptococcus thermophilus* and *L. delbrueckii* ssp. *bulgaricus* are yoghurt starter cultures, which offer some health benefits; however, they are not natural inhabitants of the intestine. Therefore, for yoghurt to be considered as a probiotic product, *L. acidophilus*, *Bifidobacterium* and *L. casei* are incorporated as dietary adjuncts.

Thus, the normal practice is to make a product with both starter organisms, e.g. *S. thermophilus* and *L. delbrueckii* ssp. *bulgaricus*, and one or more species of probiotic bacteria. The guidelines that stipulate what is required for a product to be called a probiotic were published by FAO/WHO in (2002). They require that strains be designated individually, speciated appropriately and retain a viable count at the end of their shelf life in the designated product formulation that confers a proven clinical end-point. The probiotic definition requires that the efficacy and safety of probiotics be verified and thus, assessment of this constitutes an important part of their characterization for human use. (Soccol *et al.*, 2010)

2.8.4.3 Health benefit of probiotics:

Probiotics commonly are isolated from human and animal intestinal tracts. Dead bacteria, products derived from bacteria, or end products of bacterial growth also may impart certain benefits, but these derivatives are not considered to be probiotics because they are not alive when administered. Native bacteria are not probiotics until the bacteria are isolated, purified, and proved to have a health benefit when administered. The original observation of the beneficial properties conferred by some bacteria is attributed to the Nobel Prize winner Eli Metchnikoff, who is regarded as the grandfather of modern probiotics. In the early 20th century, Metchnikoff discovered that –healthy bacteria, especially lactic acid bacteria (LAB), can have a positive influence on digestion and the immune system. Most microorganisms recognized to date as probiotics are Gram-positive, with *Lactobacillus* and *Bifidobacterium* being the main species used as treatments of intestinal dysfunctions. However, some Gram-negatives are also used as probiotics. The best example of this group is *Escherichia coli* Nissle 1917 (EcN) also known as Mutaflor, which has been used in Germany for many years in the treatment of chronic constipation and colitis. The vast majority

(90%) of the total cells in the body are present as bacteria in the colon, reaching 10¹² for every gram of large intestinal contents. Under natural conditions, a protective gut micro flora develops and there is no need for a bacterial supplement. But the changing food habits and lifestyle force us to take processed and sterile food, which affects our access to, and colonization, by certain type of bacteria. (Onyenweaku *et al.*, 2016).

Moreover, we also consume antibacterial substances ranging from vinegar to antibiotics. In the last century, many studies have reported probiotic bacteria to play important roles in the modulation of immunological, respiratory, and gastrointestinal functions (Floch *et al.*, 2011).

2.9 Criteria for classifying Microorganism as Aprobiotic:

1. It must be human origin.
2. Have nonpathogenic properties .
3. Resistance to technological processes (i.e. viability in delivery vehicle).
4. Stability in acid and bile.
5. Adhesion to target epithelial tissue .
6. Ability to persist within the gastrointestinal tract .
7. Production of antimicrobial substances
8. Ability to modulate the immune system
9. Ability to influence metabolic activities. (Onyenweaku *et al.*, 2016).

2.10 Characteristics of probiotics:

Certain physiological characteristics may be important for probiotics targeted toward particular applications. For example, resistance to stomach acid and pancreatic secretions such as bile and digestive enzymes would be important for probiotics needs to survive in high numbers through the small intestine. But if the target site for the probiotic is, for example, the

mouth, these traits would not be relevant. It is apparent from the broad range of potential probiotic targets that what is required of a probiotic depends on the specific target function. Yet some basic criteria for probiotics can be set: namely

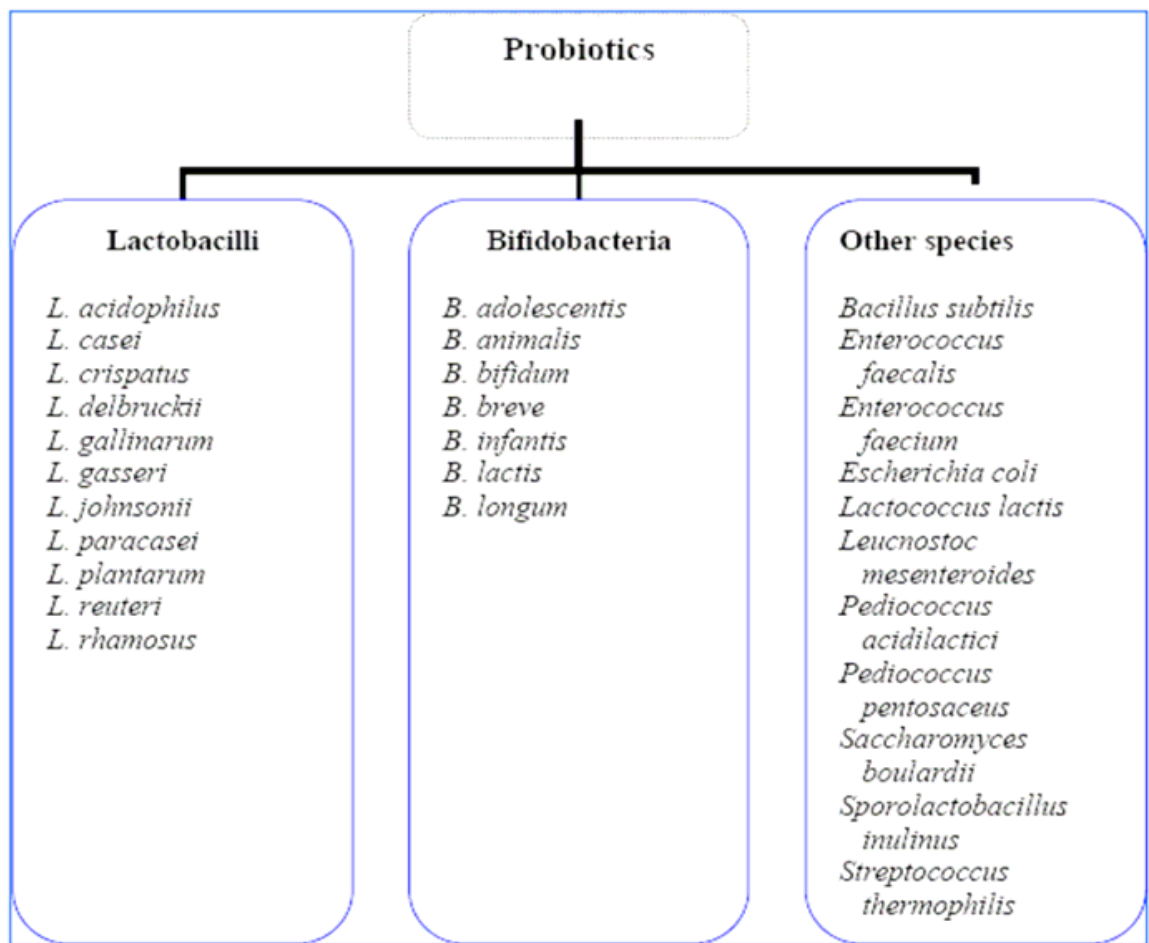
1. They are nonpathogenic, nontoxic, and free of significant adverse side effects.
2. They must be shown to exert a beneficial effect on the consumer, preferably with a mechanistic explanation of how this occurred.
3. They should retain stability during the intended shelf life of the product.
4. They should contain an adequate number of viable cells to confer the health benefit.
1. 5. Should be compatible with product format to maintain desired sensory properties (Onyenweaku *et al.*, 2016).

2.10.1 Carrier of probiotics

Yogurt is the most common source of probiotics. Yogurt consists of milk (usually from the cow, goat or sheep) fermented by bacteria that modify lactose into lactic acid. Lactic acid is responsible for giving yogurt its characteristics (sharp taste usually changed into good taste by using sweeteners and flavouring) and also denatures and precipitates casein, resulting in a semisolid consistency. “Bioyoghurts” are produced in a similar way, but bacteria used for fermentation are of different strains, usually *L acidophilus*. Fermented milk and fortified fruit juice are common sources of probiotics. Probiotics are also available in supplements consisting of freeze dried bacteria in tablets, capsules and powders. Selection of probiotic product depends on type of bacteria and type of beneficial effect expected. There are thousands of strains of probiotics and all of them

show different beneficial effects. (Iqbal *et al.*, 2014)

Fig 1: Different types of bacteria which are recognized as probiotics.
(Iqbal *et al.*, 2014)



2.11 Lactobacillus

1. There are more than 50 species of lactobacilli. They are naturally found in the digestive, urinary, and genital systems. Foods that are fermented, like yogurt, and dietary supplements also contain these bacteria. Lactobacillus has been used for treating and preventing a wide variety of diseases and conditions. Some of the lactobacilli found in foods and supplements are *Lactobacillus acidophilus*, *L. acidophilus* DDS-1, *Lactobacillus bulgaricus*, *Lactobacillus rhamnosus* GG, *Lactobacillus plantarium*, *Lactobacillus reuteri*, *Lactobacillus salivarius*, *Lactobacillus casei*, *Lactobacillus johnsonii*, and *Lactobacillus gasseri* . (Onyenweaku *et al.*, 2016).

2.12 Bifidobacterium

1. There are approximately 30 species of *bifidobacteria*. They make up most of the healthy bacteria in the colon. They appear in the intestinal tract within days of birth, especially in breastfed infants and are thought to be the best marker of intestinal health. Some of the bifidobacteria used as probiotics are *Bifidobacterium bifidum*, *Bifidobacterium lactis*, *Bifidobacterium longum*, *Bifidobacterium breve*, *Bifidobacterium infantis*, *Bifidobacterium thermophilum*, and *Bifidobacterium pseudolongum* (Onyenweaku *et al.*, 2016).

Bifidobacteria represent one of the most important bacterial groups within the Actinobacteria, usually present in the gastrointestinal tract of humans and other mammals and the hindgut of honeybees and bumblebees. They have also been isolated from waste and dairy products, where the sources could have been faecal contamination and intentional probiotic addition, respectively as lactic acid bacteria *bifidbacterium* are considered probiotic strains because of their beneficial effects and their role in

maintaining the health of their host. As has been well documented, *bifidobacteria* are generally host-species-specific bacteria; indeed, their occurrence and species composition in different animals is quite variable, suggesting a separation into ‘human’ and ‘animal’ groups (Michelini *et al.*, 2016).

2.13 Cereals

Cereals are crop plants from the grass family (*Poaceae*) and produce seeds (fruits) with high starch contents which are used for human consumption, animal feed production and industrial purposes. Among the many cultivated species of cereals, an increasingly important role is played by barley, rye and oats (Perkowski *et al.*, 2012). Cereals provide a very substantial proportion of the needs of the world's population for dietary energy, protein, and micronutrients. The major cereal crops are wheat, rice, and maize, but sorghum, millets, barley, oats, and rye are important only in some regions. Unprocessed cereals are low in fat, and a good source of fibre and phytochemicals. Cereal grains are made into a very wide range of cereal-based foods using traditional and technologically more advanced processes, which can result in changes in nutritional value (Price and Welch, 2013). Cereal itself contains high level of healthful micronutrients and macronutrients, compared to foods consumed during non-cereal breakfasts; cereal tends to facilitate consumption of other healthful foods at breakfast and replace consumption of less healthful foods; and cereal consumption may be a marker for a pattern of behaviour that includes healthful eating and high levels of physical activity throughout the day’.

Oats, maize, rye or wheat can be primarily used for the preparation of breakfast cereal and muesli. However, there are relatively few studies where the muesli cereals are triticale, barley and other cereals (Senhofa *et al.*, 2014 and Senhofa *et al.*, 2015).

2.14 The barley:

2.14.1 Scientific classification and Etymology:

Kingdom : Plantae - plants

Sub kingdom : Tracheobionta – vascular plant

Superdivision : Spermatophta – seed plants

Division : Manoliophyta – flowering plants

Class : Liliopsida – monocotyledons

Sub class : Commelinidae

Order : Cyperales

Family : Poaceae – grass family

Genus : Hordeum – barley

Species : H . vulgare

(Solomon and Weaver, 2003)

2.14.2 Barley

Barley (*Hordeum vulgare* L.) is among the most ancient of cereal crops . It was first domesticated from about 10,000 years ago in the Fertile Crescent of the Middle East .Presently , barley occupies fourth position among the cereal crops in the world. Although barley was used extensively as a food in the past, it has now been relegated to animal feed (about 60%), malt (about 30%), or seed (about 7%), with only a small amount (about 3%) for human food in most countries . Predominance of maize, wheat, and rice as main food grains has presently demoted barley to the status of “poor man’s bread” . (Das and Kaur, 2016) Besides the usual nutritional benefits of cereal grain, most importantly, barley cell wall has good amount of soluble dietary fiber, β -

glucans, chemically (1-3,1-4)- β -D-glucans distributed throughout the entire kernel, in which 30% are 1-3 and the remainder being 1-4 glycosidic linkage (Sullivan *et al.*, 2013, Spokane, 2010, Mantila, 2015). Cell walls of the starchy endosperm consist of 75% of β -glucans, 20% of arabinoxylan, 2% of cellulose and 2% of glucomannan. The walls of aleurone cells consist of 71% of arabinoxylan and 26% of β -glucans, with 3% of cellulose and glucomannan (Jamar, *et al.*, 2011). Due to β -glucans, barley as food increases viscosity in the intestinal contents, and thereby reduces absorption of glucose and trap bile acids, and function as hypoglycemic and hypocholesterolemic agent. In addition, bran in whole barley offers a source of insoluble fiber, the ingredient necessary for bowel clearance and hence to maintain colon health. Thus, use of barley as a food/food ingredient could be a preventive or controlling measure to check the alarming frequency of diabetic mellitus and other associated lifestyle disorder. (Das and Kaur, 2016)

2.14.3 Classification of Barley:

Barley is classified as spring or winter types (depending on whether they need a cold exposure, ranging from two to several weeks before making the transition to the reproductive phase of growth); two-row or six-row, based upon the fertility of the florets on the spike (in six-rowed barleys, all of the florets are fertile, leading to six vertical rows of seeds on the spike; whereas in two-rowed types only the central floret of the three at each node is fertile, and thus just two rows of seeds develop on opposite sides of the rachis); hulled (hulled barley is covered with palea and lemma, require dehulling to remove the tough inedible outer hull) or hull-less (barley has an outer hull that's so loosely attached to the kernel that it generally falls off during harvesting); and malting (high starch content) or feed (high protein) by end-use type. Based on grain composition, barley is further classified as normal, waxy or high amylose, high lysine, high β -glucans, and proanthocyanidin-free

(Das and Kaur, 2016) The number of rows on spike has no bearing on how the grain is used, that is decided mainly by regional preference. The main difference between two row and six row barley is that the latter contain more protein and therefore more nutritious (Pitzer, 2009). Hulled barley is preferred to hulless barley for malting and brewing because of the contribution of the hull to flavor development in beer and as a filtering aid during brewing .

2.14.4 Composition of Barley:

Whole barley grain consists of about 65-68% starch, 10-17% protein, 2-3% free lipids, 4-9% β -glucans and 1.5-2.5% minerals. Total dietary fiber ranges from 11-34% containing soluble dietary fiber within 3-20%. The non-starch polysachharides in barley are β -glucans, arabinoxylans, and cellulose, the major one being β -glucans; these modify the energy value of barley. (Das and Kaur, 2016) Significant differences in β -glucans content have been reported among barley types with various starch amylose contents, the average amount being 7.5% in high amylose, 6.9% in waxy, 6.3% in zero amylose waxy and 4.4% in normal starch types. The β -glucans content of barley grains is mainly determined by genetic factors and less by environmental factors. Hulless or de-hulled barley grain contains 11–20% total dietary fiber comprising 11–14% insoluble dietary fiber and 3–10% soluble dietary fiber. Waxy hulless cultivars generally exhibited much greater grain β -glucans content than normal covered cultivars, while there was no difference between two-row and six-row cultivars. Barley endosperm protein has moderate nutritional quality with protein efficiency ratio averaging 2.04 (Das and Kaur, 2016)

Amino acid composition of barley protein is similar to other cereal grains, however, lysine and threonine are the limiting amino acids followed by methionine and tryptophan. Moreover, high glutamine and proline and considerable cysteine content are its characteristics . Lipid levels in barley are

considerably low. The major fatty acids in barley triacylglycerol are palmitic acid, oleic acid, linoleic acid, and linolenic acid. Fatty acids in barley are similar to those in wheat except that barley tends to have more linolenic acid (Sullivan *et al.*, 2013) Barley is rich in fat-soluble vitamin E (tocotrienols) and contains varying amounts of vitamin B complex except vitamin B 12 the major elements in barley grain are phosphorus, potassium, calcium, magnesium, sulphur, selenium, and sodium the first two being the most abundant. Restriction of dietary oxalate intake is preferred to check kidney stone, and it is worth mentioning that barley is categorized as medium oxalate grain (Das and Kaur, 2016)

In terms of phytochemicals in barley, in addition to tocotrienols, the important ones are the sterols, flavanols, and phenolic acids. Barley grains contain much greater amounts of phenolic compounds (0.2–0.4%) than other cereal grains. The main flavanols found are the catechins, procyanidin B , and prodelfinidin B .From analysis of sixteen varieties ,the total amount of flavones ranged from 325 to 527 µg/g of fresh weight of barley flour, with no associations between proanthocyanidin levels and different barley types. also opined that the total amount of phenolic acids ranged from 604 to 1346 µg/g of fresh weight of barley flour, with ferulic acid as the dominating one. The amount of phenolic acids varied according to occurrence or lack of hull, with significantly higher levels in the hulled varieties (Das and Kaur, 2016)

2.14.5 Health Benefit and utilization in food:

Both animal studies and human clinical trials have shown a link between barley and health benefits focusing a decrease in the risk of chronic heart disease by lowering blood cholesterol, and an increased insulin response thus lowering the risk of type-2 diabetes · Blood lipids significantly reduce by diets containing barley in moderately hypercholesterolemic men , 3 g of barley β-glucans per day is a sufficient dietary intake to achieve a decrease in serum

total and low-density lipoprotein cholesterol · These beneficial effects may be attributed to the presence of β -glucans that increase intestinal viscosity leading to slow absorption of food *vis a vis* controlling blood glucose level and binding bile acids (Sullivan *et al.*, 2013) · When the bile acids are trapped in soluble fiber and subsequently excreted, stored cholesterol gets depleted to produce new bile acids · In an *in vitro* experiment, a direct logarithmic relationship between the viscosity and the β -glucans content of the acid flour extracts from 18 barley genotypes. Similar effect due to increased viscosity is also caused by arabinoxylans in barley (Das and Kaur, 2016)

CHAPTER THREE

MATERIALS AND METHODS

3.1 Raw materials:

The milk was obtained from College of Agricultural studies, Sudan University of Sciences and Technology, Department of Animals Production and immediately transfer to refergarotr till use. The Barley (local 46) was obtained from Agriculture Research Corporation Ministry of Agriculture .

3.2 Starter culture:

The strain of *befidobacterum logum* BB536 was obtained from Microbiology laboratory Department of Food Science and Technology, Sudan University of Sciences and Technology.

3.3 Chemicals:

The chemicals analytic grade was purchase from Carema Company, Khartoum.

3.4 Microbiological Media and L-cysteine.

3.5 Others

Cloves , Lab coat, Tissues ,Wiper, Aluminum foil and Packaging materials from Rodwan Super Market in Bahre

3.6 Methods:

3.6.1 Preparation of Barley powder:

The barley cleaned was prepared by manual dehulling and then ground by grinder type (KT NO 69444) at Cereals Technology Department, Food Research Center Ministry of Agriculture.

3.6.2 Preparation of Starter culture:

Bifidobacterium longum BB536 was obtained from the stock culture of Microbiology Laboratory (Department of Food Science Technology College of Agricultural Studies, SUST). The strain was maintained at 4°C in the more elaboration refrigerator and prepared by activation in milk, incubation at 37°C for 24h. Fifty ml of prepared culture add to 450ml sterilized milk (121°C for 15 min) followed by incubation at 37°C for 24 h. The conventional yoghurt starter culture was obtained from youghrt capo company

3.6.3 Yoghurt processing steps:

1. milk pasteurized in water bath at 65°C for 30minutes
2. Cooled to 45°C in case of conventional starter culture, or to 37°C in case of *Bifidobacterium longum* BB536 starter culture .
3. Increased the total soluble solid to 17 % by the added of milk powder.
4. Added of barley powder and starter culture to pasteurized milk
5. Shaked well.
6. Incubated at 45°C for 3hours for conventional starter culture and incubated at 37°C for 16 hours in case of bifidobacterium *longum* BB536.

The Samples:

A = Barley flour free and have commercial starter culture

B = Barley flour free and have Bifidobacterium starter culture

C = contains 2% Barley flour and have Bidobacterium starter culture

D = contains 4% Barley flour and have Bidobacterium starter culture

E = contains 6% Barley flour and have Bidobacterium starter culture

3.6.4 Enumeration of viable cell:

MRS media supplemented with 0.05% L.cysteine was used to enumerate *B. longum* BB536 and conventional starter culture was enumerated using the plate count technique. One ml of each yoghurt was diluted in peptone water followed by plating on Manns Rogosa agar (MRS). The plates were incubated at 37C° for 48 h the growth was calculated as colony forming unit per ml (CFU/ml)

3.6.5 Phsio-Chemical Analysis:

3.6.5.1 PH value:

The pH value of the different yoghurt was determined using a PH – meter (model HI 8521 micro process or bench PH /MV/c meter .Romani). Two standard buffer solution were added the pH meter at room temperature. The pH meter was allowed to stabilize for minute and the pH of fermented yoghurt was directly measured .

3.6.5.2 Titratable Acidity:

The Titratable acidity (TA) of the different fermented yoghurt was determined according to AOAC method (1990). Ten ml of the sample were weighted in to conical flask . Distilled water was add until the volume in the flask was 150ml .Then vigorously agitated and filtered .Twenty five milliliters of the filtrates were pipetted in porcelain dish. .Five drops of phenolphthalein was added and the sample was titrated against 0.1N NaoH till afain pink color that lasted for at least 30 second was obtained .Acidity of different yoghurt was calculated from the following equation :-

$$\text{Titrateable Acidity} = \frac{(\text{N NaoH}) * \text{mls (NaoH)} \times 0.9 \times 100}{\text{weight of sample}}$$

Were:

N=normality of NaOH

0.9=Factor of lactic acid

3.6.5.3 Chemical Determination of Total solids (TS):

Total solids =100 – moisture

3.6.5.4 Determination of lactose:

3.6.5.4.1 Preparation of standard solution:

The standard solution was prepared by dissolving 5ml lactose in 95ml of distilled water to give 5% (w/v) solution of monohydrate. One ml of this solution was diluted in 500ml volumetric flask to give 75 ml lactose /ml standard solution .The anthrone reagent was prepared by dissolving 150ml of anthrone in 100ml of 70% (w/v) sulfuric acid. The solution then cooled and stored over night .

3.6.5.4.1.1 Procedure:

One ml of milk and yoghurt was pipette in to 500ml flask with distilled water .the solution was then mixed thoroughly and 0.5ml was transferred to boiling tube (sample) standard stock solution (0.5ml) was transferred to asecond boiling (blank) . to each tube 10ml ice cooled anthrone reagent was added . the tube were then transferred to boiling water bath for 6min then transferred to an ice bath and held for 30min . The optical dencity (OD) was read at 625nm Lactose content (in mg / 100ml) was calculated as follows:

Lactose mg/100ml = OD of sample – OD of blank ÷ OD of standard –OD of blank *4.75

Where:

OD =optical density

3.6.5.5 Moisture content:

Moisture was determined according to the modified method of AOAC (1990). Five gram of the sample was weighted in dish using sensitive balance. After weighting the dish was transferred to an oven (Kat- NR-2851,Electrhelios,Sweden) at 105-+ 0.1 °C for 6 hours . Afterward the dish with sample was allows to cool at room temperature and then reweighted. Moisture content was calculated according to the following formula

$$\text{Moisture content (\%)} = \frac{M2 - M3}{M2 - M1} \times 100$$

Where :

M1 = mass of dish + cover

M2 = mass of dish + cover + sample before drying

M3 = mass of dish + cover + sample after drying

3.6.5.6 Chemical Determination of fat content:

Fat content was determined by Gerber method.youghurt samples were weighed to 10 ml in to milk butyrometer. 10 ml of sulphuric acid and 1ml of amylocohol were added. The tube was closed with toppe, the content were mixed thoroughly and immediately centrifuge at 110 rpm for 4 min . The tubes toppe were transferred down word to water bath at 65 C° for at least 3 min and read off the extending from the both of the upper flat Colum.

3.6.5.7 Chemical Determination of ash content

The ash content of youghurt ,was determined according to AOAC (1990) method. 2g of youghurt was weighted in aclean dry porcelain crucible and placed in muffle furnace model (tipofron 2A NO18203 Get Ran 1002) at 550C°for 6 hour .After that the crucible was transferred to desiccators ,

cooled to room temperature and weighted. the ash content was calculated as follow :

$$\text{Ash content (\%)} = \frac{W_1 - W_2}{\text{weight of sample}} \times 100$$

Were:

W1 = weight of crucible with ach

W2 = weight of empty crucible

3.6.5.8 Determination of protein content:

Protein content of different yoghurt was determined by Kjeldhal method according to AOAC (1990) method as follow :

1- Digestion :

Two gram of yoghurt samples was weighted in a flask and transferred to digestion flask with two tablest catalyst (mercury).

25 ml of concentrated sulphuric acid were add to the sample , the flask was placed on the digestion apparatus ,heated unit the mixture was colour less. Then the flask were allowed to cool.

2- Distillation

25 ml boric acid and three drops of bromocresol green + methy red indicator were added to each receiving flask .

The digested samples were transferred from digestion flask to volumetric flask and the volume was completed to 100 ml by distilled water. The receiving flask was placed on distillation rack with tip of the condenser extended below the surface of the acid. Immediately 5ml of the diluted sample were added from the funnel of the distillation apparatus ,then 10 ml NaOH (40%) was gently added. The distillation was continued until the volume in the receiving flask were 7ml then the flask removed from the distillatory .

3- Titration:

The sample in receiving flask were titrated against 0.1 N HCL . the color was change from green to purple . the nitrogen content was calculated as fallow :

$$N (\%) = \frac{\text{ml HCL} \times \text{Normality of HCL (0.1)} \times 0.014}{\text{sample weight}} \times 100$$

$$\text{Protein (\%)} = (N\%)*6.25$$

Where:

N = nitrogen content

0.014 = molecular weight of nitrogen / 1000

3.6.5.9 Determination of crude fiber:

It was determined according to AOAC (1990). Tow gm of defatted sample was weighted, 150ml of H₂SO₄ (conc.7.3ml/l) were added and then heated to boiling. The mixture was boiled for 30min and then filtered . The residue was washed three times with hot water, and then 150 ml of preheated KOH (12.89mg/l)were added and then heated to boiling . the system was boiled for 30min and then filtered .The residue was washed three times with hot water ,it was dried under suction and then in an oven at 150°C over night . The residues was weighed then placed in muffle furance at 550°C for 3hr till alight grey ash was formed then weigh to constant weight .

$$\text{Crude fiber \%} = \frac{(W1-W2)}{S(100-M)} \times 100$$

Where:

W1=weight of sample before ignition

W2= weight of sample after ignition

S = original weight of sample

M= moisture content of sample

Carbohydrates content = 100% - [moisture (%) + protein (%) + fat(%) + fiber (%) and ash (%)] .

3.7 Sensory evaluation of different yoghurt:

Sensory evaluation of yoghurt was carried out by selected person from staff and student of Food Science and Technology Department and from Animal Production Department at Colleague of Agricultural Studies, Sudan University of science and technology . The panelists were given a hedonic sensory test questionnaire to evaluate taste, texture, color, flavor, and overall acceptability of coded youghurt samples.

3.8 Statistical analysis:

Two sample t test and One way ANOVA tests were used to examine significant different between normally distributed data of triplicates independent measurement. Probability level of less than 0.05 was considered significant (P<0.05) all data were analyzed using version 17 Mintab Statistical Soft Ware for Windows (2007).

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 Chemical composition of raw material

Table 1 show the chemical composition of fresh cow milk and barley. The moisture content of cow's milk was 87.93% This result is similar to finding of Ahmed (2015) who reported 87.02% moisture content for fresh cow milk. Moreover, Geutouach *et al.*, (2014) also reported value of 87.2% moisture content for fresh cow milk. As presented in Table 1, the protein content of fresh cow milk was 4.01% this result was slightly higher than that reported by both Ahmed (2015) and Geutouach *et al.*, (2014). The first stated protein content of 3.62% and the latter 3.5% protein content for fresh cow milk , respectively. The slight variation in composition of different protein of milk is due to various factors, including stage of lactation, breed differences, number of calving, seasonal variations, age and health of animal, feed and management effects including number of milkings per day and herd size (Bettoni and Burlingame, 2013).

The fat content of fresh cow milk was 3.16%. However, both Ahmed (2015) and Geutouach *et al.*, (2014) had reported higher level of fat content of 3.7% and 4.36%, respectively. The lower level fat content in table 1 could be due to the higher level of protein 4.04% ,type of feeds, breed of cow and seasonal variations (Bettoni and Burlingame, 2013).

Table 1 presents ash content of 0.81% for fresh cow milk. Nevertheless, lower levels of 0.57% and 0.72% ash content for fresh milk were presented by Ahmed (2015) and Geutouach, *et.al*,(2014), respectively.

Lactose is the main sugar of milk. It is the fuel used by microorganisms during fermentation of dairy food. The lactose content of fresh milk was 4.06%. This result was comparable with the findings of Ahmed (2015) who

reported value of 4.42% and Geutouach et al., (2014) who found that lactose was content 4.9% for fresh cow milk.

The total solid of fresh cow milk was 12.07%. While Ahmed (2015) found that the total solid of fresh cow milk was 12.98%. The T.S of fresh cow milk depends on level of different component of milk.

The pH of fresh cow milk was 6.78 this result was similar to that reported by Ahmed (2015) who reported that the pH of fresh cow milk was 6.73. The titratable acidity of fresh cow milk was 0.18%, exactly as same result that reported by Ahmed (2015) found a similar acidity value of 0.18%.

Table 1 showed the chemical composition of barley. The moisture content of barley was 6.08%. This result was lower than that reported by Adhikari et al., (2015) who found moisture content 12.2 % for barley. But near to Elkarmany *et al.*, (2013) who reported 7.43% moisture content for barley. The variation in moisture content of different barley may be due to stage of harvesting and storage condition.

As presented the protein content of barley was 11.53% this result is similar to finding of Adhikari *et al.*, (2015) who reported 11.48% protein content for barley and these results it is near to Saulius *et al.*, (2016) who found 10.55% protein content for barley. While slightly lower than reported by Elkarmany *et al.*, (2013) who reported 8.88% protein content for barley.

The fat content of barley was 1.49% Both Adhikari *et al.*, (2015) and Saulius *et al.*, (2016) had reported similar result of 1.37% and 1.77% fat content respectively. Elkarmany *et al.*, (2013) reported 3.92% fat content of barley.

Ash content 2.12% for barley this result was not different from that reported by Saulius *et al.*, (2016) who reported 2.09% for barley. Adhikari *et al.*, (2015) found high value of 3.6% ash content for barley. Also Elkarmany *et*

al., (2013) reported 0.22% ash content which was lower compared with that by Adhikari *et al.*, (2015) which was 3.69% ash.

The fiber content of barley was 4.47% . Elkarmany,*et.al*, (2013) he stated 4.35% fiber for barley. While Adhikari *et al.*, (2015) presented lower level of fiber was 1.03% . Saulius *et al.*, (2016) reported higher level of 5.12% fiber content for barley. This variation in the fiber due to variation in dehulling stage.

The carbohydrates of barley was 75.22%.Same finding by Elkarmany *et al*, (2013) 75.30%. Where Adhikari *et al.*, (2015) reported 81.8% higher level of carbohydrates.

Table 1: Chemical composition of fresh cow milk and barley

Components	Fresh cow milk	Barley
Moisture	87.93 ± 0.18	6.08 ± 50
Protein	4.01 ± 0.04	11.53 ± 0.21
Fat	3.16 ± 0.13	1.49 ± 0.11
Ash	0.81 ± 0.00	2.12 ± 0.01
Carbohydrates	4.06 ± 0.08	75.22 ± 0.72
Fiber	-	4.47 ± 0.03
Lactose	4.06 ± 0.08	-
Total Solid	12.07 ± 0.18	-
pH	6.78 ± 0.04	-
Acidity	0.18 ± 0.00	-

4.2 Chemical composition of different yoghurt products

The chemical compositions of different fermented yoghurts are revealed in Table 2. There were significant ($p < 0.05$) differences in components of fermented yoghurt including moisture, protein, fat, ash, total carbohydrate, lactose and total solids. There was no significant ($p < 0.05$) differences in the moisture content between processed yoghurt A and E. Also no significant ($p < 0.05$) differences in moisture between yoghurt B and C. The highest moisture content was 84.72% obtained in yoghurt E. While the lowest moisture content of 82.86% was recorded in yoghurt D which was 82.68%.

There was significant ($p < 0.05$) differences in protein content between different types yoghurt E, B, C, and D except yoghurt A (Table 2). The highest protein content was 7.94% obtained in yoghurt E .while the lowest protein content of 6.57% obtained in yoghurt A. Elkarmany *et al.*, (2013) found protein content of 4.14% and Abdelmoneim *et.al*(2011) reported 3.22 for protein content.

The result presented in Table 2 showed no significant ($p > 0.05$) differences in fat content between all types yoghurt A, B, C, D, and E. The highest fat content was 4.97% obtained in yoghurt E. While the lowest fat content 4.66% recorded in yoghurt A. These results were comparable to findings by (Elamin ,2015)who institute that the fat content of yoghurt was 5.33%. Also Elkarmany *et al*, (2013) who reported 4.14% fat content for yoghurt.

Moreover, there was no significant ($p > 0.05$) differences in lactose content between all types of yoghurt A, B, C, D except yoghurt E (Table 2). The highest lactose content of 2.86% obtained in yoghurt A, but the lowest lactose content of 1.14% obtained in yoghurt E. During fermentation of milk lactose used by lactic acid bacteria to produce lactic acid (kmsf, 2005).

Moreover, there was no significant ($p > 0.05$) differences in total carbohydrate content between all types of yoghurt A, B, C, D except yoghurt E (Table 2). The highest total carbohydrate content was 3.05% reported for yoghurt A. While the lowest total carbohydrate content was 0.93% reported for yoghurt E. This result was comparable with Elkarmany,*et.al*,(2013) who found 7.08% total carbohydrate content which was higher.

Ash contents of yoghurt was significant ($p < 0.05$) different between different types of yoghurt. As existing in Table 2. there was no significant ($p > 0.05$) differences in ash content between yoghurt B and C. Also there was no significant difference between yoghurt D and E. The highest ash content was 1.36% obtained in yoghurt E. While the lowest ash content of 0.98% was obtained in yoghurt A. However (Elamin ,2015)and Abdelmoneim ,*et.al*,(2011) had reported a lower level of ash content which was 0.74% and 0.60% respectively moreover Elkarmany, *et.al*, (2013) found 0.94% ash content. The high level of ash due to supplementation with barley flour.

Total solids contents of yoghurt was significantly ($p < 0.05$) difference between different types of yoghurt. The highest total solid yoghurt was B (16.62%) while the lowest total solid content (15.18) was in yoghurt A. Elkarmany *et al.*,(2013) and Abdelmoneim *et al.*,(2011) had reported 17.63% and 7.10% for total solid content respectively.

Table 2: Chemical composition of different yoghurt products

Samples	Moisture	Protein	Fat	Ash	Total Carbohydrate	Lactose	Total Solids
A	84.72 ^a ± 0.25	6.57 ^d ± 0.01	4.66 ^b ± 0.21	0.98 ^c ± 0.00	3.05 ^a ± 0.27	2.86 ^a ± 0.01	15.18 ^b ± 0.04
B	83.31 ^b ± 0.10	7.72 ^c ± 0.03	4.67 ^a ± 0.012	1.22 ^{ab} ± 0.18	2.93 ^a ± 0.10	2.86 ^a ± 0.02	16.62 ^a ± 0.04
C	83.12 ^{bc} ± 0.06	7.84 ^b ± 0.02	4.91 ^a ± 0.01	1.20 ^b ± 0.03	2.90 ^a ± 0.00	2.91 ^a ± 0.01	17.20 ^a ± 0.79
D	82.86 ^c ± 0.12	7.92 ^a ± 0.01	4.96 ^a ± 0.00	1.34 ^{ab} ± 0.02	2.85 ^a ± 0.08	2.94 ^a ± 0.02	17.14 ^a ± 0.12
E	84.57 ^a ± 0.28	7.94 ^a ± 0.01	4.97 ^a ± 0.01	1.36 ^a ± 0.02	0.93 ^b ± 0.02	1.14 ^b ± 0.23	15.43 ^b ± 0.28

* Values are mean ± SD for triplicates independent analysis.

** Values that bear different superscript letter in the same column are significantly different at p<0.05.

A = Yoghurt without Barley flour fermented with commercial starter culture.

B = Yoghurt without Barley flour fermented with *B. longum* BB536.

C = Yoghurt contains 2% Barley flour fermented with strain *longum* BB536.

D = Yoghurt contains 4% Barley flour fermented with strain *BB536*.

E = Yoghurt contains 6% Barley flour fermented with strain *BB536*.

Yoghurt A without Barley flour fermented with commercial starter culture.
Yoghurt B without Barley flour fermented with *B. longum BB536*. Yoghurt C contains 2% Barley flour fermented with strain *longum BB536*. Yoghurt D contains 4% Barley flour fermented with strain *BB536*. Finally yoghurt E contains 6% Barley flour fermented with strain *BB536*.

4.3 Lactic acid bacteria and *Bifidobacterium longum* BB536 growth in yoghurt products

Table 3 showed *lactic acid bacteria* and *bifidobacterium longum* BB536 growth in different types of yoghurt significant ($p < 0.05$) as well as pH and the acidity. The highest lactic acid bacteria growth of 6.65 Log CFU/ml was in yoghurt A followed by *Bifidobacterium longum* BB536 growth in yoghurt B. While the lowest *Bifidobacterium longum* BB536 growth of 3.79 Log CFU/ml was in yoghurt E. This growths of lactic acid bacteria and Bifidobacterium were accompanied by significant ($p < 0.05$) reduction in PH and significant ($p < 0.05$) increases in acidity (Table 3). The pH of different types of fermented yoghurt ranged between 4.68 - 4.21 PH, while the acidity ranged between 1.70% - 1.36%. The lower growth level of strain BB536 in yoghurt C, D, and E may be due to raising level of barley supplement.

Table 3: *Lactic acid bacteria* and *Bifidobacterium longum* BB536 growth, pH, and acidity of different yoghurt products

Sample	pH	Acidity	Growth of <i>lactic acid bacteria</i> and <i>bifidobacterium longum</i>
A	4.68 ^a ± 0.00	1.36 ^e ± 0.00	6.65 ^e ± 0.03
B	4.59 ^b ± 0.00	1.45 ^d ± 0.01	6.57 ^a ± 0.71
C	4.51 ^c ± 0.01	1.56 ^c ± 0.00	5.63 ^b ± 30
D	4.38 ^d ± 0.04	1.60 ^b ± 0.02	4.72 ^c ± 0.02
E	4.21 ^e ± 0.01	1.70 ^a ± 0.01	3.79 ^d ± 0.01

* Values are mean ± SD for triplicates independent analysis.

** Values that bear different superscript letter in the same column are significantly different at p<0.05.

A = Yoghurt without Barley flour fermented with commercial starter culture.

B = Yoghurt without Barley flour fermented with *B. longum* BB536.

C = Yoghurt contains 2% Barley flour fermented with strain *longum* BB536.

D = Yoghurt contains 4% Barley flour fermented with strain BB536.

E = Yoghurt contains 6% Barley flour fermented with strain BB536.

4.4 Scenery properties of *lactic acid bacteria* and *Bifidobacterium longum* BB536 fermented yoghurt products

The scenery characteristic of yoghurt different products declared significant ($p < 0.05$) differences in color, flavor, taste, texture, and overall quality. For instant, Table 5 shows there was significant ($p < 0.05$) differences in color between different types of yoghurt as compared with the control yoghurt A. The highest color score was 1.22 which was excellent obtained in yoghurt A. While the lowest color score of 3.77 was in yoghurt E. It is clear that the color of yoghurt was affected by the level of barley flour supplementation. There were no significant ($p > 0.05$) differences in flavor, taste, texture and overall acceptability between yoghurt A and yoghurt B.

Table 4: Scenery characteristic of lactic acid bacteria and *Bifidobacterium* fermented yoghurt products

Samples	Color	Flavor	Taste	Texture	Overall Quality
A	1.22 ^c ± 0.42	1.55 ^c ± 0.78	1.55 ^c ± 0.61	1.61 ^c ± 0.69	1.38 ^c ± 0.60
B	2.16 ^b ± 0.85	2.05 ^c ± 0.72	2.11 ^c ± 1.02	2.11 ^c ± 1.02	2.00 ^c ± 0.84
C	2.72 ^b ± 0.95	3.33 ^b ± 1.02	3.50 ^b ± 0.78	3.05 ^b ± 0.87	3.38 ^b ± 0.91
D	3.05 ^{ab} ± 1.30	3.44 ^{ab} ± 1.04	3.55 ^b ± 0.92	3.16 ^b ± 1.33	3.66 ^{ab} ± 1.02
E	3.77 ^a ± 1.21	4.22 ^a ± 0.80	4.33 ^a ± 0.76	4.27 ^a ± 0.75	4.38 ^a ± 0.69

* Values are mean ± SD for triplicates independent analysis.

** Values that bear different superscript letter in the same column are significantly different at p<0.05.

A = Yoghurt without Barley flour fermented with commercial starter culture.

B = Yoghurt without Barley flour fermented with *B. longum* BB536.

C = Yoghurt contains 2% Barley flour fermented with strain *longum* BB536.

D = Yoghurt contains 4% Barley flour fermented with strain *BB536*.

E = Yoghurt contains 6% Barley flour fermented with strain *BB536*.

CHAPTER FIVE

CONCLUSION AND RECOMMENDATION

Conclusion:

According to the finding of this study , It is possible to manufacture yoghurt from cow's milk supplemented with barley .

Addition of high level of barley to youghrt has effect the color ,increase acidity and precipitate. However , it is possible to use 2% barely supplement yoghurt to obtain youghurt with acceptable characteristic having probiotic effect.

Recommendation:

It is recommended that more investigation are needed to:-

- 1- Encourage the use of cereals in yoghurt supplementation.
- 2- Optimize the growth of *lactobacillus* and *bifidobacterum longum* BB536 in yoghurt supplement with barley.
- 3- Further research is needed to use other sources of milk like goat's and camel's milk in yoghurt supplement with barley.

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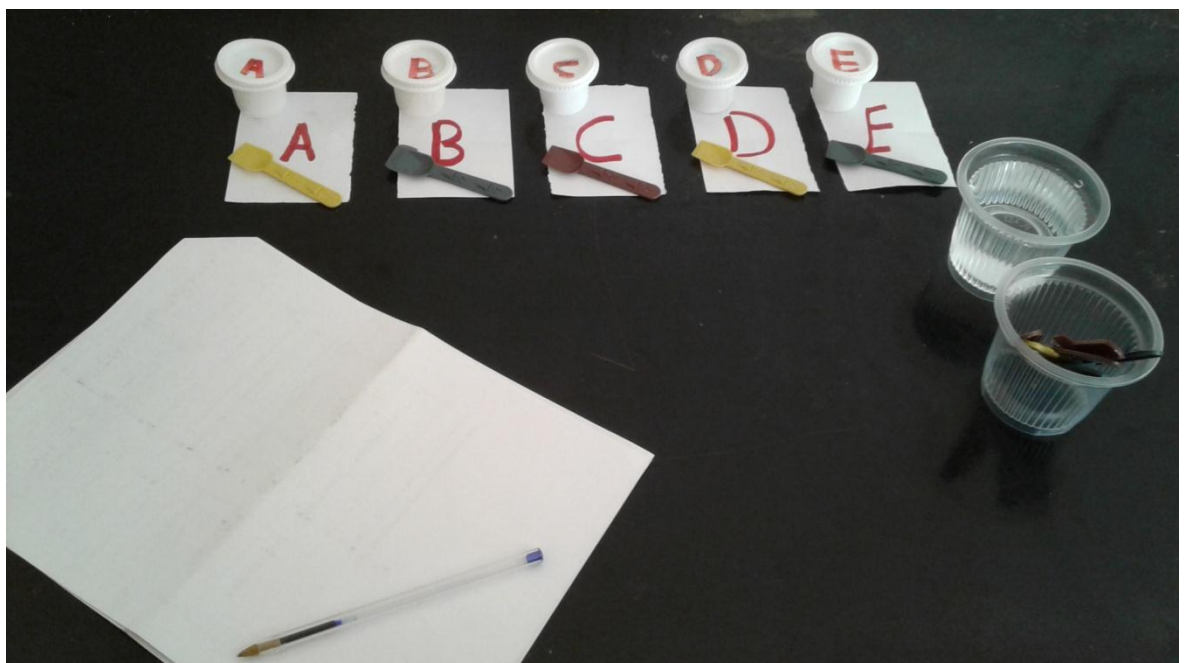
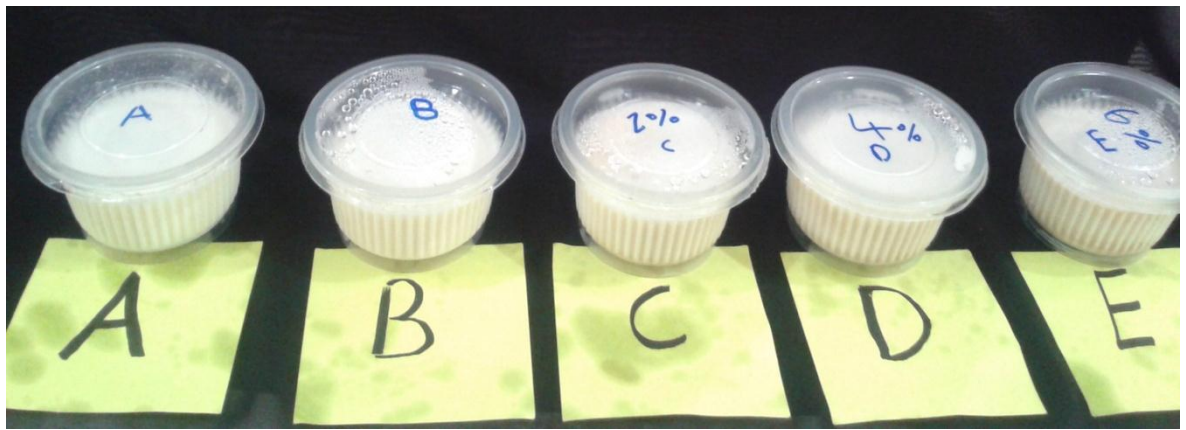
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Appendices





Sensory evaluation of bread samples

Please evaluate the following samples of supplemented Youghurt according to their colour, Flavor, taste, texture, Overall quality,. The ranking scores are given below:

1= Excellent 2= Very good 3= Good 4= Acceptable 5= unacceptable

Samples	Colour	Flavor	Taste	Texture	Overall Quality
A					
B					
C					
D					
E					